SEASONAL INCIDENCE AND MANAGEMENT OF BROOD DISEASES AND GREATER WAX MOTH (*Galleria mellonella* L.) IN HIVE BEES

Thesis

by

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Dr. YASHWANT SINGH PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY SOLAN (NAUNI) HP - 173 230 INDIA

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CERTIFICATE-I

This is to certify that the thesis titled **'Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees'** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy Entomology** in the discipline of **Plant Protection** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP)-173 230 is a bonafide research work carried out by **Ms. Sapna Devi (H-2017-06-D)** daughter of Shri Pritam Singh under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Nauni, Solan
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_____
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CERTIFICATE-II

This is to certify that thesis titled **"Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees"** submitted by **Ms. Sapna Devi (H-2017-06-D)** daughter of Shri Pritam Singh to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (HP) - 173 230 India in partial fulfillment of the requirements for the degree of **Doctor of Philosophy Entomology** in the discipline of **Plant Protection** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Place: Nauni, Solan
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LIST OF ABBREVIATIONS

%	:	per cent
/		per
@	:	at the rate
<	:	less than
>	:	greater than
°C	:	degree Celsius
°F	:	degree Fahrenheit
amsl	:	above mean sea level
CD	:	Critical difference
cm		Centimetre
df	:	Degree of freedom
E	:	East
EFB	:	European foulbrood
et al.	:	et alii (and others)
etc.	:	et. cetera (and the other things)
Fig.	:	Figure
g	:	Gram
GWM	:	Greater wax moth
H.P.	:	Himachal Pradesh
i.e.	:	id est (that is)
kg	:	Kilogram
m	:	metre
min	:	minute
ml	:	millilitre
mm	:	millimetre
N	:	North
NSKE	:	Neem seed kernel extract
SBV	:	Sacbrood virus
Spp.	:	species
w.r.t	:	With respect to

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Chapter-1

INTRODUCTION

Honey bees are highly valued resource insects which are directly beneficial to man and provide valuable products like pollen, honey, royal jelly, bee wax, propolis and bee venom. Honey bees occupy a prime position among all the pollinating agents because they can be managed in artificial nests i.e. hives and can be placed in desired numbers whenever and wherever required (Goderham, 1950). Further, Honey bees play vital role in agriculture by assisting in pollination of a wide variety of crops and help in maintaining biological diversity (Johannesmeier and Mostert, 2001). At present four species of the genus *Apis* are known for honey products and pollination of crops. Of these, *Apis cerana* F., *Apis dorsata* F. and *Apis florea* F. are native to India, whereas, *Apis mellifera* L. was introduced in the country during 1960 (Atwal and Goyal, 1973).

Honey bees are susceptible to variety of diseases, pests and environmental threats. While it is impossible to identify single factor which on its own can account for all colony losses in all regions of the world over a given time period, it is clear that several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality by adversely affecting colony health and lifespan. Among these factors, certain honey bee diseases and parasites have shown to play a significant role in increasing honey bee colony mortality and colony losses (Genersch, 2010).

The presence of honey, bee wax, brood, pollen, nectar and favourable environmental conditions available inside the hive invite a number of enemies. The bee brood constitutes a good protein source which attracts many pests, predators and diseases. During different developmental stages, it is subjected to attack by number of diseases caused by various organisms such as viruses, bacteria, fungi, mites etc. (Butler, 1945; Bailey, 1963).

Honey bee diseases like American foulbrood, European foulbrood, chalk brood, stone brood, sacbrood, Thai sacbrood virus and *Nosema* are worldwide known diseases. Among these, the American and European foulbrood diseases are of bacterial origin, affecting only the brood. From India no severe incidence of any foulbrood disease has ever been reported. Singh (1961) however, noticed the first incidence of American foulbrood in *A. cerana* colonies in Jeolikot (Nainital, UP) so far.

European foulbrood disease in the colonies of *A. mellifera* is very common in other countries but there were no reports from India till I986 (Mishra and Sihag, 1987). European foulbrood occurs world-wide wherever is *A. mellifera* (Bailey,1960). The causative bacteria responsible for this devastating disease is *Melisococcus pluton* (Greek name: Melissa - bee, coccus-berry) (Bailey and Collins, 1982). It mainly attacks on the larvae in the coiled stage. Affected larvae become slightly displaced in the comb cells. The diseased brood appears faint yellow in colour and emits sour odour (Bailey *et al.,* 1982). However, in India, there is an isolated report on the incidence of this disease in *A. cerana* from Mahabaleshwar (Maharashtra) which killed 25-30 per cent colonies during 1970 (Diwan *et al*., 1971).

It reappeared after a long period of three decades in the northwest part during 2002 (Rana *et al.* 2004). The disease was reported to have some symptoms similar to Thai sacbrood virus (TSBV) but no virus particles observed under electron microscope (Rana *et al.* 2004; Rana *et al.* 2012). Under field conditions the symptoms were, larvae pale yellowish colour, slightly displaced which died at age of 3 to 5 days and emitted vinegar-like smell. The causal organism was a bacterium, *Melissococcus plutonius*, confirmed by the Institute of Microbial Technology, Chandigarh, India (Rana *et al.,* 2012).

Virus diseases viz. sacbrood and Thai sac brood have also been posing threat to hives of *A. mellifera* and *A. cerana* in different parts of the world. American foulbrood and European foulbrood diseases are of bacterial origin killing only the brood. European foul brood disease is one of the important bacterial diseases which is prevalent throughout the country in *A. cerana* and *A. mellifera* (Rao *et al*., 2015).Thai sac brood virus (TSBV) infects larvae of the honey bee *A*. *cerana* resulting in failure to pupate and death. TSBV specifically infects *A. cerana indica* and is not known to cause infection on *A. mellifera* (Aruna *et al*., 2014).

In India Thai sacbrood appeared in *A. cerana* for the first time in 1979 in Meghalaya and later appeared in the Almora and Nainital districts in Uttar Pradesh by the end of 1982 (Kshirsagar *et al.,* 1982). Sacbrood virus disease affecting about 15 per cent *A. mellifera* colonies appeared in Himachal Pradesh in 1998. Later sacbrood virus was confirmed in other northern states. The virus primarily affects the brood resulting in perforation of sealed brood, prepupal death due to failure in pupation and accumulation of ecdysial fluid around the body and integument, forming a sac like structure. The colour of brood changes from pearly white

to pale yellow. Sacbrood virus infection appears mainly in spring when the brood rearing begins (Rana *et al.,* 2011).

The introduction of European honey bee (*A. mellifera* L.) into Asia has increased the total number of distinct species on the continent. This has completely changed the scenario of honey bee diseases for *A. mellifera* in Asia and throughout the rest of the world. Viruses have been spread by *A. mellifera* beekeepers migrating or shipping bees to new areas. In view of the fact that all bee species in Asia often occupy the same area, the problem of the disease has become especially urgent. A number of serious outbreaks of disease have already been caused in new area resulting in immeasurable economic costs to small and large beekeepers.

Another main problem of colony losses is the occurrence of several enemies such as the hive beetle, wasps, ants, termites, mites, birds, mammals and moths, causing heavy losses. Bee enemies weaken the colony, decreasing honey production and pollination. Among these bee enemies, the wax moth; *Galleria mellonella* L. resulted in a greater loss to the beekeeping industry responsible for the considerable economic losses that reach 60 to 70% of beekeepers in developing countries (Kapil and Sihang, 1983 and Hanumanthaswamy *et al.,* 2009).

The wax moth belongs to the subfamily Galleriinae of the family Pyralidae in which the females characteristically lay their eggs in beehives. This subfamily consists of two species known to be pests of the beehive, the greater wax moth *G. mellonella* and the lesser wax moth *Achroia grisella*. Both of these species have the same type of scavenging habits, but the lesser wax moth does not cause much damage, and hence is not a serious problem of beekeeping (Charriere and Imdorf, 1997). Attention will, therefore, be given to the greater wax moth.

Many people consider greater wax moth as a useful insect because its larvae are used as fish bait in many countries so much that they are raised commercially to get its larvae. However, it causes major losses to commercial beekeepers every year. Almost all colonies of Asian honey bees are prone to moth infestation (Adalakha and Sharma, 1975; Brar *et al*., 1985; Viraktamath, 1989). Normally, the wax moth attacks only abandoned beehives, or active ones in which the bee colony has been weakened. The beekeeper is more likely to see the adult moth, but it is the larval or caterpillar (worm) stage that causes damage to wax comb. They have acute sensory capability to find and exploit beeswax. Wax moths do

damage during their larval stages, destroying combs and honey, but adults do not feed since they possess atrophied mouth parts (Charriere and Imdorf, 1997).

Greater wax moth (Lepidoptera: Pyralidae, *G. mellonella*) is the ubiquitous pest of colonies of bees. They cause silken galleries in the combs and destroy the bee-wax combs where bees store pollen, honey and lay eggs. Wax moths wait for the opportunity to invade the comb and stored combs are the ideal places for their breeding. Wax moths are nocturnal in their activity and during day they hide themselves in crevices in dark places. They remain in dark, warm, poorly ventilated places where bees do not defend well (Ellis *et al.,* 2013). The weak colonies are not able to repel the moth, the attack is usually due to malnutrition, disease, large scale mortality of worker bees, loss of queen etc.

It is present throughout the world with rare exceptions in high elevations. Colonies of *A. cerana indica* have been reported to abscond due to infestation with the wax moth (Leong, 1990). During dearth and monsoon period, damage is increased many folds in *A. mellifera* L. and *A. cerana* L. colonies. In *A. dorsata* L. Moth infests all stages of brood, cells, pollen and honey region. Wax moth larvae can reduce the combs to a mass of web and debris. Severe infestation leads to suspension in brood rearing, foraging activity and ultimately desertion of colony from the nest (Thakur, 1991).

In India, it is observed that infestation of wax moth occurs throughout the year in both higher and lower altitudes, but peak infestation was recorded during May to September. Brar *et al.* (1985) recorded 16 to 19 per cent infestation in *A. mellifera* colonies in north India. It causes the greatest damage in apiaries which lead to financial losses every year, beside damaging wax combs by larval feeding, and destroying frames and wooden parts in the hive. Severely infested combs are reduced to web mass and debris. Summer, monsoon and autumn are the most vulnerable time periods of the year for wax moth activity on stored combs in India. The prevalence of *G. mellonella* infestation is common, however, the intensity depended on colony strength and prevailed ecological conditions during various seasons at different regions (Raghunandan and Basavarajappa, 2014).

Keeping in view the importance of different pests and diseases which affect and pose a threat to beekeeping industry, the present studies were carried out with the following objectives:

b) Objectives:

i) To study the seasonal incidence of brood diseases, mites and greater wax moth in hive bees in relation to colony and weather parameters

ii) To study the management of greater wax moth in stored combs and in field conditions

iii) Studies on management of brood disease in hive bees

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Chapter-2

REVIEW OF LITERATURE

Honey bee colony is a rich source of food with brood, adults, honey, pollen and bee-wax attracting a variety of pests, predators, and diseases. Honey bees are susceptible to a variety of diseases and pests; trophyllatic behaviour and frequent interaction among colony bees facilitate the spread of pathogens. Absconding, swarming, and drifting behaviours enable disease to spread within the colony, colony to colony, and apiary to apiary with more ease (Bailey, 1963).

American foulbrood, European foulbrood, Sac brood virus, Thai sac brood, Kashmir bee virus, and varroasis are all important honey bee diseases. *Melissococcus plutonius*, a bacterium, causes European foulbrood disease in honeybee's young larval stages (Bailey and Collins, 1982).

Greater wax moth (*G. mellonella*) one of honey bees natural enemy, causes massive damage by tunnelling into combs and gradually weakening colonies (Singh, 1962 and Shah, 1983).The attack of the wax moth might sometimes result in the destruction of the entire colony. In weaker colonies, this pest becomes more severe.

The available literature on *A. mellifera* and *A. cerana* colony parameters, diseases, particularly Thia sacbrood disease, sacbrood disease and European foulbrood, Ectoparasitic mites in relation to weather and hive parameters and wax moth seasonal incidence along with its management in the stored combs and apiary is presented under following headings:

2.1 Colony Strength (*A. cerana* and *A. mellifera*)

2.2 Brood diseases and mites in *A. mellifera* and *A. cerana*

2.2.1 European foulbrood

2.2.2 Thia sacbrood and sacbrood

2.2.3 Management of brood diseases

2.2.4 Ectoparasitic mites

2.3 Wax moth

2.3.1 Seasonal incidence of greater wax moth (*G. mellonella*)

2.3.2 Studies on management of greater wax moth in stored combs under laboratory conditions

2.3.3 Studies on management of greater wax moth in apiary

2.1 COLONY STRENGTH

Chhuneja (1991) reported that pollen storage and colony size have a linked but independent influence on brood rearing in *A. mellifera* colonies. Blaschm *et al.* (1999) studied the effect of poor weather on the development of a honeybee colonies brood area and pollen storage. The amounts of food stored and brood was measured during three periods of good weather (each lasting six days) and three periods of severe weather (5 days each). The number of large larvae declined dramatically and heavily during bad weather periods, while the number of small larvae increased. The number of large larvae in the colony changed in lockstep with the amount of pollen in the colony. It indicated that a decrease in pollen stocks during adverse weather may affect nurse bees brood-feeding behaviour, resulting in underfed larvae that may be sealed at a lesser weight.

Puskadija *et al.* (1999) demonstrated that climatic factors such as air, temperature, relative humidity, amount of precipitation, maximum and minimum air temperature, wind and wind force, have a considerable impact on *A. mellifera's* daily activity.

Rana and Goyal (1994) reported that the pollen and brood areas were at their peak in the summer, while colony strength was at its peak in the autumn. The amount of pollen in the colonies indicates their health and can be used to forecast the honey yield at the end of the season. Strong colonies (10.50 kg/colony) generated more honey than weak colonies (7.17 kg/colony).Summer was the best time for brood rearing in both strong and weak colonies.

Sharma (2002) studied that the egg laying rate, brood survival, brood rearing efficiency, relation between sealed brood and bee population, presence of pollen on frames, and swarm forecasting of the indigenous honey bee *A. cerana* in the Katrain area of District Kullu and the Nauni area of District Solan of Himachal Pradesh. In the months of April and March, respectively, the maximum egg laying rate was 523.37 and 319.3 eggs per day at Katrain and Nauni. Even within the same colony, brood survival rates fluctuated throughout the year. However, during March and August, 2002 the average adult emergence on an egg basis at Katrain was 55.22 and 61.22 per cent, respectively. While, it was just 19.68 per cent in Nauni during April, 2001. There was no correlation between sealed brood and bee popupation. The presence of pollen and brood on the same frame, however, was found to have a strong positive correlation.

Shawer *et al.* (2003) reported that summer season yielded maximum brood in both strong and weak colonies. Pollen trapping had a negative impact on colony development, as brood rearing was reduced by 25.16 per cent and 50.72 per cent in two consecutive years, respectively and the usage of traps reduced clover and cotton honey output by 50.61 per cent and 78.05 per cent and by 85.39 per cent and 98.14 per cent, respectively.

Bhusal and Thapa (2004) observed that in Chitwans during the litchi flowering season (February to April, 2003), researchers studied the effects of initial colony strength of *Apis mellifera* L. on honey production with four levels of initial populations: 4 frames (9,800), 6 frames (14,700), 8 frames (19,600), and 10 frames (24,500) of adult honeybees per colony replicating 5 times. Colony parameters including colony strength, brood rearing, comb construction, foraging activity, and honey output all had a significant positive linear correlation.

Gowda *et al.* (2005) studied the foraging and hive profiles of the Indian honey bee *A. cerana* and discovered that pollen foragers were most active between 9.00 and 11.00 hours while nectar foragers were most active between 13.00 and 16.00 hours. From January to April, the number of nectar foragers were at its peak. However, in November and December the number of pollen foragers were highest followed by March and April. During the same time period, the maximum brood, pollen and honey stores were also recorded.

Neupane and Thapa (2005) studied pollen gathering, storage, and its impact on *Apis mellifera* brood production throughout the year in Nepal's Terai region. During different seasons the number of pollen foragers, the amount of pollen stored as beebread and the amount of brood in the colony varied dramatically. During the spring season the highest number of pollen foragers (117.5 bees/hive/5 min), amount of pollen as beebread (2439.0 gm/hive) and number of brood cells (14787.2 brood cells/hive) were observed. While in rainy season the lowest number of pollen foragers (38.1 bees/hive/5 min.), least amount of beebread or pollen store (152.5 gm/hive) and lowest number of bees were observed. Pollen collection and brood production were normal during the autumn, winter, and summer seasons but starvation and nutritional deficiencies caused by a severe lack of pollen during the rainy season were the primary cause of bee population reduction or collapse before the honey flow season.

Neupane *et al.* (2007) evaluated the effect of the original strengths of honey bee colonies supered in different ways for honey production by *A. mellifera* bees in the Terai region of Nepal. Three distinct ways were used to super the bee colonies of three different strengths in which the bees covered 5, 10, and 20 combs. Honey production was shown to be substantially associated with the amount of worker brood cells in the colonies. Farmers in Nepal used 5 comb initial strength (CIS) colonies, which generated the least amount of honey (30.1 kg per annum). Bees in 10 CIS colonies with deep supers produced twice as much honey (62.2 kg) and colonies of 20 CIS with deep supers produced even more honey (74.5 kg).

Mohapatra *et al.* (2008) investigated the causes of absconding in *A. cerana indica* F. in coastal Orissa. The brood area was maximum in April - May (313.4 - 398.4 cm^2) and November-December (331.7 - 372.5 cm^2), according to the average of two years' data. In April-May and November-December, respectively the maximum pollen stores (54.6-63.6cm^2 and 55.8-73.7cm^2) were recorded. However, during periods of higher brood rearing i.e., April-May (12.0-30.5/5 minutes) and November-December (25.0-26.0/5 minutes), a relatively large number of pollen foragers were observed. Before the commencement of desertion, a comparison of the normal and absconded colonies in terms of the foregoing characteristics revealed that suitable measures were required to maintain the brood area, pollen store, and honey stores throughout April-May, the period corresponding to the end of the honey flow season.

Shahi *et al.* (2010) reported that under stationary and migratory conditions in *A. mellifera* L. and *Apis cerana indica* F., the comparative honey yield potential during 2007 and 2008 at Farmer's apiary indicated more honey in *A. mellifera* (32.640 and 55.660 kg/colony) than *A. cerana indica* (7.600 and 10.570 kg/colony) colonies. In the following year (2008), a similar trend was seen in the production of honey by both species under both the conditions, however the quantity of honey produced was lesser than in the previous year of research. Both species generated more honey in migratory conditions than in stationary conditions. *A. mellifera* honey production was more during both stationary and migratory condition than *A. cerana indica*.

Taha and Al-kahtani (2013) conducted research at the Agriculture and Veterinary Training and Research Station in Saudi Arabia on the relationship between colony strength and stored pollen, worker brood rearing, colony population density, and honey production of

A. mellifera. The strong colonies had much more stored pollen and worker sealed brood, colony population size, and honey yield than the weak colonies ($p<0.001$). During clover and sidir seasons, the strong colonies produced 286.80 and 291.67 per cent more honey than the weak colonies, with an average increase of 289.24 per cent. The research findings suggest that weak colonies should be united to become strong in order to achieve high rates of stored pollen and brood production, as well as a high honey yield.

Naik and Hugar (2014) studied the seasonal inicdence of natural enemies in honey bee colonies in five places in the Uttar Kannada district of Karnataka: Honnavar taluk, hilly region-Badal (Kumta taluk), malnad region- Nanikatta (Sirsi taluk), plains- Malagi taluk (Mundgod taluk) between July 2012 and June 2013. During one-year study period, no cases of Thai sac brood virus were found. Throughout the study period, pests such as *Vespa cincta*, *G. mellonella*, *Oecophylla smaragdina*, and *Camponotus compressus* were seen and honey yields ranged from 30.42 to 38.95 kg/5colony.

Chaand *et al.* (2017) investigated the impact of different colony strengths (6, 8, and 10 frames hive) on colony performance including as brood area, pollen, and honey storage in *A. mellifera* colonies in Jammu From June 2013 to May 2014. In all of the test colonies with varying bee strength, the colony parameters in terms of brood area, pollen area, and honey stores fluctuated. From October to June, the highest brood development was seen in 10-frame beehives. The highest pollen area was found in all three colonies from January to June, with the highest colony strength 10-frame beehive. From March to August, the highest honey stores in all three colonies was reported. Depending upon the level of adaptation and colony strength, the foraging activity showed a rhythmic pattern. The incoming and outgoing bees were not in equilibrium at any time. For maximal honey production and colony strength, the study clearly demonstrated that enough levels of brood, pollen, and honey reserves should be present in the colonies initially.

Chaudhary *et al.* (2017) conducted an experiment at PAU Ludhiana and PAU Regional Research Station at Bathinda during February-April 2014, to determine the effect of bee strength (5, 10, 15, 20, 25, and 30 bee-frames) on the performance of *A. mellifera* colonies under stationary and migratory beekeeping on Eucalyptus blooming. Under both stationary and migratory situations, there was a constant increase in worker brood rearing, pollen, and honey reserves as bee strength increased. More worker brood (15850.9 cm^2 in migratory, 14513.0 cm^2 in stationary) was reared in 30 bee-frame colonies, as were pollen

stores (2765.4 cm^2 in migratory, 2685.7 cm^2 in stationary), honey production (5.87 kg in migratory, 3.58 kg in stationary), and bee strength (32.0 bee-frames in migratory, 30.6 bee-frames in stationary). Over 5 bee-frame strength, under migratory and stationary conditions, the 30 bee-frame strength colonies, bee strength, worker brood rearing, and honey stores increased by 21.9 bee-frames, 11,206.9 cm^2, 2,234.1 cm^2, 9.85 kg and 17.3 bee-frames, 10482 cm^2, 1818.9cm^2, 3.70 kg and 17.3 bee-frames, 10482 cm^2, 1818.9cm^2, 3.70 kg, respectively. In migratory conditions, colony development and productivity were significantly higher than in stationary conditions.

Brar *et al.* (2018) reported that experimental colonies of *A. mellifera* gained strength during February (4.2 bee frames/ colony) and March (4.6 bee frames/ colony) when the build-up flora was in bloom at Nauni, Solan so the colonies became stronger, averaging 6.2 and 6.6 bee frames per hive in June and May, respectively. The average brood area was much higher in May (3143.6cm^2), followed by June (2497.6 cm^2) and April (2497.6 cm^2). In the month of April, the maximum pollen area (160 cm^2) was reported. Throughout the year, average honey storage in *A. mellifera* colonies at Nauni remained low. Under migratory conditions, in March, when honey bees foraged for nectar and pollen on the *Brassica juncea* crop at Hisar, the increased average colony strength (8 bee frames), brood area (5399.2 cm^2), and pollen area (520 cm^2) was observed. So the condition of stationary beekeeping was improved in terms of criteria such as bee strength and brood area, although pollen area and honey store remained low. So migratory beekeeping is more profitable in terms of food reserves.

Naveen and Yadav, 2021 studied the colony records of *A. mellifera* colonies under stationary conditions. In the first fortnight of November 2019, they recorded the minimum colony strength (3.30 bee frames/colony) and the maximum number of bee strength (8.30 bee frames/colony) in the second fortnight of January 2020. In the second fortnight of October 2019, brood area was significantly low (1160.34 cm^2 /colony) and was significantly high (4013.67 cm^2 /colony) in the first fortnight of January 2020. In the first fortnight of November 2019, pollen area was at its minimum (345.0 cm^2 /colony) and it was maximum (1720.71 cm^2 /colony) in the first fortnight of February 2020. In the second fortnight of January 2020, the honey store reached its peak (1660.50gm/colony).

2.2 BROOD DISEASES AND MITES IN *A. mellifera* AND *A. cerana*

2.2.1 European foulbrood

European foulbrood disease in *A. mellifera* has long been documented in the United Kingdom (Cheshire and Cheyne, 1885) and the United States of America (White, 1907). It

has been reported in Canada (Katznelson *et al.,* 1952), Switzerland, France, England, USA (Morgenthaler, 1955), Argentina (Camugli, 1962), Nepal, and Thailand where *A. mellifera* exists (Thapa *et al.,* 2000).

Bacillus alvei or a combination of *B. alvei* and *Streptococcus apis* was once supposed to be the source of European foulbrood. *Bacillus pluton*, a lanceolate Gram-positive bacteria, was later named and described as the causal organism. This is the first of several organisms that have been discovered in diseased larvae. Later, it was thought to be a dissociant form of *B. alvei*, *Bacterium eurydice*, or both (Bailey, 1956).

The European foulbrood disease initially appeared in India in 1970 in *A. cerana*, killing 25-30% of colonies in the kharad district of Maharashtra (Bailey, 1974). It was discovered in Himachal Pradesh and the Dharwad district of Karnataka in 1998, killing 25-40% of colonies in some apiaries (Viraktamath, 1998).

M. plutonius was the cause of European foulbrood disease. It was found in North and South America, Europe, Japan, Australia, India, China, and South Africa, and was of major economic importance (Jamaludin *et al.,* 2002).

Engles *et al.* (2004) reported that because of the development of fungal diseases throughout the winter honey bee colonies were weakened, which was the main cause of European foulbrood disease. They also stated that environmental factors have a significant influence in the onset of diseases. The rise and fall in temperature in the spring reduced the hive's ability to maintain an appropriate microclimate, and the brood in peripheral combs was reared at temperatures below the optimal. This reduced larvae resistance and provided the ideal environment for harmful microorganisms to flourish, causing European foulbrood disease.

Rana *et al.* (2004) reported that *A. cerana* brood died during prepupal and pupal stages due to European foulbrood disease with symptoms similar to Thai sac brood sickness and mite infestations. The larvae had a 15% mortality rate, were pale yellowish in colour and somewhat displaced, died after 3 to 5 days, and emit a vinegar-like odour (Rana *et al*. 2012). The confirmation of the causal organism i.e., a bacterium *M. plutonius* was confirmed by the Institute of Microbial Technology, Chandigarh, India.

According to Somerville (2004), European foulbrood disease was caused by a change in seasonal conditions as well as other stress-related factors such as dietary deficits, bee shifting and dominance by older workers, especially in the early spring season.

Russenova and Parvanov (2005) found that the European foulbrood often appeared in the spring and first half of the summer season. The spread of European foulbrood disease in the colony was caused by bees removing diseased or dead bee larvae.

Abrol and Ball (2006) conducted a survey in various apiaries in the Jammu division to monitor European foulbrood disease and its diagnosis during the years 2003-2004. They reported that the disease was present in 10-15% of the colonies. Sudden weakening of the colonies occured due to this disease. However, the brood pattern was scattered with larvae that were twisted and have creamy-white guts visible through the body wall. The gram-positive bacterium *M. plutonius* lives in the intestine and competes for food with the larva. Both larva and bacteria survive if food is sufficient. When food is scarce, however, the bacteria scavenge it, causing the larvae to starve and die. During dearth period the disease was noticed and higher infestation was found when the larvae were less than 48 hours old and died while still in coiled stage. In unsealed cells, dead coiled larvae were present and dead and dried scales were stiff and rubbery. Infected larvae, prepupae and occasionally pupae altered colour from dazzling white to yellowish brown then blackish dark brown. The dead brood cell cappings were punctured, sunken, and convex. Some pre-pupae and pupae had tongues that protruded outwardly. The adult bees were lethargic, and unable to fly, and they died shortly after emerging.

Fathy *et al*. (2012) performed a survey in four districts of the Dakahlia governorate (Mansura, Metghamer, Al-Met salsil, and Bilgas) from September 2009 to August 2011. The American and European foulbrood disease which affected honey bee larvae was found to be widespread in all districts. During the autumn and winter, AFB disease was one of the most damaging diseases of the honey bees in all apiaries and it spread during the spring. EFB disease was a bacterial infection that affected honey bee larvae before they reached the capped stage, and it spreaded throughout all districts over the spring and summer.

Brar *et al.* (2019) studied the seasonal incidence of diseases and enemies of *A. mellifera* under stationary and migratory conditions. Under stationary conditions incidence of European foulbrood disease ranged from 17.00 to 29.00 per cent. Highest incidence (29.00

%) was observed in the month of September 2015 which was statistically at par with June 2016 (27.00) and July 2016 (26.00%). The disease reduced significantly in the month of August (7%) and disappeared from October, 2015 to April, 2016 when brood was healthy in experimental colonies at Nauni, Solan. Under migratory conditions the incidence of European foulbrood disease was high from July, 2015 to September, 2015 (26.33 to 38.00 %) which reduced subsequently in the month of October, 2015 (16.78%). The European foulbrood incidence was observed in *A. mellifera* colonies in different winter months which were statistically low being 4.90, 1.92 and 2.32 per cent, respectively during November 2015, January and February 2016.

European foulbrood disease incidence was statistically maximum (23.00%) in the month of July, 2016 when temperature, relative humidity and rainfall were high. Maximum incidence of Thai sacbrood disease was recorded during the month of May, 2016 (2.33%) when humidity was low (46.00%). Thus the external weather parameters also influenced the disease incidence in the hive of *A. cerana* (Negi *et al.,* 2018).

Naveen and Yadav (2021) demonstrated that no incidence of EFB disease in *A. mellifera* was observed from first fortnight of August to first fortnight of September 2019 and first fortnight of November 2019 to first fortnight of February 2020 in Morena, Madhya pradesh. The incidence of EFB was found Maximum at 16.0 percent during study period in the first fortnight of October 2019. Incidence of EFB had significantly negative association with colony strength (r= -0.522) and brood area (r= -0.627). The incidence of European foulbrood was found positively correlated with temperature (r= 0.341), and negatively correlated with rainfall (r= -0.144) and relative humidity (r= -0.494).

2.2.2. Thai sacbrood and sacbrood disease

The Sacbrood virus affects honey bee brood and causes larval mortality (Ritter, 1996). Symptoms of sacbrood disease in *A. mellifera* colonies included partially perforated, scattered brood, prepupae with typical elevated heads, brood mortality in the prepupal stage, and the formation of a sac-like structure in afflicted brood.

Furthermore, symptoms such as inability of prepupae to pupate and a change in colour of prepupae from white to pale yellow, light brown, and eventually dark brown, which dry into soft scales, have also been reported (White, 1913, 1917; Griffin, 1953, Fyg,1962 ;

Bailey, 1981, Chandel *et al.,* 1999 ; Hornitzky and Anderson, 2003, Shen *et al.,* 2005, Berenyi *et al.,* 2006).

White (1913) proved for the first time that sacbrood disease was produced by a filterable agent, a virus which he introduced in healthy *A. mellifera* colonies by feeding them a mixture of sugar syrup and bacteria-free water extract of diseased larvae. Later, using an electron microscope virus-like particles were discovered with varying shapes and sizes in a water extract of a diseased brood, he observed round or slightly oval particles with a diameter of about 60 um.

The sacbrood virus mostly affected honey bee brood and caused larval mortality. Symptoms of sacbrood disease in *A. mellifera* colonies included partially perforated brood scattered among capped brood, prepupae with typical elivated heads, brood mortality in the prepupal stage, and the formation of a sac-like structure in diseased brood. Furthermore, indications such as inability of prepupae to pupate and a change in colour of prepupae from white to pale yellow, light brown, and eventually dark brown, which dry down to soft scale were present (Griffin,1953). Bailey (1963) discovered a new strain of Sac brood virus, which he named Thai sacbrood virus (TSBV).

Bailey (1969) reported that immature worker bees in *A. mellifera* affected with sacbrood disease, live for only three weeks, ate less pollen, and have a shorter lifespan.

Bailey *et al.* (1982) found that *A. mellifera* sacbrood virus had single-stranded Ribose Nucleic Acid (ssRNA) with a molecular weight of $2.8x10^6$ Dalton, sedimented at 160S, and generated one broad band with a molecular weight of 29,000 to 34,000 in 0.01M phosphate buffer. It had a buoyant density of 1.3520±0.001N in CsCI (pH=7).Thai sacbrood virus in *A. cerana* was also isometric, measuring 30 nm in diameter, containing ssRNA with a molecular weight of $2.8x10^6$ Dalton, and sedimenting at 160S in 0.1 M KCI. When 0.01M phosphate buffer was used, it sadimented at 150S and created three close but clearly defined bands in gel with molecular weights of 30,200, 34,000, and 38,700 with 1.351±0.002 buoyant density.

Verma and Joshi (1985) demonstrated that Thai sac brood disease was more prevalent during the winter months in Uttar Pradesh, India, low temperatures played a key role in the severity of the disease and also reported that the disease increased when brood rearing was at its peak.

In the Kashmir region, Abrol and Bhatt (1990) detected symptoms of Thai sac brood virus (TSBV) in *A. cerana*. According to their survey report, TSBV killed over 94% of bee colonies in movable-frame and wall hives. According to Abrol and kakroo (1996), about 13,000 *A. cerana* colonies have been eradicated in Jammu & Kashmir due to TSBV disease.

Sacbrood caused larvae to fail to pupate, and ecdysial fluid rich in sac brood virus (SBV) accumulated beneath their unshed skin, forming the sac. Infected larvae changed colour from pearly white to light yellow, then dried out and form a dark brown scale shortly after death. It happened most often in the spring, when the colony was growing quickly and there were a lot of larvae and young adults around (Grabensteiner *et al.,* 2001).

According to Devanesan and Jacob (2001) all four larval instars of *A. cerana indica* were vulnerable to TSBV. The highest mortality (100%) was seen in one-day-old larvae, whereas the lowest mortality (72-74%) was observed in four-day-old larvae. Infected larvae were seen in the cells stretched out on their backs, their heads pointed outwards and turned upwards like a boat's prow. When the dead larvae were raised up, they seemed to be a sac filled with milky white fluid that easily ruptured, releasing the milky fluid. The study also discovered lower egg laying rates, foraging activity, and the presence of workers inside the hive.

Rana (2008) reported incidence of sacbrood virus in colonies of western honey bee (*A. mellifera* L.) in the North Western Himalayan region of India. The virus killed honey bee brood at the prepupal stage (10 days old) on the second day after brood sealing. SBV was discovered in two places in Himachal Pradesh in 2003, It was originally discovered in colonies in the Solan district of Nauni during the spring and summer (March to May), when it affected 0.39 percent to 5.20 percent of the brood. The disease was also found in the second location, Kangra district, Jachh (Himachal Pradesh), throughout the spring and summer (March to June), when it affected 0.23 % to 2.10 % of brood.

Rana *et al.* (2011) observed that the Sac Brood Virus primarily affected the *A. mellifera* brood, caused perforation of the sealed brood, pre-pupal death due to failure in pupation, and an accumulation of ecdysial fluid around the body and integument, forming a sac-like structure. Brood changed colour from pearly white to light yellow, and deceased brood turned into dark brown scales and its infection was most common in the spring, when brood rearing started in the colonies.

Sacbrood virus caused sacbrood disease which was spread by infected water, pollen, or nectar and affected worker bee larvae in *A. mellifera*. Shortly after capping, infected larvae died and create a fluid-filled sac. Infected brood was seen among healthy brood, with discoloured, sunken, or punctured cappings. Sacbrood virus lived for up to four weeks in dead larvae, honey, or pollen (Anonymous, 2013).

According to Srinivasan *et al.* (2014), the devastating outbreak of Thai sacbrood virus (TSBV) disease resulted in extinction of more than 90% of *A. mellifera* bee colonies in South India causing significant decline in honey output. They reported that Kanyakumari was the beekeeping centre in South India with over 2 lakh colonies reared by roughly 20,000 beekeepers and the disease infected 5 to 30% of the colonies. Like Thai sacbrood virus, sacbrood Virus (SBV) infected *A. mellifera* colonies all over the world but was less virulent than TSBV. Both viruses are mostly found in honey bee brood and cause larval death. Infected larvae changed colour from pearly white to pale yellow, then dry down and form a dark brown scale shortly after death.

According to Rao *et al.* (2015), 95% of *A. cerana* colonies in India were destroyed by Thai sacbrood virus (TSBV) disease, while only 15% colonies were affected by sacbrood virus (SBV) disease in *A. mellifera* colonies. In *A. cerana*, the symptoms of Thai sacbrood virus can be confused with those of the bacterial disease European foulbrood (EFB). TSBV and SBV are strains that are species specific but closely related. Both are unstable and are isometric in shape, with a diameter of 30 nm.

Aruna *et al*. (2016) reported that the seasonal incidence of TSBV disease was most prevalent during the winter (October to January) season, lasting until the spring (late January to March) season, and was influenced by brood rearing. Maximum temperature (34.3°C) and low relative humidity (RH 65%) and rainfall (62.7 mm) decreased the TSBV disease incidence in April (2/30 colonies infected, 80 cells infected/colony).The disease incidence was zero in the following four months namely; May, June, July, and August. The disease incidence started again in September and peaked in November (9/30 colonies infected, 342 cells infected/colony), when the mean maximum temperature was low (28.6°C), relative humidity was high (82%) and rainfall was high (191.3mm). As a result, low temperature was shown to be strongly related with high disease incidence, while high relative humidity and rainfall were found to be non-significantly correlated. The onset of the disease also coincided with the active brood rearing season (November to March).

Negi *et al.*(2018) studied the impact of weather parameters on seasonal incidence of diseases and enemies in *Apis cerana* at university apiary, Nauni, Solan during April, 2016 to March, 2017 and reported that disease incidence was statistically maximum (2.33%) in the month of May, 2016 when temperature, relative humidity and rainfall were high.

Brar *et al.* (2019) documented the seasonal incidence of diseases and enemies in *A. mellifera* under stationary and migratory conditions. They evidenced sacbrood with 8 per cent brood infestation only in the month of April, 2016 under stationary conditions. The colonies were found free from this disease during the rest of months from July, 2015 to June, 2016 in both stationary and migratory conditions.

Sacbrood incidence was reported minimum (2.0%) in second fortnight of July 2019 and maximum (7.30%) in second fortnight of February 2020. No incidence of sacbrood was found from first fortnight of August 2019 to second fortnight of January 2020. The incidence of sacbrood disease was found significantly negatively correlated with brood area (r= -0.560) and non-significantly with relative humidity. The incidence of sacbrood disease showed positive correlation with colony strength, average temperature and rainfall, though non-significant (Naveen and Yadav, 2021).

2.2.3 Management of brood diseases

Gochnauer (1954) found that (0.5-1.0 g) oxytetracycline (OTC) dissolved in 500 ml concentrated sucrose syrup sprinkled over smaller to larger bee cluster in the hive was effective in controlling the EFB disease in warm weather. Cornejo (1967) demonstrated that the disease was effectively controlled by spraying of combs with 500 ml sugar syrup containing 62.5 mg either of OTC, neomycin or chlortetracycline per hive. Corner and Gochnauer (1971) conducted studies on control of EFB and concluded that in honey bee colonies tetracycline appeared to be useful as protection against bacterial infections. Moeller (1978) recommended 3.5 grams oxytetracycline in one gallen of sugar syrup for the treatment of bee colonies having the epidemic type European foul brood for at least three consecutive years.

Peng *et al.* (1996) reported that the antibiotic tylosin was quite effective for prevention and control of American foulbrood disease (AFB). Honey bee larvae could tolerate a wide range of antibiotic doses in their diet demonstrated by a research conducted on immature worker bees kept in the lab. At a concentration of 1.5 x 10^8 spores/ml of food,

doses of tylosin protect very young larvae from becoming infected by *Bacillus* larvae. The results of protection against American foulbrood infection were compared to those obtained with 200 mg Terramycin. The colonies were protected for up to 3 weeks with both 200 mg Terramycin and 100 mg tylosin. The colonies were free from disease for another week after receiving a 200-mg dosage of tylosin.

According to Thompson and Brown (2001), in England and Wales after oxytetracycline therapy the rate of reoccurrence of European foul brood disease in colonies was reported to be between 26 and 27 percent.

Bahman and Rana (2002) reported that Treatment with oxytetracycline (OTC) before the onset of the disease in the apiary was more effective. The results of feeding oxytetracycline (5.0 per cent a., i. veterinary grade) to *A. mellifera* colonies at 200 mg/colony in 300 ml concentrated sugar syrup was effective.

Elzin *et al.* (2002) observed that controlling American foulbrood with the antibiotic tylosin in honeybee (*Apis mellifera* L.) colonies, (Tylan) was tested for control of oxytetracycline-resistant American foulbrood (*Paenibacillus* larvae). A 200mg powdered sugar combination containing tylosin was applied as a dust against American foulbrood symptoms. The application of Tylan per 20 g powdered sugar at weekly intervals for three weeks resulted in a reduction of diseased colonies.

Rana *et al.* (2004) reported that in *A. cerana*, European foul brood was also controlled by feeding tetracycline (5.0% a.i., veterinary grade) @ 200 mg/ colony in 300 ml 50 per cent sugar syrup.

Gende *et al.* (2008) studied cinnamon's (*Cinnamomum zeylanicum*) essential oil physicochemical properties and antibacterial activity. The bioactivity of this essential oil against *Paenibacillus* larvae was investigated using in vitro techniques, including the tube dilution method and bioautography, a chromatogram-based approach for locating antibacterial activity. Against *Paenibacillus* larvae, cinnamonaldehyde and eugenol were found to exhibit antibacterial properties. For all bacterial strains the minimal inhibitory concentration (MIC) and minimal bactericide concentration (MBC) for *C. zeylanicum* essential oil were 25-100 g/ml and 125-250 g/ml, respectively. Essential oil, particularly two of its primary components, has inhibitory activity against *Paenibacillus* larval strains.

Fuselli *et al.* (2010) observed that the bacterial strains were inhibited at the lowest doses tested in in- vitro experiments of *Melaleuca viridiflora* and *Cymbopogon nardus* oils, with minimum inhibitory concentration (MIC) mean values of roughly 320 mg/l for both oils, respectively. This property could be related to the type and proportion of the oils' constituents. *M. viridiflora* had the most terpinen-4-ol (29.09 per cent), -pinene (21.63 per cent), and limonene (17.4 per cent), whereas *C. nardus* had the most limonene (24.74 per cent), citronelal (24.61 per cent), and geraniol (15.79 per cent). The use of these essential oils contributes to the screening of alternative natural compounds to control American foul brood in apiaries; toxicological risks and other undesirable effects would be avoided as resistance factors developed by the indiscriminate use of antibiotics.

Gehtori *et al*. (2011) investigated the agar disc diffusion and micro broth dilution methods, researchers studied the effects of distinct organic extracts of three different bryophytes and a standard drug (positive control) on the bacterium in vitro. Against the test pathogen, all of the extracts showed good antibacterial activity. The highest activity of the acetone extract of *Metrosideros polymorpha* and the chloroform extract of *D. undulatum* (AI 15.51 and 15.56 mm, respectively) was comparable to that of the standard drug.

Roussenova (2011) studied the antibacterial activities of eleven essential oils against *Paenibacillus* larvae (15 field strains and the reference BCCM LMG 9820 strain) by the disc diffusion method and the method of serial dilutions in agar. The minimal inhibitory concentration (MIC) of essential oils was determined within 1%-0.015% v/v. Highest activity i.e. MIC 0.06-0.015% v/v was shown by essential oils of cinnamon, thyme, clove, peppermint, lemongrass, sage and oregano. Variable activity exhibited marjoram and tee tree oils. Citrus essential oils showed the lowest inhibitory effect with MIC 0.12- 1.0% v/v for mandarin oil and > 0.25-0.5% v/v for grape fruit oil. This further encourages the research to include essential oils as an alternative means in the measures for prevention and control of American foulbrood without the use of antibiotics.

Kamal *et al*. (2013) observed the effectiveness of tylosin and three kind of ethanolic extract of propolis (Chinese, Egyptian and old wax comb extract propolis) for controlling AFB disease in honey bee colonies under field conditions. Identification of Phenolic composition of the ethanolic extract samples were investigated by high performance liquid chromatography (HPLC) instrument. Field assays showed that the treatment of beehives

affected with AFB disease by tylosin 1% and Egyptian ethanolic extract in concentration of 0.1 and 0.05% had elimination of clinical symptoms at 100% of reduction rate.

Tiwari *et al.* (2014) evaluated the eco-friendly formulations i.e. spraying of cow urine, plant decoctions prepared in cow urine, cow dung cake, cow dung ash powders, ajwain seed powder and compared to an antibiotic, terramycin sugar syrup and synthetic chemicals, sulphur and thymol powder with two applications in a month. The data revealed that the cow urine sprays @ 50, 75 and 100% reduced the disease infection to below detectable limit in 10 to 14 days, respectively, as in terramycin treated infected colonies only 50-55% recovery was seen in EFB infection.

The antimicrobial activity of different extracts of *Flourensia riparia, F. fiebrigii* and *F. tortuosa* against *P. larvae* were studied by using agar diffusion technique. It was reported that chloroform and ethyl ether extracts of *F. riparia* and *F. fiebrigii* inhibited *P. larvae* successfully (Reyes *et al.,* 2013), Dichloromethane and methanol extracts from inflorescence of various species of *Hypericum* genus also presented antibacterial activity against *P. larvae* (Hernandez-Lopez *et al.,* 2014). The antimicrobial activity of *Azadirachta indica* and *Vitex trifolia* crude aqueous extracts at different concentrations (20, 40, 60 mg/ml per disk) was also studied against *P. larvae* and found successful results (Anjum *et al*., 2015).

Patruica *et al.* (2017) analyzed the effect of using plant extracts of various medicinal plants *viz., Allium sativum, Echinaceae, Ganoderma,* and *Salvia officinalis.* They fed bee colonies with protein candy supplemented with different plant extracts. Their study showed that these extracts stimulated queen brood laying capacity as well as reduce bacterial infection.

Hassona (2018) has evaluated the natural products like honey, cinnamon (*Cinnamomum zeylanicum*), cloves (*Syzygium aromaticum*), propolis, and thymol (*Thymus vulgaris*) against AFB and EFB bacteria in laboratory and field. In laboratory, thymol had the highest effect on AFB with different concentrations and had a total mean of 3.37±1.03cm of circle around disc diffusion. In contrast, thymol had the lowest effect on EFB with a total mean 0.33±0.15cm.The cloves had the highest effect on EFB with a total mean of 4.50±2.00 cm and fourth level effect on AFB with a total mean of 0.73±0.20 cm. Propolis had the second level effect on AFB and EFB with total mean 1.15±0.93 cm on AFB and 1.49±0.95 cm on EFB. In Apiary, thymol found to be highly effective with a rate of 25.8% increase of

capped brood through all the treated period contrast with EFB. On the other hand, cloves had increased the capped brood by 25.3 through all treated periods.

Aruna *et al.* (2017) conducted an experiment on the effectiveness of plant products against sac brood infections, and it was discovered that combining 2 g of *Phyllanthus niruri* L. extract in 250ml sugar solution with a modified shook swarm method resulted in the lowest number of infected larvae per thousand brood cells. Then treatments with extracts of *Ficus religiosa* L., *Carica papaya* L., and *Azadirachta indica* L. were given to the colonies. In honey bee colonies that had recovered from the disease, supplementary sugar feeding resulted in an increase in the adult population. They concluded that, honey bee colonies fed with 2g of *P. niruri* in 250ml sugar solution combined with modified shook swarm method was effective for both the recovery of disease and increase in brood rearing and adult population.

Stamets *et al.* (2018) reported that Honey bee deformed wing virus (DWV) and Lake Sinai virus (LSV) can be cured by using extracts from amadou (*Fomes*) and reishi (*Ganoderma*) fungi in a dose dependent manner. Colonies fed *Ganoderma* extract exhibited a 79-fold reduction in DWV and a 45,000-fold reduction in LSV compared to control colonies in field trials.

Topal *et al*. (2020) reported that herbs and essential oils like thyme, clove, mint, lemongrass, cinnamon, grapefruit, rosemary, marigold, laurel, eucalyptus, and tea tree had fatal effects against mites and bacteria. Aromatic oils had no harmful effect on the development of bee colonies when administered in acceptable doses.

2.2.4 Mites

a) *Varroa destructor*

Varroa destructor feeds on haemolyph of larvae, pupae and adult bees. Movement of infested colonies of bees for pollination and importation of queen bees from infested areas, led to the rapid spread of mite. Kumar *et al.* (1988) firstly reported *V. desructor* from Himachal Pradesh on *A. mellifera* and Gatoria *et. al.* (2004) and Rana *et al.* (2004) reported it in Punjab and later again in Himachal Pradesh, the virulent haplotype of *V. destructor* causing serious damage to colonies was reported.

Ball (1994) reported that the reproduction in *Varroa* takes place within the sealed brood cells and adult female of *V. destructor* feeds on haemolymph of adult bee and pupae. During feeding, mites can reduce the life expectancy of the bees and cause the colony to decline by transmitting virus during feeding. In the countries where it became established *V. destructor* had a significant influence on beekeeping (Sanford, 1996).The mite *V. destructor* is also a vector for various viruses, enhancing the pathogenicity within the hive.

The *V. destructor* mite is a major ectoparasite of the European honeybee, *Apis mellifera* (Sammataro *et al.,* 2000). It originated in Asia and spread worldwide in recent years, inflicting considerable damage to *A. mellifera* colonies. The presence of the mite and various viruses, including the Israel Acute Paralysis Virus (IAPV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV) and the Acute Bee Paralysis Virus (ABPV) has been linked to colony loss in several nations (Denholm, 1999; Lanzi *et al.,* 2006; Chen and Siede, 2007; Rosenkranz *et al.,* 2010).

According to Chaudhary (2005), the percentage of *V. destructor* infestation in *A. mellifera* colonies in Haryana ranged from 3.9 to 29.3%. *Varroa* infestations were found in 29.3% of colonies in Haryana's primary beekeeping zone with varying degrees of severity among apiaries and colonies. 3.9 per cent of colonies had a moderate level of infection (<5% brood infestation), 6.5 percent had a medium level of infestation (5% to 10% brood infestation), and 18.9 percent had a severe level of infestation (>10% brood infestation). Kurukshetra has the highest *varroa* incidence of 44.1 per cent among different migratory sites in different districts of the state (Haryana) followed by Karnal (36.8 per cent). On a colony basis, Yamunagar and Kaithal have lower incidence. In Karnal, where all colonies were badly damaged (brood infestation level >10 percent), the infestation was the most severe. Kurukshetra was the second most badly infested district, with 29.4% of total colonies severely affected and 11.5 and 3.2 per cent of colonies with medium and low levels of infestation, respectively. The reduction in the size of hypopharyngeal glands in parasitized honey bees throughout their growth may diminish their ability to generate royal jelly and induce abnormal brood development (Wegener *et al.,* 2009). The mite may interfere with the development of numerous internal and exterior structures while parasitizing the bee during its pupal stage causing damage to its host (Wahab *et al.,* 2006).

Le Conte *et al.* (2010) in France found that *A. mellifera* colonies producing honey may tolerate the mite infestation. They also noticed that an increase in mite infestation occured during the colder months as a result of honey bee population decline in the colonies.

In the Shivalik hills of Himachal Pradesh, Sharma *et al.* (2011) found the highest incidence of the ectoparasitic mite *V. destructor* in November. Seasonal incidence of parasitic mite i.e. *V. destructor* in *A. mellifera* colonies revealed that the peak infestation occurred in April (12.10±0.57) in 2008-09.While during 2009-10, the peak of *Varroa* infestation was seen in October (72.00±0.86), November (80.10±1.58), and December (72.50±1.27).

Deosi and Chhuneja (2012) reported that *V. destructor* mite population on adult bees peaked in March and minimum infestation was found in September to October at PAU Ludhiana during 2008-2009. In April-May the mite fall was at its maximum and in July-August it was minimum. They came to the conclusion that colony strength was correlated to the incidence of *V. destructor*.

Asha *et al.* (2013) observed that the highest incidence of Varoasis (8%) in adults of *A. mellifera* L. was observed in the second fortnight of May, 2008. Adult bee deformity was modest ranging from 0.0 to 3.0 per cent with an average of 0.52 per cent infestation. The sticky paper method which was used for the estimation of *V. destructor* on *A. mellifera* colonies revealed a substantial positive correlation (r= 0.81) between per cent infestation and per cent deformity which implies that an increase in mite incidence in honey bees led to an increase in the number of deformed bees. Mite infestation levels ranged from 0% to 9%, with an average of 1.58 %. Mites first appeared on adult bees in first fortnight of May (7.5%), increased to 9% in the second fortnight of May and then progressively decreased to 0% in the second fortnight of August but reappeared in the month of September (5%) observed by sticky paper method sampled colonies. Infestation levels was highest in the second fortnight of May (9%) and lowest in the second fortnight of August (0 per cent).

Kotwal *et al.* (2013) recorded that the seasonal incidence of ectoparasitic mite *V. destructor* found throughout both the years during 2006-08. They reported *Varroa* mite infestation was maximum in March and minimum in June.

Poonia *et al.* (2014) investigated the impact of environmental factors on the population of *V. destructor* and revealed that the highest number of mites (38 and 51 mites/per hive) was observed in the second fortnight of May, with a significant positive correlation with maximum (r= 0.659) and minimum (r= 0.648) temperature. However, relative humidity (r= -0.416) and sunshine hours (r= -0.023) had a negative correlation with *V. destructor* infestation, although rainfall (r= 0.019) had no significant correlation with *V. destructor* population. They hypothesized that mite population in *A. mellifera* colonies rise during the summer months, when temperature was high and flora was less for foraging.

Brar *et al*. (2019) recorded the incidence of ectoparasitic mites, *V. destructor* and *Tropilaelaps clareae* was recorded by three different methods viz. sticky paper, per 100 bees and visual examination and reported that the incidence of *V. destructor* and *T. clareae* was more when the temperature was high and relative humidity was low under stationary conditions and no mite infestation was found during migratory conditions.

b) *Tropilaelaps clareae*

Singh (1957) demonstrated that ectoparasitic mites, *V. jacobsoni*, and *T. clereae*, are not a problem in the case of *A. cerana* colonies. *Acarapis woodi*, an endoparasitic mite is the sole one that causes acarine disease in *A. cerana*. It was reported firstly in India on *A. cerana* colonies in the Katrain area of Himachal Pradesh. According to Atwal (1967), about 1% of *A. cerana indica* in the Himalayan region was infected with this mite (*A. woodi*). According to Atwal and Sharma (1970), acarine infestation causes the loss of 25-40% of *A. cerana* colonies each year, and it is disseminated via infected swarms.

Chahal *et al.* (1986) observed maximum infestation of *T. clareae* twice a year i.e. February to May (33.7-51.7%) and September to November (26.8-42.0%) which was correlated with the peak of brood rearing activity in Ludhiana, Punjab. Whereas high infestation rate of *T. clareae* during March to April (21.75-29.80%) and October to November (13.80 and 14.23%) at Hisar, Haryana was observed (Aggarwal and Kapil, 2013).

Woyke (1987 a; b) studied the *T. clareae* infestation level on brood and workers in *A. mellifera* colonies. The infestation level was reported more on brood (32.5 %) than on workers (1.7 %) in *A. mellifera* in North Vietnam (Woyke 1987a). The infestation level in apiaries located in Afghanistan and southern Vietnam was 36.10 per cent in brood and 1.5 per cent in workers (Woyke 1987b). Camphor and Martin (2009) reported that the sealed brood

infestation level was reported to fluctuate in relation to the amount of available brood maximum in the April to May in Pakistan.

The maximum incidence of *T. clareae* has been recorded to occur during October-November by Gatoria *et al.* (1995) and September to November by Jhajj *et al.* (1996) in Punjab. Nagaraja and Rajagopal (2003) showed that peak period of brood infestation by *T. clareae* mite was between September to November at Bangalore and during October to November in Pakistan.

Baker *et al.* (2005) reported that tropical bee mites *T. clareae* and *T. koenigerum* feed on bee larvae and pupae causing brood malformation and the death of bees. *T. clareae* occurred on five species of bees-*A. cerana*, *A. dorsata*, *A. florea*, *A. laboriosa* and *A. mellifera*.

Camphor and Martin (2009) observed population changes of *T. clareae* mites in *A. mellifera* colonies over a 14 month period in Islamabad, Pakistan. The environmental conditions resulted in honey bee brood being present throughout the year which allowed *T. clareae* to breed continuously. The phoretic period of *T. clareae* was very short as the infestation of the brood (8.1 %) was 20 times greater than that of the adult workers (0.4 %). There were rapid increases in the *T. clareae* population during March and April corresponding with the expansion of the honey bee sealed brood and an increase in the number of falling mites. The mite infestation level of worker brood (mean = 8.1 ± 4.3 %) was always significantly greater (<0.001) than the infestation level of the adult workers (mean = 0.4 ± 0.4 %) in *A. mellifera* colonies.

In Mysore Karnataka, during different seasons of the year 2008-2010 the *T. clarae* infestation was recorded on the Asian giant honey bee, *A. dorsata*. The study revealed that, during monsoon, post-monsoon and winter seasons the mites infestation was found on the foraging worker bees, hive bees and dead bees nearby *A. dorsata* colonies. The rate of incidence of mite infestation varied significantly ($X2 = 5.99$; $P=0.05$) between the seasons. The mite's density ranged between 3 to 17% and was found on legs and thoracic region of the body. Interestingly, thoracic region had recorded highest density of mites that was followed by the abdomen and there existed a significant variation ($X2 =7.80$; $P<0.05$) (Basavarajappa *et al.,* 2010).

Sharma *et al.* (2011) recorded the seasonal variation of *T. clareae* mite in Shivalik hills of Himachal Pradesh. Seasonal incidence of *T. clareae* mites revealed that mite population was maximum in the month of September (56.00±0.31) and October (43.00±7.40) in *A. mellifera* colonies during 2009 and 2010, respectively. Infestation rates during these periods were 31.49% in 2008-09 and 25.28% in 2009-10. During autumn there are favorable conditions of temperature and relative humidity for *T. clarae* mite infestation so mite population showed positive correlation with temperature (r=0.592, 0.698; P≤0.01) and relative humidity (r=0.997; 0.856; P≤0.01) during autumn.

Padhi and Rath (2012) observed the infestation level of ectoparasitic mite *T. clareae* on brood and adult bees at Bhubanewar, Odisha in *A. mellifera* colonies. The infestation of *T. clareae* was maximum in the month of October followed by November. While on worker brood of *Apis mellifera* 10-20 per cent mite infestation was visualized throughout the year, at 30-40 per cent infestation level in bee hives *T. clareae* do not produce any abnormality in the adult bees but when the infestation level crossed 40 per cent, abdominal length in bees decreased and weight loss occurred in bees. They further reported that an infestation level of 50-60 per cent of *T. clareae* caused pupal death in bees.

Brar *et al*.(2019) demonstrated that ectoparasitic mite *T. clareae* was recorded by three different methods viz. sticky paper, per 100 bees and visual examination and found that the mite incidence was maximum 12.00% and 4% in the month of June (per hundred bees method and per cent brood infestation methods).

2.3. GREATER WAX MOTH (*G. mellonella*)

2.3.1 Seasonal incidence of greater wax moth (*G. mellonella)*

G. mellonella, often known as the greater wax moth, belongs to the Lepidoptera order and is a major beekeeping pest (Laridon, 2006).With their primary hosts; the honey bees *A. mellifera* L., *A. cerana indica* F., and *A. florea* F., the wax moths maintain a scavenger-parasitic (ecto-symbiotic) relationship (Wilson, 1971 and Singh *et al.,* 1983). In Japan (Okada, 1988), species-specific relationships of wax moths and honey bees were discovered; *A. grisella* F. was found connected with *A. cerana japonica*, while *G. mellonella* L. was found associated with *A. mellifera*. The wax moth is one of the most damaging natural enemies of the bees causing significant losses to the beekeeping industry. It is one of the most destructive pest (Jafari *et al.,* 2010). When wax moth larvae feed on honey, pollen and wax

they form tunnels and destroy honeycombs (Saraswathy and Kumar, 2004). They attack the wax of the combs create tunnels in to it destroy wax combs and also contaminating the honey (Knoxfield, 2006).

Various workers have done studies on seasonal incidence of greater wax moth recording several overlapping generations of wax moth in a year. Stored combs and weak or poorly maintained colonies are continuous source of wax moth population. Wax moths incidence were observed from March to October with its peak in September and after that incidence decreases (Brar *et al.,* 1985; Swamy, 2000 and Sohali *et al.,* 2017).

According to Brar *et al.* (1985), the wax moth posed a serious problem during monsoon (dearth period) when a large number of colonies absconded due to infestation in *A. mellifera* apiaries in Punjab. The greater wax moth infestation started in June and gradually increased to a peak in September, then decreased to a minimum in November.

Shylesh (1987) observed wax moth in *A. cerana* throughout the year in Bangalore, with a maximum larval population of 336.36 per infested hive, whereas at Dharwad, (Karnataka), egg laying of *G. mellonella* was observed from March to August, with an average of 312 eggs per colony found in three bee colonies in July. The pest's larval stage was discovered throughout the year, with a high population from May to August, which coincides with the area's floral dearth period. During July-August in Bangalore, a high incidence of *G. mellonella* infestation was reported in *A. dorsata* colonies, resulting in absconding of bee colonies. The abandoned combs with high pest infestation rates provided as a source of infestation for new bee colonies.

Thakur (1991) reported that all larval stages of wax moth affect the brood combs, cells, pollen, and honey stores. Severe infestation causes a halt in brood rearing, foraging activities, and eventually colony desertion. When compared to strong colonies, weak colonies are more susceptible to wax moth infestation (53 per cent).

The peak invasion of this pest was observed in South India during the season of floral dearth. In stored combs, the pest hibernated in larval (about 70%) and pupal (roughly 30%) stages. During different seasons the tropical climate may have supported overlapping generations. As a result it is of great economic importance of controlling the wax moth in hive bees (Turker *et al.,* 1993).

Abrol and Kakroo (1996) showed that the peak infestation was observed between August and October in (*A. mellifera* apiaries in Punjab).The population dynamics of the greater wax moth, *G. mellonella* L. differed depending on the bee species. From May to December, wax moth larvae, pupae, and adults were seen in *A. mellifera* colonies.

Swamy (2000) observed that the population of wax moth larvae in *A. cerana* varied during different months in a year. In Bangalore the wax moth attack in bee colonies was 90.68 in June 1996, 199.33 in April 1997, and only 49.55 in March 1998.

Ansary *et al.* (2001) reported that *G. mellonella* infestation in *A. cerana* combs was maximum in June (59.33 per cent) in Bangladesh (Dhaka). However, a significant outbreak of *G. mellonella* in *A. mellifera* colonies was found in Himachal Pradesh (India) in August 2002, with an average of 46 larvae per comb.

In South India, the pest was most prevalent during the period of floral dearth, which coincided with weak colonies. Because of the overlapping generations in a year, the pest infestation occurred throughout the season in various geographical regions. Infected combs and weak colonies were reservoirs for *G. mellonella* population (Viraktamath *et al.,* 2005).

Swamy (2008) found greater wax moth *G. mellonella* infestation in both strong and weak *A. cerana* colonies. In the weaker colonies, more wax moth infection was detected throughout the year, with the maximum percentage of infestation occurring from October to February. During August, however, the infestation was observed to be decreased. Furthermore, wax moth infestation was high in strong colonies during March, whereas the lowest per cent infestation was seen during December and September.

Varshneya *et al.* (2008) investigated the seasonal incidence of the greater wax moth (*G. mellonella* L.) in European honey bee colonies (*A. mellifera* L.) and found that the greater wax moth started infecting bee colonies in July i.e. in the early rainy season. The larval infestation increased gradually from January to September, reaching its climax in September. In the larval stage, they feed on the wax of the honey combs, causing extensive damage. Wax moth weakened the honey bee colonies decreasing honey production.

Kumari and Jha (2013) reported that the pest infestation was found in the bee hive as well as stored combs. In both cases, the maximum infested area was detected in September, while the lowest was reported in June (3.42 cm^2, 4.41cm^2), respectively in 2011 and 2012. It

could be due to the high temperature and humidity. Due to the self-defense behaviors of worker bees frames fully covered with bees recorded least infestation.

According to Raghunandan and Basavarajappa (2014), colonies with low population were more susceptible to *G. mellonella* infestation, which was more prevalent in the semi-arid zone during the summer (30.80% of colonies), followed by the rainy season (23.40 per cent).In Malnad, Mysore (Karnataka) however, the infestation was less 11.00 and 6.60 per cent during the summer and winter seasons, respectively.

Kebede *et al.* (2015) investigated the prevalence of wax moth in modern hive colonies in four villages of Kafta Humera (Ethiopia) from April 28 to May 30, 2009. According to the study, the degree of the infestation is divided into three categories: light, moderate, and seriously damaged colonies with 11.4 per cent, 15.3 per cent, and 0.65 per cent infestation, respectively and the overall prevalence of wax moth larvae in modern bee hives is 27.4%.

Sohali *et al.* (2017) investigated the seasonal abundance of *G. mellonella* larvae in honey bee hives in Sargodha area (Punjab in Pakistan). The highest moth abundance was seen during the regional dearth season, which lasted from May to November. August was the month with the highest abundance of wax moth larvae (14.8±3.9 wax moth larvae per hive).

Lalita *et al*. (2018) demonstrated that the population of wax moth fluctuated every month in both the years (2016-2017). Seasonal incidence of *G. mellonella* was started from the April and the highest number of wax moth population was recorded in July after that population decline till March. Least number of greater wax moth larvae, pupae and adults were recorded in March in all frame strength during both the years of study. There was no population of larva, pupa and adult of *G. mellonella* was recorded during February to March in colony with highest frame strength.

2.3.2 Studies on management of greater wax moth (*G. mellonella*) in stored combs

Sattigi *et al.* (1990) found that the use of lime sulphur paste to seal the cracks and crevices of the bee hive to reduce wax moth infestations was a successful measure. Their findings revealed that only one colony out of ten was found to be infected resulting in 90 per cent protection in 1989 compared to zero protection in untreated colonies. In 1990, two out of nine treated colonies and all the untreated colonies were found to be infested. They further reported that the effectiveness of each treatment persisted for 5- 6 months and throughout the

year two treatments were done during May and December resulting 78 per cent protection from wax moth attack.

Kuusik *et al.* (1993) investigated the effects of plant extracts on the muscular and respiratory activity patterns of greater wax moth (*G. mellonella*) pupae. Visual observations and the recording patterns of a micro respirometer actograph, which also documented the oxygen consumption rate and the external gas exchange rhythms, were used to describe the several forms of periodic and regular stereotyped abdominal movements in *G. mellonella* pupae. The actograph, infrared gas analyser, and differential thermocouple calorimeter were used to study muscle and respiratory responses after treatment with insecticidal plant extracts. The majority of plant extracts worked as respiratory depressants and muscle contraction inhibitors.

Verma (1995) used the biological insecticide Dipel to suppress the greater wax moth, *G. mellonella* in *A. cerana* colonies. The colonies were given a comb sprayed with 10% Thuricide, which remained effective for 5 months and 17 days, had the highest mortality of larvae of all the treatments.

Calderone (2000) described the use of various chemicals to suppress the attack of wax moth larvae and adults, including paradichlobenzene, sulphur fumigation, acetic and formic acids. Formic acid was found to be less effective against *G. mellonella* L. and *A. grisella* F. than para-dichlorobenzene, with the lowest reduction in infestation of bee combs under storage conditions.

Gowda and Roopa (2001) Purified *Bacillus thuringenesis var. kurstaki* HD-73 protein and was introduced to the diet of larval and adult honey bees. The highest *B. thuringenesis var. kurstaki* HD-73 protein test concentration was one hundred times the maximum detectable level expression of *B. thuringenesis var. kurstaki* HD-73 protein in pollen and nectar of genetically engineered *B. thuringenesis var. kurstaki* HD-73 cotton (0.2 ppm). The active and inactive *B. thuringenesis var. kurstaki* HD-73 proteins were compared to untreated larvae exposed to distilled water and the results were recorded. On larval and adult bees, active and inactive *B. thuringenesis var. kurstaki* HD-73 protein had no negative effects.

Dipel, Delfin, Biobit, Biolep, and Bioasp, five commercial *B. thuringiensis* formulations were investigated for efficacy against different instar larvae of the greater wax

moth. All of the formulations were poisonous to wax moth larvae, but their potency varied greatly. The level of mortality varies depending on age of the larvae, producing up to 100% mortality in the first instar and 56-88 percent mortality in the seventh instar (Viraktamath *et al.,* 2005).

Zaitoun (2007) investigated the effects of ethanolic extracts of twenty-one medicinal herbs on the growth of the greater wax moth (*G. mellonella*) as well as honey bee workers. He found that feeding the wax moth larvae with these plant extracts increased the duration of the larval stage by 2-40 days as compared to the control. Six extracts out of the twenty-one extended the pupation duration by 2-5 days compared to the control. The wax moth larvae and pupae got killed by extracts of *Abrus precatorius*, *Laurus nobilis*, *Petroselimum satinum*, and *Plantago pzyllium*. Except *A. precatorius*, these plant extract killed 100% or 95% of tested wax moth larvae respectively, with no deleterious effects on worker bees. Few of the extracts employed had a detrimental effect on worker honey bees; the most dangerous was *Cicer arietinum*, followed by *Myristica fragrans* and *Raphanus sativus* and 80, 70, and 55 per cent of the experimental bees were killed by these substances, respectively. Some of the plant extracts employed appear to be insect growth regulators and toxicants, and could be used to efficiently manage wax moth populations.

Ahmed *et al.* (2008) investigated the effects of aqueous Neem Seed Extract (NSKE) at various concentrations (0.5, 2.3, and 4 per cent) on *G. mellonella* larvae and found that after spraying with 4 per cent aqueous NSKE, mean mortality of the pest larvae was (83.33%), followed by 73.33, 56.67, 50.00, 50.00% with 3.0, 2.0, 1.0, and 0.5 per cent NSKE, respectively (3.33). At various post-treatment periods, there was a statistically significant difference in mortality at different concentrations. Weight loss in treated combs was decreased with neem seed kernal extract at 3 and 4 per cent compared to all other lower concentrations.

Sankara *et al.* (2009) tested on the greater wax moth *G. mellonella* larvae, the pathogenic effect of an indigenous entomopathogenic nematode, *Heterorhabditis indica*, and commercial biopesticides of three fungal pathogens (*Metarrhizium anisopliae*, *Beuveria bassiana,* and *Trichoderma viride*) and one antagonistic bacteria (*Pseudomonas fluorescence*). The biopesticide's efficacy was tested alone and in combination with *H. indica*. At each twelve-hour interval the pathogenic interaction of *H. indica* and biopesticides on *G. mellonella* larvae was examined. The percentage of larval mortality was found to differ

significantly between the biopesticide treatments. When compared to other biopesticides, *B. bassiana* caused more mortality on host larva (40%) when evaluated in isolation. While, the combination of *P. fuorescence* and *H. indica* proved to be the most effective, killing *G. mellonella* completely after 24 hours of storage.

Surendra *et al.* (2010) reported the use of natural plant products for the control the greater wax moth (*G. mellonella* L). The experiment was conducted in a laboratory and the results demonstrated that the larval mortality of wax moths varied dramatically depending on the concentration of three different plant components. The mortality rate with Neem (*Azadirachta indica*) seed extract was significant, ranging from 84.8±12.7 to 93.65± 3.25 per cent at various doses. Different doses of tulsi (*Ocimum sanctum*) leaf extract resulted in moderate larval mortality for different instars, ranging from 65.36± 4.36 to 73.41± 4.46 per cent. Pongamia (*Pongamia pinnata*) seed extract had a low mortality rate of 31.10± 3.38 to 52.1± 19.85. Tulsi had a mild effect, while pongamia had the lowest mortality. Under all of the experimental conditions, Neem performed better than the other two plant products.

Sezer and Ozalp (2011) demonstrated the effects of *Azadirachtin* on the percentage of glycogen content in *G. mellonella* larvae. The effects of *Azadirachtin* extracts derived from the ripe fruits, seeds, trunk, and flower of *Melia azedarach* and *A. indica* on the total glycogen content of *G. mellonella* were investigated. When the larvae reached their final larval stage after being fed 1.00, 2.00, 4.00, and 6.00 ml/100g of *Azadirachtin*-containing food, total glycogen synthesised was measured, and compared to control, 33 per cent, 42 per cent, and 50 per cent decrease was observed in individuals fed 2.00, 4.00, and 6.00 ml/100g of *Azadirachtin*-containing feed, respectively.

Katna *et al.* (2012) tested the sulphur fumigation, neem leaf powder, neem oil, *Bt*, neem seed kernel extract(NSKE), karanj oil and deep freezer for management of wax moth (*G. mellonella* L.). Out of these, the most effective treatment against wax moth damage under storage conditions was found to be combs stored in by deep freezer treatment (1.33 %) for 5h and sulphur fumigation (6.74%). The data indicated that with the progress of time of storage, the combs are badly damaged due to wax moth. In the control infested area was recorded to be 4.16%, 30.00 DAT which increased to 80.00% at 60 DAT and become 100. 00% , 90 days after treatment. In case of sulphur fumigation, infestation was 3.16% at 30 DAT which increased to some extent (15.35%) in 90 Days after treatment.

Bhopale *et al.* (2013) evaluated the effects of botanicals and microbial pesticides on wax moth (*G. mellonella* L.) management under storage conditions. The treatment of *B. thuringlensis var. kurstaki* caused the highest mortality of the greater wax moth (*G. mellonella* L.) (Lepidoptera: Pyralidae), followed by pongamia oil (3 per cent) and neem oil (3 per cent). *B. thuringiensis* local strain-2 and dried neem leaf caused the lowest mortality. For most of the treatments, the maximum mortality was found in third instar larvae.

Pitan *et al.* (2015) tested the efficacy of methanol, n-hexane, and aqueous leaf extracts of *O. gratissimum*, *Carica papaya*, *Tithonia diversifolia*, *Ageratun conyzoides*, and *A. indica* under laboratory conditions against bee pests: acrobat ant (*Crematogaster lineolata*) and small hive beetle (*Aethina tumida*). The contact toxicity of the extracts was determined by applying 2ml of 10 per cent w/v treatments to four insects using a conventional procedure. All of the insects died within 6 hours after being exposed to N-hexane extracts from all of the plants. Except for the small hive beetle (*Aethina tumida*), methanol extract caused 90% to 100% mortality in acrobat ant (*Crematogaster lineolata*). The acrobat ant (*C. lineolata*) was killed completely by aqueous extracts of all the plants. The mortality rate of both the pests treated with aqueous extracts of the plants ranged from 15% to 100%.

Kwadha *et al.* (2017) reported The greater wax moths developmental cycle can be disrupted by exposing beekeeping equipment and combs to temperature above (heating technique) or below (freezing technique) of greater wax moths tolerance range. In large-scale farming, infested combs are removed from the hive, insulated in second-hand houses, and exposed to higher temperatures of 45-80 °C for 1-4 hours, whereas in small-scale farming, the combs are kept in hot water for 3-5 hours. Cold rooms or refrigerator equipment, such as home freezers set at -7°C to -15°C for 2-4.5 hours can also be used to treat infested combs. These strategies are helpful since the greater wax moth growth and development is influenced by environmental factors such as temperature.

Vijaykumar *et al.* (2019) studied the incidence of *G. mellonella* in two *Bt* products, V-*Bt* (commercial product) and HD-1(local *Bt* product) treated combs. Irrespective of the concentration of *Bt*, in V-*Bt* treated combs *G. mellonella* infestation was significantly lower than that observed in HD-1 treated combs. Out of the two *Bt* products and V-*Bt* (commercial product) and HD-1(local *Bt* product), the highest larval mortality with less comb damage was recorded in case of the former.

2.3.3 Studies on management of greater wax moth in Apiary

a) Cultural methods & mechanical methods

Wax moth incidence can be reduced by sealing the cracks and crevices of the hive and cleaning the hive at regular intervals (Fletcher, 1911; Morrison, 1948). Placing of split reeds in the hive serves as egg trap can be removed twice a week to kill the egg and larvae collected in it (Surface, 1913).

Paddock (1918) tried light traps and decoy hives for attracting the moths. Wax moth attack can be lowered by maintaining colonies strong (Franssen, 1930). Paddock (1918) and Nessa *et al.* (1980) suggested cleaning of hives and keeping colonies in good condition to keep the colony free from wax moth infestation. Whitecomb (1936) and Kannagara (1940) advocated that the wax moths were attracted to propolis and bur combs for oviposition which are also a shelter for the larvae, so removal of propolis and bur combs at regular interval of time can protect the colonies. Reduction in size of entrance and regular cleaning of the hives can prevent the wax moth infestation (Adamson 1943). Cherian and Ramchandran (1943) stated that uniting the weak colonies to make them strong, artificial feeding during dearth period, inter changing the combs, providing brood combs, removal of old combs, preventing cracks and sanitation are the major measures to be adopted to keep the apiary free from wax moth infestation.

Williams (1976) demonstrated that wax moth management in apiary involved maintaining the strong bee populations and removal of wax and debris from hive bottom boards. Colony management included a combination of cultural, physical, chemical and biological controls.

Gela *et al*. (2017) evaluated the effect of strengthening honey bee colonies via feeding, removing the supers and unoccupied combs, the combination of these practices along with trapping wax moths that are intended to enter beehive for their effectiveness to prevent wax moth infestation for three years and compared with control. All these management packages (feeding, reducing super and unoccupied frames) significantly low wax moth infestation and high honey yield was recorded. It reduced the wax moth infestation level by 82.3%.

Madhu (2013) reported the placement of delta trap fitted with *A. dorsata* comb on top cover of the *A. cerana* hive prevented the wax moth infestation in *A. cerana* colonies. Greater wax moths were attracted towards delta trap fixed with *A. dorsata* comb when compared to *A. cerana* comb.

Vijaykumar *et al.* (2019) used old and unused brood combs of *A. dorsata*, *A. cerana* and *A. florea* to form sticky traps for attracting adults of greater wax moths. Yellow sticky traps with different combs were placed in different apiaries to ascertain the preference of combs by wax moth. They observed the efficiency of sticky traps in trapping the wax moth adults and concluded that placing yellow sticky trap fitted with *A. dorsata* comb on top cover of the *A. cerana* hive helped in better prevention of the wax moth infestation in *A. cerana* colonies.

b) Chemical methods

Wax moth infestation can be controlled up to two months by treating the cracks and crevices with lime sulphur paste (Shylesh, 1987).

Metwally *et al.* (1982) demonstrated that sealing the cracks and crevices with the lime sulphur paste results in 78% protection against wax moth infestation. The effectiveness of the treatment lasted for about 5-6 months and throughout the year if two treatments were done during May and December. Basically it acted as a repellent and thereby prevented the egg laying.

Gillard, (2009) used carbon dioxide as fumigant to control wax moth in storage chambers. At 100°F with 50 per cent RH the level of CO_2 was highest and killed all stages of wax moth within 4 hours and in addition to this PDB (Para dichlorobenzene) also used in storage chambers as fumigant.

Madhu (2013) reported that out of two *Bt* products V-*Bt* (commercial product) and HD-1(local *Bt* product), the highest larval mortality with less comb damage was recorded in case of V-*Bt* (commercial product). The IPM practices comprising cultural (periodic cleaning), Mechanical (oviposition trap installation) and chemical (lime sulphur application) methods when integrated helped in total protection of *A. cerana* bee colonies from the attack of greater wax moth up to three months.

Telles *et al.* (2020) evaluated the efficiency of four natural products Neem oil (*Azadirachta indica*), eucalyptus oil (*Eucalyptus spp*.), tobacco extract (*Nicotiana tabacum*), and malagueta pepper extract (*Capsicum frutescens*) for the control of greater wax moth. They observed their effects on population growth of colonies and adult bees also. In vitro bioassays were done on the 4^{th} instar wax moths larvae and adult bees for different concentrations of the products. They sprayed the natural products on bee colonies to evaluate their effects on population growth and reported that the neem and eucalyptus oils caused wax moth mortality at low concentrations, but did not affect colony population growth.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled **"Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees"** was conducted during January, 2019 to August, 2021. For seasonal incidence of the diseases, mites and greater wax moth, the studies were carried out under stationary conditions of *A. mellifera* L. and *Apis cerana* F. colonies maintained by the Department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, which is situated at 33.3°N latitude, 70.70°E longitude and 1256 m above mean sea level (amsl) and migratory conditions of *A. mellifera* L. from the university apiary to the plains at Hisar (Haryana) situated at 29.1°N latitude, 75.46°E longitude and 215 m above mean sea level (amsl). Experiments on management of greater wax moth (*G. mellonella* L.) and brood diseases were carried out at Apiculture field and Apiculture Laboratory of the Department of Entomology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. during 2020-2021.

3.1 TO STUDY SEASONAL INCIDENCE OF BROOD DISEASES, MITES AND GREATER WAX MOTH (*Galleria mellonella* L.) IN RELATION TO ENVIRONMENTAL FACTORS

The incidence of brood diseases, mites and greater wax moth in relation to environmental factors were studied under stationary conditions in both *A. mellifera* and *A. cerana* colonies and migratory conditions only in *A. mellifera* colonies.

3.1.1 Colony Records

Data on following parameters of experimental colonies under stationary and migratory conditions was recorded at an interval of 21 days for a period of two years. Healthy ten colonies of *A. mellifera* and five colonies of *A. cerana* of almost equal strength were selected from the existing stock at university apiary. The colonies of *A. cerana* were maintained at university apiary and five out of ten colonies of *A. mellifera* were kept under stationary condition at university apiary and five colonies were migrated to Hisar for four months, during winter season in both the years of study.

Colony Strength

The strength of each selected colony for the experiment was estimated by observing the number of frames covered with bees.

Brood area and Pollen area

Brood area and pollen area were measured with the help of measuring grid having squares, each square measuring one square inch (6.45 cm^2) on both side of the frame. The brood area was converted into cm^2 by multiplying the number of squares with a factor of 6.45.

Honey/nectar stores

Honey stores were estimated visually on the assumption that one Langstroth frame sealed with honey contains 2 kg of honey in *A. mellifera* and 1.7 kg in *A. cerana* colonies.

3.1.2 Diseases of *Apis mellifera* and *Apis cerana*

The incidence of diseases appeared in the colonies during the period of experimentation was recorded at monthly interval in the experimental colonies both under stationary and migratory conditions.

Incidence of Brood Diseases

Hundred brood cells (50 sealed and 50 unsealed) in each of selected colony were examined for recording incidence of brood diseases. During disease incidence, larvae showing the standard disease symptoms were counted.

European foulbrood disease (EFB)

The brood of *A. mellifera* and *A. cerana* colonies was visualized for the presence of different symptoms of European foulbrood disease. Field diagnosis for EFB infected colonies was carried out on the basis of symptoms viz., brood pattern, placement and change in colour of diseased larvae in cells and discoloured appearance of brood capping.

Thai sacbrood and Sacbrood diseases (TSBV and SBV)

The disease suspected colonies of *A. mellifera* and *A. cerana* were visualized for different symptoms of sacbrood disease. Field diagnosis for TSBV and SBV diseased

colonies was carried out on the basis of symptoms like, scattered brood pattern, sunken and perforated cappings, change in colour and shape of larvae.

3.1.3 Incidence of ectoparasitic mites

The incidence of ectoparasitic mites (*Varroa destructor* and *Tropilaelaps clareae*) in brood and adults were recorded in selected honey bee colonies by using two different methods of mite estimation (visual examination and per 100 bee methods) (Asha *et al.,* 2013 and Poonia *et al*., 2014) during January, 2019 to December 2020 in the university apiary, Nauni, Solan both under stationary and migratory conditions at an interval of 21 days. The incidence of ectoparasitic mites in brood and adults was recorded in selected colonies of *A. cerana* by visual examination only and in *A. mellifera* colonies by both visual examination of brood cells and per hundred bees method.

Observations recorded

Estimation of mite population in brood cells by visual examination method

For determining the mite populations in experimental colonies 100 brood cells of *A. mellifera* and *A. cerana* colonies were observed in each selected colonies with the help of hand lens.

Estimation of mites per 100 bees

Random samples of about 100 bees from brood nest of selected colonies were collected and put into open mouthed glass bottle. Thereafter, powdered sugar (5g) was dusted over these bees in order to motivate them for grooming and slightly agitating them. After 4-5 minutes, the jar was inverted on a paper and dislodged mites falling on paper were counted.

3.1.4 Incidence of greater wax moth (*G. mellonella* L.)

For recording the seasonal incidence of greater wax moth in *A. mellifera* and *A. cerana* colonies, the colonies with equal bee strength were selected. Selection was made from the colonies with the same queen age. All the colonies were equalized with respect to the bee strength, brood (unsealed and sealed) and stores (pollen, nectar and honey). For the purpose each comb after brushing off the bees were held against the sunlight and observed for the presence of larvae, pupae and adults. The debris at the bottom board was also observed for

the presence of wax moth stages. The cracks and crevices were also observed for the presence of pupae. Observations were made at an interval of 21 days.

3.1.5 Studies on impact of weather parameters

To study impact of environmental factors on brood diseases, mites and wax moth incidence in honey bee colonies, the data on temperature, rainfall and humidity was procured from Department of Environmental Science, UHF Nauni, Solan and Department of Agricultural Meteorology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana for stationary and migratory colonies, respectively. The incidence of brood diseases and enemies in honey bee colonies during 2019-2021 was correlated with weather (temperature, relative humidity and rainfall) and colony (brood area and colony strength) parameters.

3.2 REARING OF *G. mellonella* ON ARTIFICIAL DIET UNDER LABORATORY CONDITIONS

The larvae of greater wax moth (*G. mellonella)* were collected from infested bee combs in Apiculture field of the Department of Entomology, Dr. Y S Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. The larvae of wax moth were reared on artificial diet and the culture was maintained under laboratory conditions.

Maintenance of pure culture of greater wax moth

Preparation of artificial diet

The artificial diet for larval rearing was prepared and used as and when needed. The ingredients used in the preparation of artificial diet were corn flour, wheat flour, milk powder, yeast tablets, honey and glycerine. The ingredients were mixed thoroughly in a container by stirring or mixing well with hands covered by surgical gloves (Plate 1).

Ingredients of artificial diet for rearing *Galleria mellonella* (Metwally *et al*., 2012)

Sr. No.	Ingredients	Quantity (g or ml per kg)	Quantity %
1	Wheat flour	350	35.00
2	Corn meal	200	20.00
3	Milk powder	130	13.00
4	Yeast tablets	70	7.00
5	Honey	100	10.00
6	Glycerine	150	15.00

Plate 1. Preparation of artificial diet for wax moth (*Galleria mellonella*)

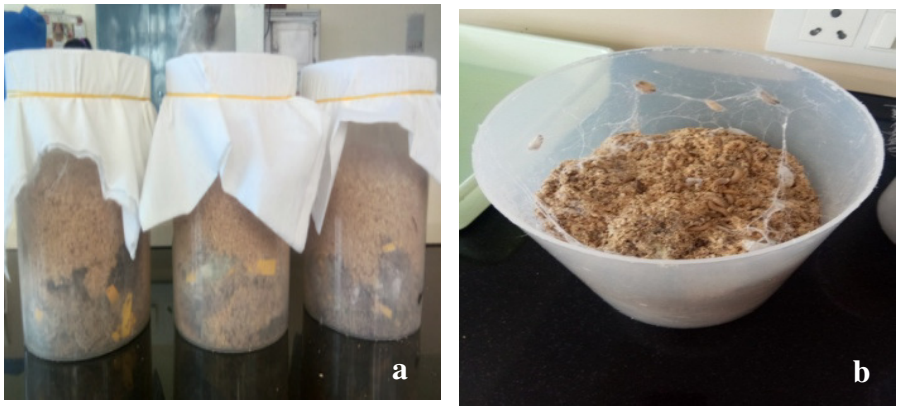


Plate 2. a) Culture of greater wax moth (*Galleria mellonella)* in artificial diet maintained under laboratory conditions and b) Feeding of larvae of greater wax moth on artificial diet

Plate 3. Wax moth adults in insect rearing cages

The collected wax moth larvae were released on artificial diet in plastic jars (Plate 2a & b). The culture was maintained in the laboratory conditions. The larvae were pupate, the pupae were collected and kept in mating cages where adults emerged and these adults were fed with honey syrup (1:1) on the cotton swab and allowed for mating (Plate 3). Zigzag folded paper strips were inserted in the cages to facilitate egg deposition. The eggs were seeded with freshly made artificial diet. The emerged larvae started feeding and acted as pure culture for various experiments.

3.3 MANAGEMENT OF GREATER WAX MOTH (*G. mellonella*) UNDER LABORATORY CONDITIONS

3.3.1 Treatment Details

No of combs	60
Comb's dimensions	17×8 cm^2
No of treatments	15
No of replications	4

Treatments (Plate 4) were **T1**: Spray with neem oil, **T2**: Dried neem leaf powder, **T3:** Spray with 5% Neem Seed Kernel Extract (NSKE), **T4**: Spray with karanj oil (3%), **T5:** Alsi (*Linum usitatissimum*) seed extract, **T6:** Pumpkin (*Cucurbita moschata*) seed extract, **T7:** *Bacillus thuringiensis var.kurstaki* spray, **T8:** *Trichoderma viridae*, **T9***:* *Bauveria bassiana*, **T10:** *Metarhizium anisopliae*, **T11:** Acetic acid , **T12:** Formic acid , **T13:** Sulphur fumigation, **T14:** Deep freezing at -8°C to - 10°C, **T15:** Control. The combs were air dried after application of various treatments. However, in case of treatment **T14:** (Keeping the frames in deep freezer at -8°C to - 10°C), there was no need to dry the combs, the larvae were directly transferred to the fresh combs. Ten third instar larvae were released on the treated combs in four replications (Plate 5). The jars were covered with muslin cloth and were kept in isolation at room temperature and relative humidity. The number of larvae were kept up to ten to avoid cannibalism. Observations on damaged area and change in weight of the combs were recorded at 7, 14 and 21 days of treatment.

Preparation of treatment

T1: Neem oil (3%) spray

The neem oil spray (3%) was prepared by mixing commercially available neem oil with water. Then 2-3 ml of Teepol was added to one liter of solution as sticker. The

prepared solution was sprayed over the combs with the help of a hand sprayer. The combs were left to dry for about 20 minutes.

T2: Dusting with dried neem leaf powder

Neem leaves were collected and left to dry under shade in laboratory conditions. After 15-18 days, when the leaves were completely dried, they were ground with the help of electronic grinder and fine powder was made**.** Each of four replications were applied with 8.33g neem leaf powder (Plate 6a).

T3: Spray with 5% Neem Seed Kernel Extract (NSKE)

Five per cent (5%) neem seed kernel extract was prepared by using commercially available NSKE powder. The extract was prepared by adding 5gm of NSKE powder in 95ml of water and mixed by stirring with the help of a glass rod and sieved through the double layer of a muslin cloth. The combs were treated with this extract by spraying it with the help of hand sprayer and were allowed to dry for 15-20 minutes.

T4: **Spray with karanj oil (3%)**

A spray solution of Karanj oil (3%) was prepared by adding 3 ml of Karanj oil to 97 ml of water. Few drops of Teepol were added as a sticker to let the oil mix in water properly. The solution was mixed properly by stirring with a glass rod. The combs were treated with Karanj oil solution and allowed to get dry for 15-20 minutes.

T5: Alsi (*Linum usitatissimum*) seed extract

For the preparation of Alsi seed extract, seeds were procured from local market and dried under shade. After drying, seeds were ground into powdered form by an electronic grinder, separately. 20 g of powder was mixed with 100ml of acetone (85%) and kept for 10 minutes. The plant- solvent mixture was stirred gently with glass rod. The plant solvent mixture was filtered using 125 mm filter paper. The mixture was then strained in the flask. The residual was pressed gently with hand and discarded later and liquid was clarified by filtration or decantation. The decanted solution again filtered by filter paper and finally prepared solution was used for experimental purpose.

Plate 4. Complete set of treatments for the control of greater wax moth *G. mellonella* in *A. mellifera* and *A. cerana* combs in laboratory conditions

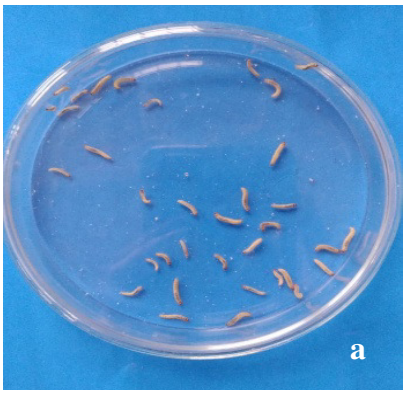


Plate5. a) Third instar larvae and b) Transfer of third instar larvae to *A. mellifera* and *A. cerana* comb

T6: Pumpkin (*Cucurbita moschata*) seed extract:

Pumpkin seeds were ground into powder and 20 g of powder was mixed with 100 ml of acetone (85%). The solution was kept as such for 10 minutes. This mixture was stirred gently with glass rod and was filtered. The mixture was then strained in the flask. Residue was pressed gently with hand and was clarified by filtration. The decanted solution again filtered by filter paper and final solution was used for spraying the combs.

T7: *Bacillus thuringiensis* var. *kurstaki* spray

Spray suspension was prepared by mixing 5 g of *Bacillus thuringiensis* var. *kurstaki* (Dipel) in one liter of water and 50 ml of solution was sprayed on individual comb covering both the sides of combs (Plate 6b).

T8: *Trichoderma viridae* (1 X 10^9 spores/ml)

2 ml of *Trichoderma viridae* was dissolved in 1litre of water**.** The prepared solution (50ml) was sprayed over the combs with the help of a hand sprayer.The combs were left to dry for about 20 minutes.

T9: *Bauveria bassiana* (1 X 10^9 spores/ml)

For the preparation of *Bauveria bassiana* spray solution, 2 ml of the suspension was dissolved in 1litre of water and 50 ml of this solution was used to spray the pieces of the combs.

T10: *Metarhizium anisopliae* (1 X 10^9 spores/ml)

Spray solution of *Metarhizium anisopliae* was also prepared by dissolving 2 ml of the biopesticide suspension in 1litre of water and 50 ml was sprayed on the combs.

T11: Acetic acid spray

Acetic acid solution (80%) was prepared by dissolving 80 ml of acetic acid (100%) in 20 ml of water further, 20ml of acetic acid (80%) was dissolved per 10 liter of water and 50 ml was sprayed on each piece of the comb.

T12: Formic acid spray

Comb was sprayed with formic acid (85%) solution (8ml formic acid per 10 liter of water). 50 ml of solution was sprayed on both sides of the comb for further experiments.

T13: Sulphur fumigation

The experiment with sulphur fumigation was established with four replicates. Sulphur fumes were produced by burning sulphur @ 0.5 g on live charcoal (Plate 7a). The treatment with the sulphur requires air tight conditions. The jars in which sulphur fumigation was done were kept in air tight conditions with the help of polythene sheet, bound with an adhesive tape (Plate 7b).

T14: Deep freezing

Ten larvae were transferred to combs of each of the four replications and the combs were kept in jars. The jars covered with muslin cloth were kept in deep freezer maintained at **-**8°C to -10°C temperature. Observations were recorded after 4 to 5 hours of treatment.

T15: Control

All four replications in control were supplied each with ten larvae of *G. mellonella*. The combs were kept free of any treatment and were protected from any external infestation by keeping the jars in isolated conditions.

3.3.2 Testing the effect of different testing materials on the development of wax moth larvae

To test the effect of above mentioned treatments on the development of wax moth larvae, multi-well insect rearing trays were used. Combs of *A. mellifera* and *A. cerana* were cut into 1 cm^3 pieces and dipped in selected concentrations of the testing materials. These pieces of combs then dried and ten pieces were placed in each tray. Ten third instar larvae were released on these pieces of the combs. Each treatment was replicated four times (Plate 8 & 9). Rearing trays were daily observed for dead or any unusual sign appearing on the larvae or pupae until the adult emergence.

3.3.3 Observations Recorded

To evaluate the effect of various treatments on the combs and wax moth larvae, pupae and adults following observations were recorded:

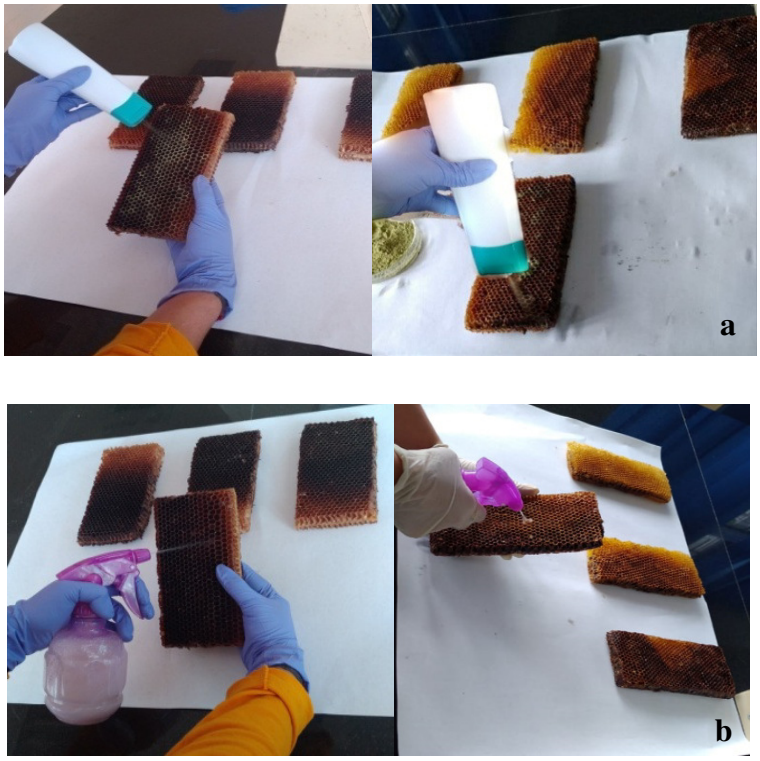


Plate 6. a) Dusting of *Apis mellifera and A. cerana* comb with neam leaf powder b) Spraying of *A. mellifera and A. cerana* comb with *Bt*

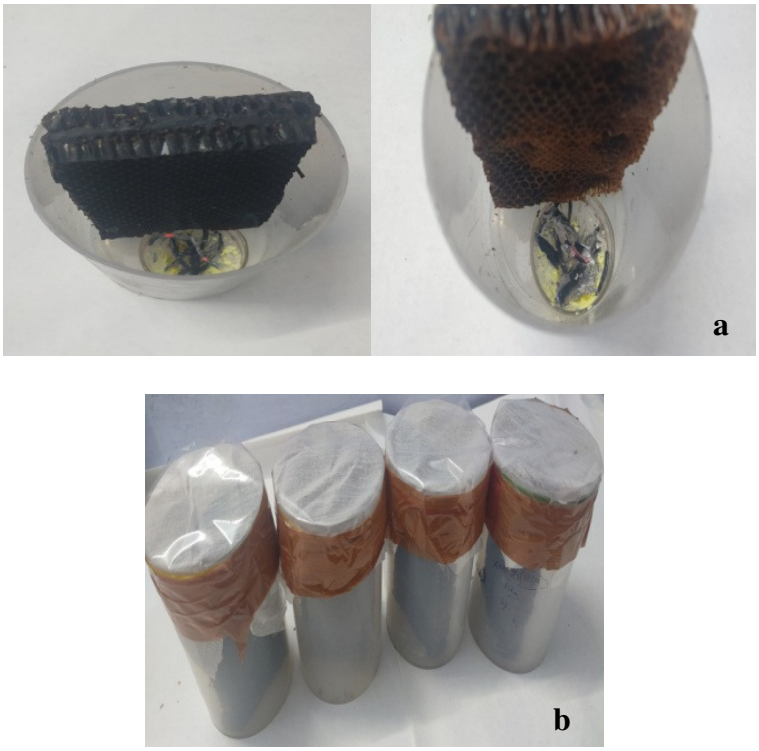


Plate 7. a) Sulphur fumigation in *A. mellifera and A. cerana* combs and b) Suphur fumigation in jars covered with polythene sheets

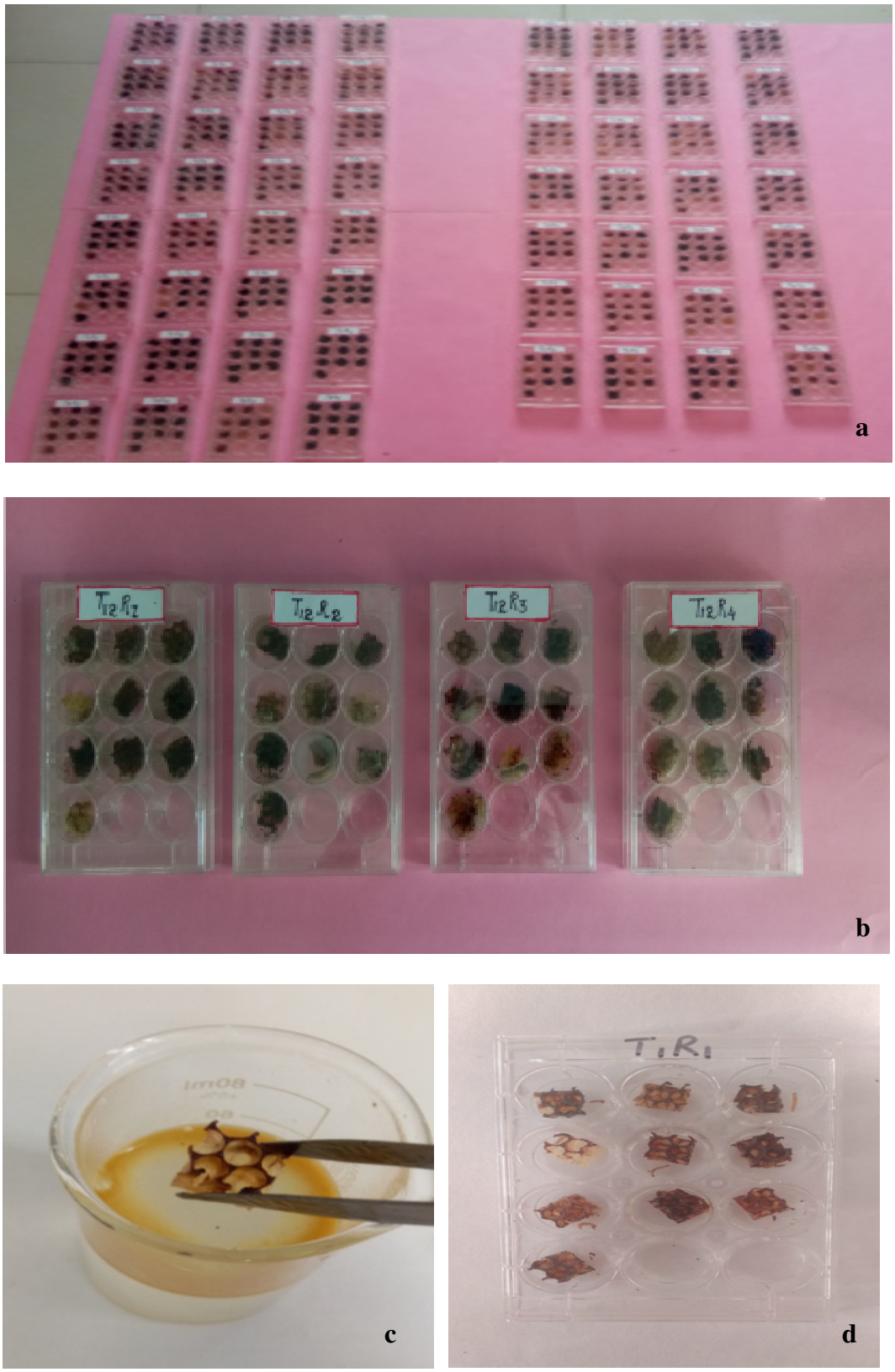


Plate 8. a) Complete set of experiment to check larval, pupal mortality and adult emergence in *A mellifera* treated combs in insect rearing trays, b) Four replications of formic acid treatment , c) Dipping of the comb pieces in the neem seed kernal extract and d) Third instar larvae in the treated comb pieces

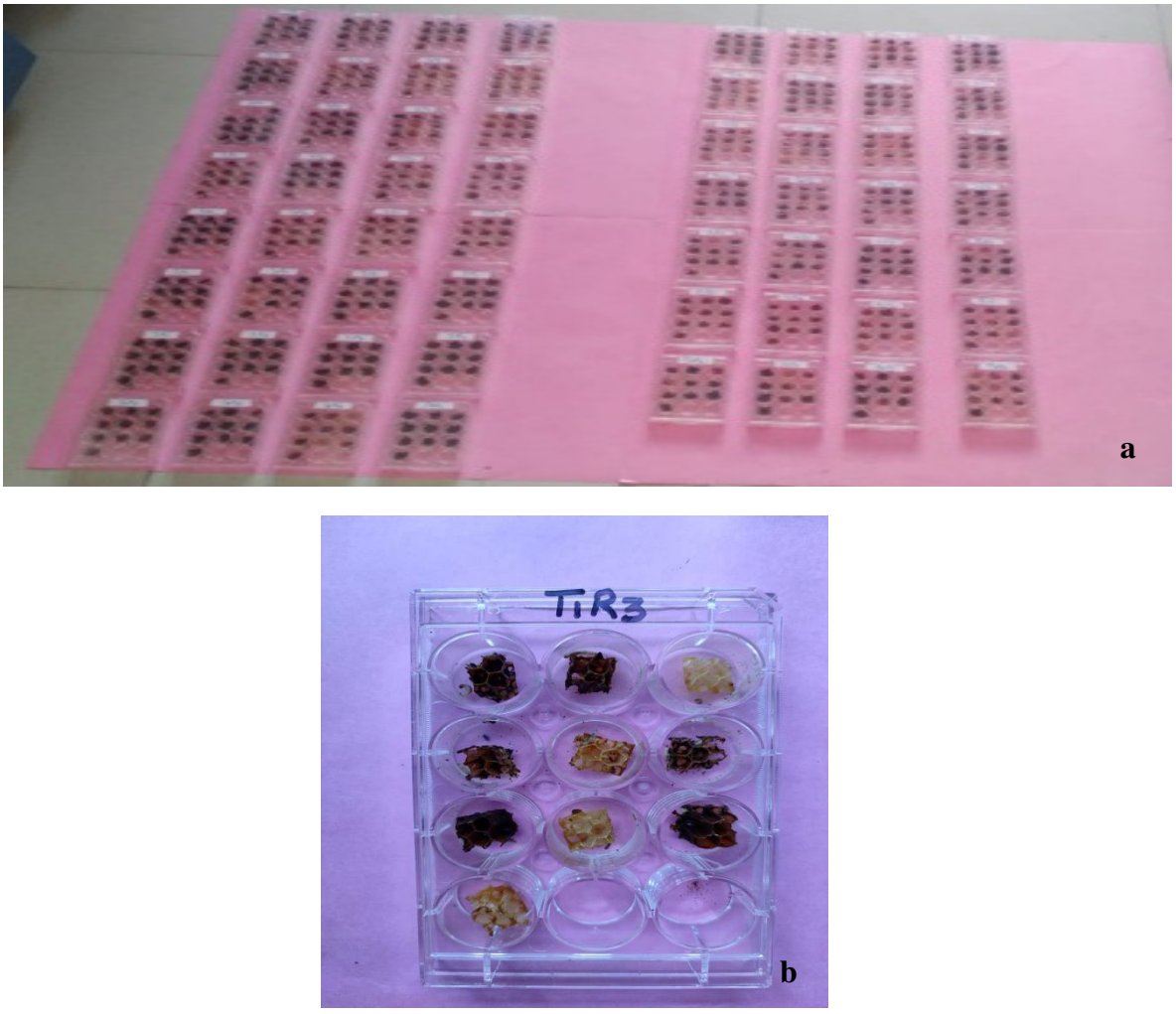


Plate 9. a) Complete set of experiment to check larval, pupal mortality and adult emergence in *A cerana* treated combs in insect rearing trays b) Third instar larvae in the treated combs and c) Four replications of neem oil treatment

1) Per cent infestation in the combs

Per cent infestation area was measured on both sides of pieces of comb with the help of measuring grid having squares, each square measuring one square inch. The damaged area was converted into cm^2 by multiplying the number of squares with a factor of 6.45.

2) Weight of the wax comb before and after treatment

The weight of wax combs before treating and after treatment was recorded to measure the reduction in weight as a result of wax moth larval feeding at 7, 14 and 21 days after treatment. Average of all four replications was taken to calculate per cent reduction in the weight of the combs.

3) Per cent larval, pupal mortality and adult emergence

During the whole period of development from larvae up to adult emergence, the number of dead larvae were counted to calculate per cent larval mortality. The number of dead pupae were eventually calculated by counting the number of emerged adults. Per cent larval and pupal mortality was calculated for each replication and average per cent mortality was calculated for each treatment. Similarly, the number of adult emerged in each replication was counted and per cent adult emergence was calculated for each treatment.

3.4 STUDIES ON MANAGEMENT OF GREATER WAX MOTH (*Galleria mellonella*) IN FIELD CONDITIONS

a) Biological, chemical and non- chemical, cultural and IPM methods in live honey bee colonies at university apiary

The better performing treatments under laboratory conditions *viz., Bt*, NSKE, neem oil and sulphur along with cultural practices and mechanical method were evaluated with integration of these treatments (9 treatments) and observations were taken at an interval of one month. Twenty seven colonies were selected having natural infestation of wax moth and each treatment was replicated three times. The treatments were repeated at an interval of thirty days.

1) Effect of *Bacillus thuringiensis* treatments on brood combs of hive

The efficiency of *Bacillus thuringiensis* var. *kurstaki* @ 3, 6 and 9 g/ l was tested on live colonies in apiary. Spray suspension was prepared by mixing 3, 6 and 9g of *Bt* in one litre of

water. 70 ml of suspension was used to spray *A. cerana* brood combs and 100 ml was used in *A. mellifera* combs. The trial was replicated three times (Plate 10a & b).

2) Neem products

The efficiency of different neem products; neem oil@ 3% and NSKE @ 5% was tested on live colonies in apiary. The formulations were prepared in the same way as described in treatment details.

3) Treating cracks and crevices of hive with lime sulphur

Preparation of lime Sulphur : 2.25 litre of water was boiled in a tin vessel. Then 200 g of quick lime and 550 g of sulphur was added to boiled water, stirred well and boiled again for one hour. The resultant liquid was strained and allowed to settle. The clear water settled above was decanted and the thin paste was used for treating the hives (Ayyar, 1940).

In this treatment, all cracks and crevices of the hive were sealed with lime sulphur paste to prevent the wax moth egg deposition in the managed colonies (Plate 10c & d).

4) Periodical cleaning of hives

The hives were cleaned once in 30 days. The eggs, larvae or debris present on the bottom board were cleaned thoroughly using a hive tool. A control was maintained without any cleaning to know the incidence of wax moth.

5) Integrated management of wax moth

Here all above said treatments like periodical cleaning, *Bt* spray, sealing cracks and crevices with lime sulphur along with delta traps with *A. cerana*, *A. mellifera* and *A. dorsta* combs were integrated and replicated thrice.

6) Control

Finally for all above said treatments the control colonies were maintained without carrying out any treatments on combs or hives to compare the effect of various treatments.

b) Mechanical method

Trapping of greater wax moth adults into delta traps fitted with *A. dorsata, A. cerana* and *A. mellifera* combs and wax moth trap

Delta trap with old brood combs of *A. dorsata*, *A. cerana and A. mellifera* were used to trap adult females of greater wax moth (*G. mellonella*). The wax moth trap used to trap both male and female was prepared according to the standard trap mixture having one peeled

Plate 10 .a) Spraying of *A. mellifera* comb and bottom board with *Bt* , b) Spraying of *A. cerana* comb and bottom board with *Bt* c) Sealed cracks and crevices of *A. mellifera* hive with lime sulphur paste (inside and outside view) and d) Sealed cracks and crevices of *A. cerana* hive with lime sulphur paste (inside view and outside view)

banana along with water, sugar and vinegar (1:1:0.5) contained in 2L plastic bottle. This mixture was used to attract the adults after fermentation for a few days. For application, the plastic bottle was drilled with an inch wide hole at its slope below the neck and hanged on a branch closer to the hive and was observed for its attractant effect to adult wax moths before getting into the hive to breed. The traps were installed in front of the hives in apiary for trapping greater wax moth adults (Plate 11) with four replications for a period of three months**.** Observations were taken to record trapped wax moths in delta trap on monthly basis.

3.5 MANAGEMENT OF BROOD DISEASES IN HIVE BEES UNDER FIELD CONDITIONS IN *A. mellifera*

The field experiments on management of brood diseases were conducted in the colonies of *A. mellifera* in university apiary in different months of the years 2020-2021, during the appearance of the disease.

3.5.1 MANAGEMENT OF SACBROOD VIRUS DISEASE

The field experiment was conducted in *A. mellifera* colonies which were naturally infected with sacbrood virus. For the control of sac brood virus in honey bee colonies of *A. mellifera*, fungal and plant extracts were used.

(a) Maintenance of pure culture and preparation of fungal extract of *Ganoderma lucidum*

Pure culture of *Ganoderma lucidum* was procured from Directorate of Mushroom Research, Chambhaghat, Solan, Himachal Pradesh. 20 ml of sterilized potato dextrose agar medium was poured in sterilized petri plates. Each petri plate was then inoculated with pure culture bit of *G. lucidum* (Plate 12 a, b). The plates were incubated at 25℃ for four to five days for growth (Plate 12 c) and were stored at 4℃. For culture maintenance disc of 4 mm diameter of mycelial mat of already grown culture was cut with the help of pre-sterilized cork borer and put in the 100 ml sterilized potato dextrose broth in 250 ml flask incubated at 25℃ for 5-7 days (Plate 12 c). After seven days the grown mycelium was separated from media through Whatmann filter paper, washed with distilled water dried with the help of lyophilizer (Plate 12 d) and ground to powder using a grinder (Plate 12 e). The aqueous extract of *G. lucidum* was prepared by mixing of 100 mg of powdered mycelium with 1 ml of sterile distilled water (Plate 12 f) then it was centrifuged at 10000 rpm for 10 minutes (Plate 12 g). The extract was then stored in a refrigerator at 4℃ (Krupodorova *et*

al., 2015). This supernatant was used for the management of experiment. *G. lucidum* extract (1ml and 3ml) was dissolved in 250 ml of 50% sugar solution and fed to the colonies three times at an interval of 4 days.

(b) Preparation of plant extract

Aqueous extracts of *Phyllanthus niruri, Azadirachta indica, Curcuma longa,* and *Carica papaya* were prepared from different plant parts as mentioned in the table below. The samples were dried, crushed and two grams of the powdered plant part was added to 250 ml of sugar solution (1:1). This extract was fed to the colonies by filling it in frame feeder at four days of interval (Plate 13).

Plant and fungal extracts used for the management of sacbrood virus in *A. mellifera* colonies (Plate 14)

Sr. No.	Plant/ fungus species	Part of plant/ fungus used	Dose (in grams or ml /250 ml of 50%sugar solution)
1.	*Ganoderma lucidum*	Mycelium	1.0
2.	*G. lucidum*	Mycelium	3.0
3.	*Phyllanthus niruri* (Bhumi amla)	Matured whole plant	2.0
4.	*Azadirachta indica* (Neem)	Bark	2.0
5.	*Curcuma longa* (Turmeric)	Rhizome	2.0
6.	*Carica papaya* L.(Papaya)	Leaves	2.0
7.	Untreated diseased control	-	-

Observations recorded

The number of infected larvae per 1000 brood cells were recorded prior to treatment and four days after each treatment.

Per cent reduction in disease with respect to control was calculated by the following formula given by Henderson & Tilton:

$$\% \text{ disease reduction} = \frac{100 \times [1 - n \text{ in Co before treatment} \times n \text{ in T after treatment}}{n \text{ in Co after treatment} \times n \text{ in T before treatment}}$$

Where, n= larval population, T= Treated, Co= control

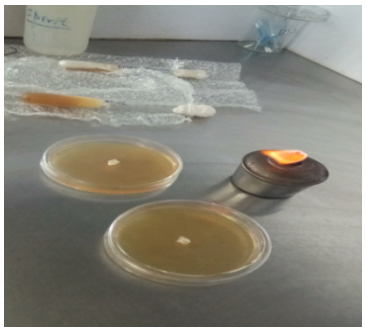
Colony strength and brood area was also recorded after 15, 30 and 60 days after treatment.

3.5.2 MANAGEMENT OF EUROPEAN FOULBROOD DISEASE

The field experiment was conducted in honey bee, *A. mellifera* colonies naturally infected with European foulbrood (EFB) disease (Plate 15a). The field experiment was conducted in different months of the years 2020-2021 during the period of disease

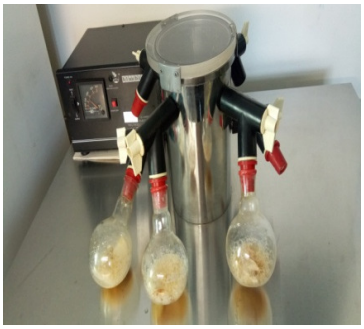
Plate 11. a) Delta trap and wax moth trap installed in *A. mellifera* apiary, b) Delta trap and wax moth trap installed in *A. cerana* apiary, c) Wax moth trap installed in front of the hives of *A. mellifera* and *A. cerana* colonies and d) Delta trap in front of *A. mellifera* and *A. cerana* colonies

a) Inoculation of *Ganoderma lucidum*

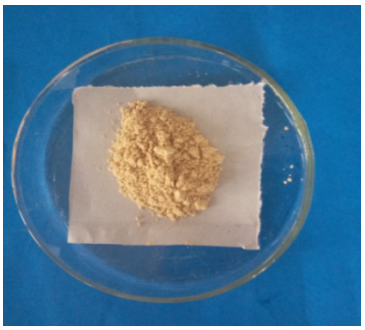


b) Inoculated plates

c) Growth of *Ganoderma lucidum* in petriplates and flasks



d) Lyophilization



e) Powder of *Ganoderma lucidum* after lyophilization

f) Dissolved Powder of *Ganoderma lucidum* (100mg in 1 ml of water)

g) Solution of *Ganoderma lucidum* after centrifugation

Plate 12. Preparation of the *Ganoderma lucidum* fungal extract

Plate 13. a) Brood pattern of sacbrood infected comb b) Feeding of *Ganoderma lucidum* , c) *Phyllanthus niruri,* d) A*zadirachta indica*, e) *Carica papaya* and f) *Curcuma longa* extract to *A. mellifera* colonies

a) Dried leaves and powder of *Phyllanthus niruri*

b) Bark and powder of *Azadirachta indica*

c) Dried leaves and powder of *Carica papaya*

d) Rhizome and powder of *Curcuma longa*

Plate 14. Plant materials used for treatment of sacbrood disease in *A. mellifera* colonies

appearance in the colonies. The formulations of essential oils were made by mixing them in sterile distilled water and propylene glycol 5% v/v as an emulsifier. Two spray of treatments 100 ml/bee frame and *G. lucidum* (1ml and 3ml /250 ml of 50% sugar solution per colony by feeding) were given to the infected colonies (Plate 15b & c) and the treatments were done two times at an interval of seven days.

Plant, fungal and bee-products used for the management of European foulbrood disease in *A. mellifera* colonies

Sr. No.	Treatments	Dose g or ml / 100 ml distilled water or 250 ml of sugar solution
1.	Mycelial extract of *G. lucidum*	3.00 ml
2.	Mycelial extract of *G. lucidum*	1.00 ml
3.	Bee honey	1.00 g
4.	Propolis	1.00 g
5.	Cinnamon (*Cinnamomum zeylanicum*) powder	1.00 g
6.	Cloves (*Syzygium aromaticum*) oil	1.00 ml
7.	Thymol (*Thymus vulgaris*) oil	1.00 ml
8.	Untreated diseased control	-

Observations recorded

Observations were recorded on number of European foulbrood infected larvae to the total number of cells observed after seven days of first and second treatment. The percent infection can be calculated by using formula:-

$$\text{Per cent infection} = \frac{\text{Number of diseased larvae}}{\text{Total number of cells observed}} \times 100$$

Percent reduction in disease with respect to control was calculated as per Henderson & Tilton's formula:

$$\%\ \text{disease reduction} = \frac{100 \times [1 - \text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}$$

Where, n= larval population, T= Treated, Co= control

3.6 STATISTICAL ANALYSIS

The data obtained from these investigations were analyzed statistically by using MS-Excel and OPSTAT. The mean value of data was subjected to statistical analysis as described by Gomez and Gomez (1986) by applying Completely Randomized Design (CRD) and Randomized Block Design (RBD).

Plate 15. a) Brood pattern of European foulbrood infected comb, b) Feeding of *Ganoderma lucidum* extract to the colony and c) Spraying of the European foulbrood infected colony with cinnamon extract

Chapter-4

RESULT AND DISCUSSION

The experimental results and discussion of present investigation entitled "**Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees"** are presented and discussed in this chapter.

4.1 SEASONAL INCIDENCE OF BROOD DISEASES, MITES AND GREATER WAX MOTH (*G. mellonella*) IN *A. cerana* COLONIES

4.1.1 COLONY RECORDS

a) During 2019

Colony records in *A. cerana* colonies under stationary (Nauni, Solan) conditions were recorded at monthly interval during January, 2019 to December, 2019. During the study period five experimental colonies of equal strength of *A. cerana* in the stationary conditions were selected in the university apiary at Nauni, Solan.

The data on colony records of *A. cerana* under stationary conditions during January, 2019 to December, 2019 are presented in Table 1. Maximum average colony strength was recorded in the month of April (5.24 bee frames) which was statistically at par with May (5.02 bee frames) and October (5.00 bee frames) followed by March (4.72 bee frames) which was at par with February (4.44 bee frames). The average colony strength in July was 3.98 bee frames which was statistically at par with September (3.88 bee frames) and August (3.65 bee frames) followed by November (3.60 bee frames) and June (3.08 bee frames). Minimum average colony strength was observed in the month of January (2.36 bee frames) which was statistically at par with December (2.45 bee frames).

The average brood area was maximum during the month of April (1849.86 cm^2) which was statistically at par with March (1510.59 cm^2) followed by average brood area in February (1153.26 cm^2) which was at par with May (1137.78 cm^2), September (957.18 cm^2), June (921.06 cm^2), November (906.87 cm^2), July (890.10cm^2) and October (878.49 cm^2). The average brood area in the month of December was 675.96 cm^2 which was statistically at par with August (647.58 cm^2). Significantly minimum average brood area was observed in the month of January (145.77 cm^2).

Table 1. Colony records of *A. cerana* during January to December, 2019 at Nauni

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2019	2.36	145.77 (12.08)*	52.89 (7.20)	122.00 (11.09)
February	4.44	1153.26 (33.97)	95.46 (9.79)	224.00 (15.00)
March	4.72	1510.59 (38.87)	122.55 (10.99)	452.00 (21.28)
April	5.24	1849.86 (43.02)	181.89 (13.49)	678.00 (28.44)
May	5.02	1137.78 (33.68)	245.10 (15.65)	808.00 (26.06)
June	3.08	921.06 (30.18)	135.45 (11.59)	556.00 (23.60)
July	3.98	890.10 (29.81)	140.61 (11.88)	408.00 (20.22)
August	3.65	647.58 (25.23)	79.98 (8.94)	596.00 (24.43)
September	3.88	957.18 (30.89)	69.66 (8.34)	702.00 (26.51)
October	5.00	878.49 (29.44)	185.76 (13.66)	714.00 (26.74)
November	3.60	906.87 (29.90)	112.23 (10.52)	368.00 (19.21)
December	2.45	675.96 (25.79)	61.92 (7.89)	244.00 (15.65)
C.D $_{0.05}$	0.33	(5.18)	(1.56)	(4.52)

*Figures in parentheses are square root (x+1) transformed values

Significantly maximum average pollen area was recorded in the month of May, 2019 (245.10 cm^2) followed by October (185.76 cm^2) which was statistically at par with average pollen area in the month of April (181.89 cm^2).The average pollen area in the month of July was 140.61 cm^2 which was statistically at par with June, March and November i.e. 135.45 cm^2, 122.55 cm^2 and 112.23 cm^2, respectively followed by February (95.46cm^2) which was statistically at par with August (79.98 cm^2) and September (69.66 cm^2). Minimum average pollen area (52.89 cm^2) was observed in the month of January which was statistically at par with December, (61.92 cm^2) and September (69.66cm^2).

Maximum average honey store was observed in the month of May (808.00 g) which was statistically at par with October (714.00 g), September (702.00 g), April (678.00 g) and August (596.00 g) followed by June (556.00 g) which was statistically at par with March (452.00 g), July (408.00 g) and November (368.00 g) which was further at par with February (244.00g). The minimum average honey store (122.00 g) in the present study was observed in the month of January which was statistically at par with honey store in the month of December (244.00 g).

b) During 2020

The data on colony records of *A. cerana* under stationary condition during January, 2020 to December, 2020 is presented in Table 2. Significantly maximum average colony strength was recorded in the month of April (5.51 bee frames) followed by May (4.44 bee frames) which was statistically at par with July (4.24 bee frames), March (4.24 bee frames) and October (4.09 bee frames). The average colony strength in August was 3.85 bee frames which was statistically at par with February (3.72 bee frames), September (3.60 bee frames) and June (3.55 bee frames) followed by November (2.76 bee frames). Minimum average colony strength was observed in the month of December (2.00 bee frames) which was statistically at par with January (2.08 bee frames).

The average brood area was maximum during the month of April (1780.20 cm^2) which was statistically at par with average brood area in the month of March (1379.00 cm^2) followed by February (1179.06 cm^2) which was statistically at par with November (968.79 cm^2), September (957.18 cm^2), May (936.54 cm^2), June (839.79 cm^2) and October (801.09 cm^2). The average brood area in the months of July was 727.56 cm^2 which was statistically at par with August (664.35 cm^2) and December (509.55cm^2). Whereas, in the year 2020, significantly minimum average brood area was observed in the month of January (245.10 cm^2).

The data on pollen area in *A. cerana* colonies revealed that pollen area was significantly high during the month of April (232.20 cm^2) followed by October (174.15cm^2) which was statistically at par with average brood area in the month of May (171.57 cm^2), July (148.35cm^2) and June (144.48 cm^2). The brood area in March was 129.00 cm^2 which was statistically at par with November (127.71 cm^2) and February (107.07 cm^2). Least average pollen area 64.50 cm^2 was observed in the month of January which was statistically at par

with pollen area in the month of September (68.37 cm^2), December (70.95 cm^2) and August (72.24 cm^2).

Data in Table 2 further revealed that maximum average honey store was observed in the month of May (904.00 g) being statistically at par with October (722.00 g) and September (690.00 g) followed by April (650.00 g) which was statistically at par with August (604.00 g), June (584.00 g) and March (472.00 g). The honey stores in the month of July was 416.00 g which was at par with November (304.00 g) which was further at par with February (232.00 g) and December (210.00 g). The minimum average honey store (112.00 g) was observed in the month of January which was statistically at par with December (210.00 g).

Table 2. Colony records of *A. cerana* during January to December, 2020 at Nauni

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2020	2.08	245.10 (15.02)*	64.50 (8.09)	112.00 (10.63)
February	3.72	1179.06 (33.80)	107.07 (10.36)	232.00 (15.26)
March	4.24	1379.00 (37.15)	129.00 (11.39)	472.00 (21.75)
April	5.51	1780.20 (42.20)	232.20 (15.27)	650.00 (25.51)
May	4.44	936.54 (30.5)	171.57 (13.12)	904.00 (30.08)
June	3.55	839.79 (28.94)	144.48 (12.03)	584.00 (24.19)
July	4.24	727.56 (26.81)	148.35 (12.18)	416.00 (20.42)
August	3.85	664.35 (25.57)	72.24 (8.48)	604.00 (24.60)
September	3.60	957.18 (30.87)	68.37 (8.28)	690.00 (26.29)
October	4.09	801.09 (28.20)	174.15 (13.20)	722.00 (26.89)
November	2.76	968.79 (30.67)	127.71 (11.33)	304.00 (17.46)
December	2.00	509.55 (22.18)	70.95 (8.48)	210.00 (14.52)
C.D $_{0.05}$	0.42	(5.79)	(1.17)	(3.98)

*Figures in parentheses are square root (x+1) transformed values

c) Pooled data (2019-2020)

Pooled colony records of *A. cerana* under stationary conditions during January, 2019 to December, 2020 are presented in Table 3 and Fig. 1. Significantly maximum average colony strength was recorded in the month of April (5.38 bee frames) followed by May (4.73 bee frames) which was statistically at par with October (4.55 bee frames) and March (4.48 bee frames). The average colony strength in July was 4.11 bee frames which was statistically at par with February (4.08 bee frames), August (3.75 bee frames) and September (3.74 bee frames) followed by June (3.32 bee frames) which was at par with November (3.18 bee frames). Minimum average colony strength was observed in the month of January (2.22 bee frames) which was statistically at par with December (2.23 bee frames).

Pooled data in Table 3 and Fig. 2 revealed that the average brood area was significantly maximum during the month of April (1815.00 cm^2) followed by March (1444.80 cm^2), February (1166.16 cm^2) which was statistically at par with May (1037.16 cm^2) and September (957.18 cm^2). The average brood area in November was 937.83 cm^2 which was statistically at par with June (880.42 cm^2), October (839.79 cm^2) and July (808.83 cm^2). The average brood area in the months of August was 655.96 cm^2 which was at par with December (592.75 cm^2). Significantly minimum average brood area was observed in the month of January (195.43 cm^2).

Significantly maximum average pollen area (Fig. 3) was recorded during the month of May (238.65 cm^2) followed by October (179.96 cm^2) which was statistically at par with average brood area in the month of April (176.73 cm^2).The average brood area in the month of July was 144.48 cm^2 which was statistically at par with June (139.97 cm^2) and March (125.78 cm^2) followed by November (119.97 cm^2) which was statistically at par with February (101.27 cm^2). Minimum average pollen area (58.70 cm^2) was observed in the month of January which was statistically at par with December (66.44 cm^2) and September (69.02 cm^2).

Maximum average honey store (Fig. 4) was observed in the month of May (856.00 g) which was statistically at par with October (718.00 g), September (696.00 g) and April (664.00 g) followed by August (600.00 g) which was statistically at par with June (570.00 g) and March (462.00 g). The honey store in the month of July was 412.00 g which was at par with November (336.00 g) and was further at par with February (228.00 g) and December

(227.00 g). Significantly minimum average honey store (117.00 g) was observed in the month of January.

Table 3. Pooled data on colony records of *A. cerana* during January, 2019 to December, 2020 at Nauni

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January	2.22	195.43 (14.02)*	58.70 (7.73)	117.00 (10.86)
February	4.08	1166.16 (34.16)	101.27 (10.11)	228.00 (15.13)
March	4.48	1444.80 (38.02)	125.78 (11.26)	462.00 (21.52)
April	5.38	1815.00 (42.61)	176.73 (13.33)	664.00 (25.79)
May	4.73	1037.16 (32.22)	238.65 (15.48)	856.00 (29.27)
June	3.32	880.42 (29.69)	139.97 (11.87)	570.00 (23.90)
July	4.11	808.83 (28.46)	144.48 (12.06)	412.00 (20.32)
August	3.75	655.96 (25.63)	76.11 (8.78)	600.00 (24.52)
September	3.74	957.18 (30.95)	69.02 (8.37)	696.00 (26.40)
October	4.55	839.79 (29.00)	179.96 (13.45)	718.00 (26.81)
November	3.18	937.83 (30.64)	119.97 (11.00)	336.00 (18.36)
December	2.23	592.75 (24.37)	66.44 (8.21)	227.00 (15.09)
C.D $_{0.05}$	0.38	(3.51)	(0.93)	(4.01)

*Figures in parentheses are square root (x+1) transformed values

In our studies maximum colony strength in *A. cerana* colonies was recorded in the month of April whereas, minimum was in the month of January statistically at par with December. December and January months are second dearth period as there is very low temperature and flora so very low foraging activity was observed. Our findings got support from earlier studies carried out in the same apiary of *A. cerana* at Nauni (Negi, 2017 and Verma, 2005). However, maximum colony strength in *A. cerana* colonies was found in the

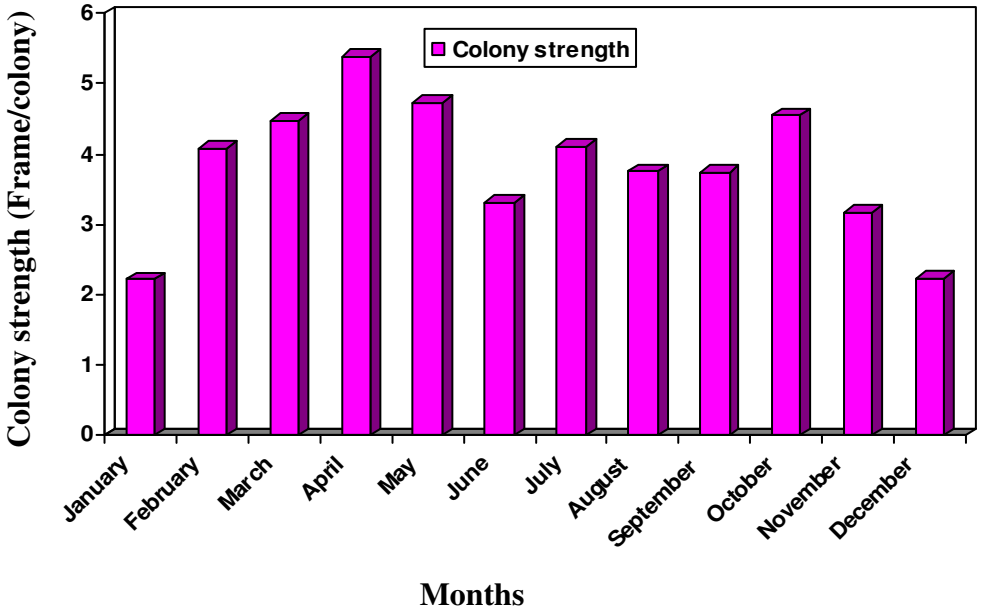


Fig 1. Colony strength of *A. cerana* (2019-2020)

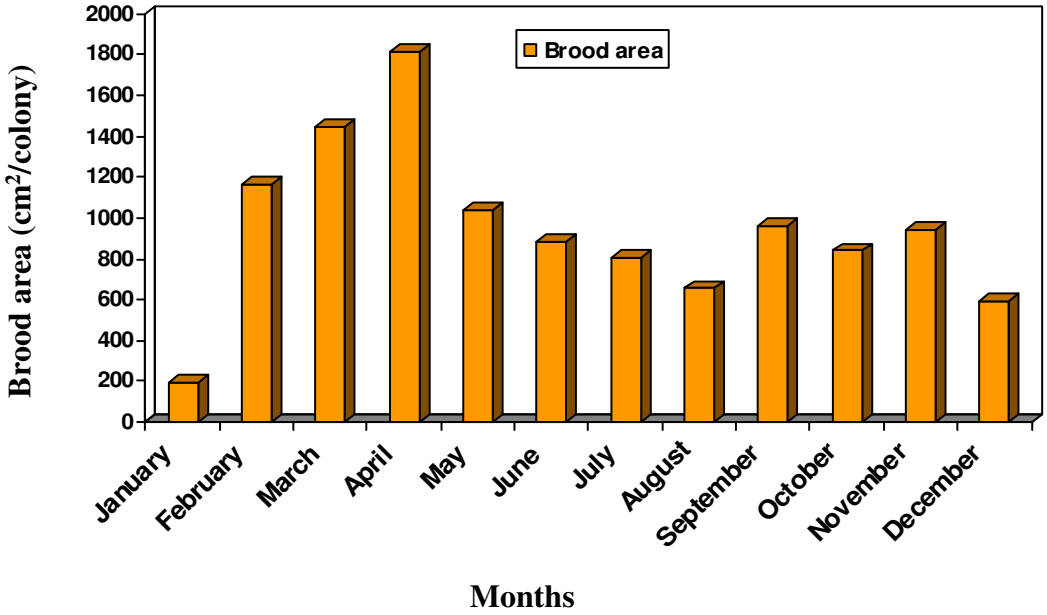


Fig 2. Brood area of *A. cerana* (2019-2020)

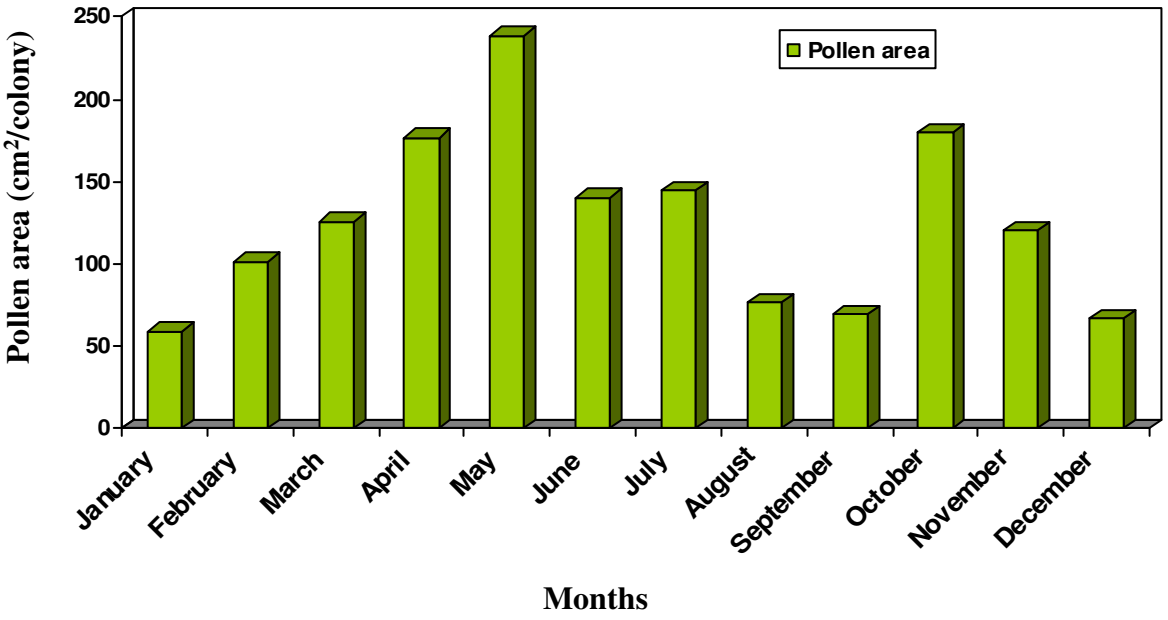


Fig 3. Pollen area of *A. cerana* (2019-2020)

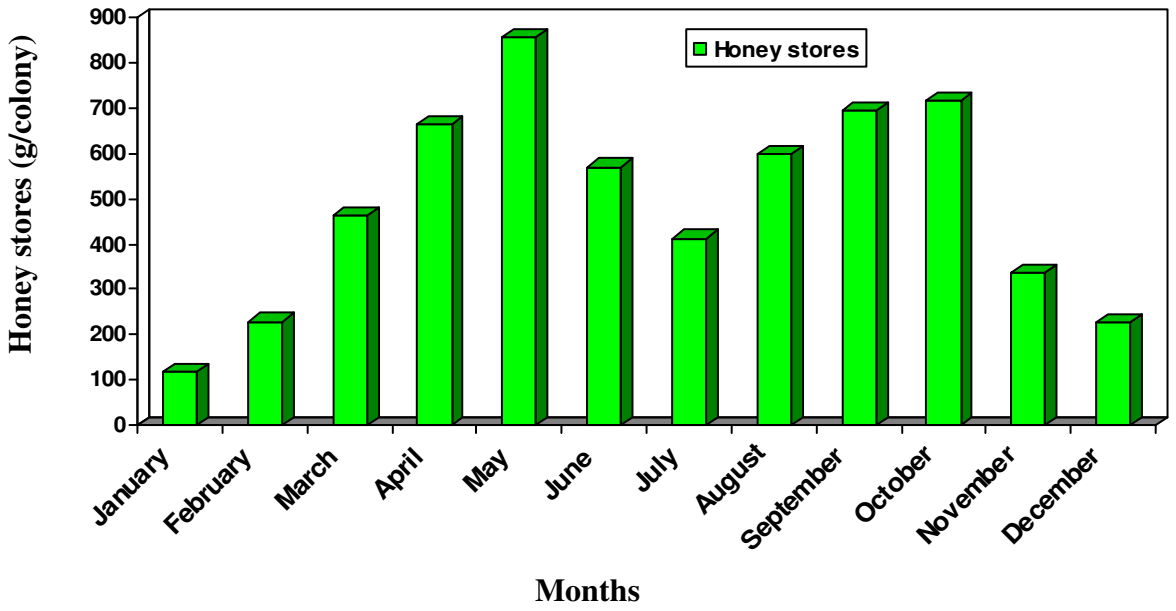


Fig 4. Honey stores of *A. cerana* (2019-2020)

month of September and minimum in the month of February at Katrain, Kullu (Rao, 2009). It may be due to different environmental conditions and location of study.

Spring and summer are most active seasons of brood rearing while autumn and winter are least one (Ismail *et al.,* 2015). The average brood area was found maximum in April followed by March and February which was statistically at par with May and September. Our findings are in close proximity with Negi (2017) who observed maximum brood area in March statistically at par with April (1326.67 cm^2/colony) and February (1217.33 cm^2/ colony). Whereas Rao (2009) recorded maximum brood area in the month of September in Katrain, Kullu. Gowda *et al*. (2005) reported maximum brood area during April, 2001 at Karnataka whereas, Rao (2009) observed in September, 2007 (2250.13 cm^2/colony).

Maximum average pollen area was found in May (238.65 cm^2) followed by October (179.96 cm^2) which was statistically at par with April (176.73 cm^2). These results are in corroboration with Sharma (2002) and Negi (2017) who observed maximum pollen area in the month of May and April, respectively. More pollen is required for brood rearing in the month of April and May as observed in present studies. Similarly, Gowda *et al*. (2005) found maximum pollen area (868.75 cm^2) in *A. cerana* during April, 2001 at Karnataka. This was mainly due to blooming of abundant pollen flora, which in turn increased pollen stores. Because there is a direct relationship between pollen flow and brood rearing activity. The greater flow of pollen results in increased brood rearing activity, which further provides more foraging force for pollen collection. In our studies minimum average pollen area (52.89 cm^2) was observed in the month of January statistically at par in December, (61.92 cm^2) and September (69.66 cm^2). Negi (2017) also showed minimum brood area in the month of December, 2016 (43.33 cm^2) which was at par in the month of January, February, March August, September and November. December and January are the dearth period due to prevailing low temperature, there are no flowering plants, so brood and foraging strength decreases. Shahi *et al*. (2010) found minimum pollen store (490.05 cm^2) in *A. cerana* in the month of November, 2007 at Bihar. Gowda *et al*. (2005) observed that pollen grains washed away due to continuous heavy rains for several days and cloudy weather during the rainy season thereby making them unavailable for bees. Moreover, continuous rain for many days reduces or stops foraging activity for longer time. Mishra and Sharma (1998) reported that

the worker bee loose ability to move at temperature below10℃ and they further said that workers stop foraging below 14℃ temperature.

In the present investigation maximum average honey stores were observed in May at par with October, September and April. The bee flora, *Eucalyptus* and *Callistemon* is found in the Nauni during March to May which is a good source of nectar and these studies supported by Negi (2017) who found maximum average honey stores in May, 2016 (1408.33 g) statistically at par with June, July and November. Earlier study conducted by Sharma (2002) also observed maximum honey store during September in *A. cerana* at Nauni. Gowda *et al.* (2005) reported maximum honey store (1300.75 g) of *A. cerana* in the month of April, 2001 at Karnataka. They found that increased number of nectar collectors resulted in the increased honey stores in the experimental colonies. Significantly minimum average honey store (117.00 g) in the present study was observed in the month of January. The present study further got support from Negi (2017) who also found minimum honey stores in the month of February statistically at par with January. Whereas, Gowda *et al*. (2005) reported minimum honey store (84.81 g) of *A. cerana* in the month of May at Karnataka, due to different environmental parameters.

4.1.2 DISEASES

4.1.2.1 Incidence of Thai sacbrood

Thai sacbrood disease of *A. cerana* was recorded from Thailand in 1976 (Bailey *et al.,* 1982). In India, Thai sacbrood disease was noticed from Meghalaya in 1978 (Kshirsagar *et al.,* 1981) which killed 95% of the colonies throughout the country by 1996 (Rana *et al.,* 1987; Phadke and Wakhle, 1996). The brood was observed and symptoms found were; scattered pattern of brood area, tongue-like projection, change in colour of the brood, and sac-like structure of brood (Plate 16) (Devaneson and Jacob, 2001; Srinivasan *et al.,* 2014).

a) During 2019

The data on incidence of Thai sacbrood disease observed in *A. cerana* colonies is presented in Table 4. The incidence varied from 0.60 to 6.60 per cent during January, 2019 to December, 2019.

Plate 16. Thai sacbrood infected larvae with tongue like projection and sac like structure in *A. cerana*

Plate 17. European foulbrood infected larvae and prepuapae in *A. cerana* colonies

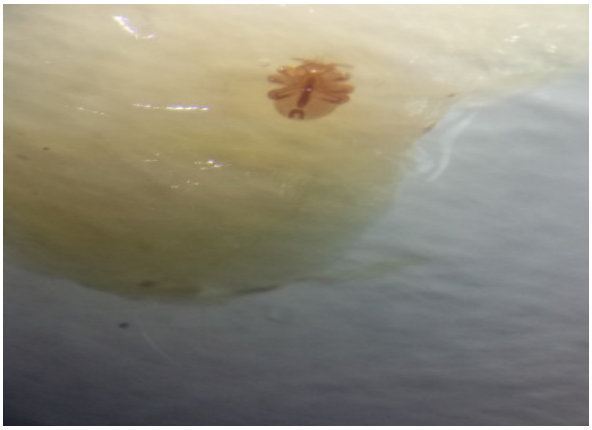


Plate 18. Ectoparasitic mite (*Tropilaelaps clareae*) on *A. cerana* larva

Table 4. Incidence of Thai sacbrood disease in *A. cerana* colonies during January to December, 2019

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	1.60 (1.61)*	2.36	145.77 (12.08)*	8.85	59.00	73.00
February	2.00 (1.73)	4.44	1153.26 (33.97)	10.34	63.00	103.10
March	0.80 (1.34)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	4.20 (2.28)	5.24	1849.86 (43.02)	20.03	49.00	36.80
May	6.60 (2.76)	5.02	1137.78 (33.68)	22.61	44.00	21.30
June	1.20 (1.48)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	2.60 (1.90)	3.98	890.10 (29.81)	23.82	79.00	218.10
August	1.80 (1.67)	3.65	647.58 (25.23)	24.43	79.00	225.80
September	3.00 (2.00)	3.88	957.18 (30.89)	23.48	77.00	151.40
October	0.00 (1.00)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	0.60 (1.26)	3.60	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	2.45	675.96 (25.79)	10.49	58.00	33.20
C.D $_{0.05}$	0.67	(0.33)	(5.18)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Thai sacbrood incidence = 0.42
Relative Humidity× Thai sacbrood incidence = -0.26
Rainfall × Thai sacbrood incidence = 0.05
Colony Strength × Thai sacbrood incidence = 0.28
Brood Area× Thai sacbrood incidence = 0.36

Maximum incidence was observed in the month of May, 2019 (6.60%) when average colony strength and brood area were 5.02 bee frames and 1137.78 cm^2 which was statistically at par with April (4.20%) followed by September (3.00%) which was statistically at par with July (2.60%), February (2.00%), August (1.80%), January (1.60%), June (1.20%) and March (0.80%). During the month of May when the incidence of Thai sacbrood virus was maximum the temperature, relative humidity and rainfall were 22.61°C, 44 per cent and 21.30 mm, respectively. The temperature, relative humidity and rainfall during the whole disease incidence period varied from 8.85 to 25.74°C, 44 to 79 per cent and 5.6 to 225.80 per cent, respectively. Whereas, minimum incidence was recorded in the month of November, 2019 (0.60%) when average colony strength and brood area were 3.60 bee frames and 906.87 cm^2 and temperature, relative humidity and rainfall were low i.e. 15.50°C, 62.00 per cent and 32.20 mm, respectively which was statistically at par with March (0.80), June (1.20%), January (1.60%), August (1.80%), February (2.00%) and July (2.60%). No incidence of Thai sacbrood disease was observed in the month of October and December. The data on correlation of Thai sacbrood incidence with colony and weather parameters in *A. cerana* colonies indicated negative correlation with relative humidity (r= -0.26).

b) During 2020

In the present study maximum incidence of Thai sacbrood disease was observed in the month of May, 2020 (7.20%) when temperature, rainfall and relative humidity were high i.e. 22.05°C, 53.00 mm and 74.80 per cent, respectively and average colony strength and brood area were 4.44 bee frames and 936.54 cm^2 which was statistically at par with April (5.40%) followed by July (3.40%) which was statistically at par with September (2.60%) and February (2.20%) (Table 5). There was no incidence of Thai sacbrood virus in the month of December, 2020 when the temperature and rainfall was 12.20°C and 23.80 mm, respectively. Whereas, minimum incidence was recorded in the month of October, 2020 (0.20%), when average bee strength and brood area was 4.09 bee frame and 801.09 $cm^2$and temperature, rainfall and relative humidity were 20.40°C, 0.00 mm and 65.00 per cent, respectively. It was statistically at par with March (0.40%), June (0.60%), November (0.80%) and August (1.00%). Thai sacbrood disease had negative correlation with relative humidity (r=- 0.39).

Table 5. Incidence of Thai sacbrood disease in *A. cerana* colonies during January to December, 2020

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	1.80 (1.67)*	2.08	245.10 (15.02)*	9.10	68.00	168.30
February	2.20 (1.79)	3.72	1179.06 (33.80)	12.15	57.00	38.50
March	0.40 (1.18)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	5.40 (2.53)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	7.20 (2.86)	4.44	936.54 (30.5)	22.05	53.00	74.80
June	0.60 (1.26)	3.55	839.79 (28.94)	24.10	69.00	58.70
July	3.40 (2.10)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	1.00 (1.41)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	2.60 (1.90)	3.60	957.18 (30.87)	24.10	77.00	6.00
October	0.20 (1.10)	4.09	801.09 (28.20)	20.40	65.00	0.00
November	0.80 (1.34)	2.76	968.79 (30.67)	14.60	68.00	37.70
December	0.00 (1.00)	2.00	509.55 (22.18)	12.20	62.00	23.80
C.D $_{0.05}$	(0.42)	0.42	(5.79)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Thai sacbrood incidence = 0.28
Relative Humidity× Thai sacbrood incidence = -0.39
Rainfall × Thai sacbrood incidence = 0.08
Colony Strength × Thai sacbrood incidence = 0.44
Brood Area× Thai sacbrood incidence = 0.36

c) Pooled data (2019-2020)

Pooled data during 2019 and 2020 on Thai sacbrood disease incidence observed in *A. cerana* colonies is presented in Table 6 and Fig. 5. Thai sacbrood disease incidence varied from 0.10 to 6.90 per cent during 2019 to 2020.

Significantly maximum incidence was observed in the month of May (6.90%) when average colony strength and brood area were 4.73 frames and 1037.16 cm^2, respectively

followed by April (4.80%), July (3.00%) which was statistically at par with Thai sacbrood disease incidence in September (2.80%) and February (2.10%). The Thai sacbrood incidence in the month of January was 1.70% which was statistically at par with August (1.40%) and June (0.90%). During the maximum incidence of Thai sacbrood disease the temperature, relative humidity and rainfall were 22.33°C, 48.50 per cent and 48.05 mm, respectively. Whereas, minimum incidence was recorded in the month of October (0.10%) which was statistically at par with March (0.60%) and November (0.70%). No incidence of Thai sacbrood disease was observed in the month of December. The data on correlation of Thai sacbrood incidence with colony and weather parameters in *A. cerana* colonies indicated negative correlation with relative humidity (r= -0.33).

Studies conducted on Thai sacbrood disease revealed that colonies remain affected from January to November and no infection was observed during December. Maximum incidence was observed in May (6.90%) followed by April and July which was statistically at par with September and February. Disease firstly appeared in January with 1.70 per cent infection. Our findings are in line with Negi *et al.* (2018) who observed maximum incidence of Thai sacbrood disease in *A. cerana* in May, 2017. She had reported less percentage of brood infection (0.27% to 1.27%) in *A. cerana* colonies as compared to our studies may be because of different weather conditions. These investigations also got support from Rana (2008) who demonstrated incidence of sacbrood disease during April to May and maximum incidence in May with 1.6 to 15.0 per cent brood infection and 20 to 60 per cent colony infection when colony strength and brood area started increasing at faster rate. During present studies the brood area and bee strength were high in February, March, April and May. The disease in the present study started appearing in the month of January. The occurrence of viral disease in *A. mellifera* in build- up period is due to the existence of greater proportions of susceptible young adults and larvae in colonies (Bailey, 1968). Sharma (2002) reported the incidence of Thai sacbrood disease during August to October, 2001 and again during March and July 2002 in *A. cerana* at Nauni, Solan. The occurrence of disease during spring to summer have also been reported by different workers (Chandel *et al.,* 1999, Hornitzky and Anderson, 2003; Rana and Rana, 2015). Kshrisagar *et al*. (1982) also recorded the incidence of Thai sacbrood virus during winter in addition to early summer. No incidence of Thai sacbrood disease was observed in the month of December in both the years. Verma and Joshi (1985) reported minimum incidence of Thai sacbrood disease in *A. cerana* colonies in the month of October, 1984 in hills of Uttar Pradesh. Negi *et al.* (2018) observed the minimum

incidence of Thai sacbrood disease in the month of November, 2016 (0.23%) in *A. cerana* colonies in the same apiary when the temperature and humidity were low i.e.15.80℃ and 40.50 per cent, respectively and no rainfall.

Table 6. Pooled data on incidence of Thai sacbrood disease in *A. cerana* colonies during January, 2019 to December, 2020

Months	Thai sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	1.70 (1.64)*	2.22	195.43 (14.02)*	8.98	63.50	120.65
February	2.10 (1.76)	4.08	1166.16 (34.16)	11.25	60.00	70.80
March	0.60 (1.26)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	4.80 (2.41)	5.38	1815.00 (42.61)	19.59	50.00	42.25
May	6.90 (2.81)	4.73	1037.16 (32.22)	22.33	48.50	48.05
June	0.90 (1.38)	3.32	880.42 (29.69)	24.92	58.50	78.60
July	3.00 (2.00)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	1.40 (1.55)	3.75	655.96 (25.63)	24.69	82.50	187.20
September	2.80 (1.95)	3.74	957.18 (30.95)	23.79	77.00	78.70
October	0.10 (1.05)	4.55	839.79 (29.00)	19.44	65.00	2.80
November	0.70 (1.30)	3.18	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	2.23	592.75 (24.37)	11.35	60.00	28.50
C.D $_{0.05}$	(0.29)	0.38	(3.51)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Thai sacbrood incidence = 0.35
Relative Humidity× Thai sacbrood incidence = -0.33
Rainfall × Thai sacbrood incidence = 0.05
Colony Strength × Thai sacbrood incidence = 0.41
Brood Area× Thai sacbrood incidence = 0.37

4.1.2.2 Incidence of European Foulbrood

The advertences were recorded on seasonal incidence of European foulbrood diseases in *A. cerana* colonies under stationary conditions from January, 2019 to December, 2020. In

A. cerana, death of the brood generally recorded at pupal stage and rarely in prepupal and larval stages which resulted perforations in sealed brood and uncapped brood (Plate 17). However the colour of the brood didn't change. This is possibly due to less anaerobic conditions inside the gut of pupae (5th instar larvae) (Rao, 2009), similar observations have reported by Rana *et al*. (2004) and Singh (2005) in *A. cerana* colonies.

a) During 2019

Seasonal incidence of European foulbrood disease under stationary conditions in *A. cerana* colonies from January, 2019 to December, 2019 is presented in Table 7.

Table 7. Incidence of European foulbrood disease in *A. cerana* colonies during January to December, 2019

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	1.80 (1.67)*	2.36	145.77 (12.08)*	8.85	59.00	73.00
February	1.00 (1.41)	4.44	1153.26 (33.97)	10.34	63.00	103.10
March	0.60 (1.26)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	5.00 (2.45)	5.24	1849.86 (43.02)	20.03	49.00	36.80
May	19.80 (4.56)	5.02	1137.78 (33.68)	22.61	44.00	21.30
June	13.40 (3.79)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	11.80 (3.58)	3.98	890.10 (29.81)	23.82	79.00	218.10
August	7.20 (2.86)	3.65	647.58 (25.23)	24.43	79.00	225.80
September	4.80 (2.41)	3.88	957.18 (30.89)	23.48	77.00	151.40
October	3.20 (2.05)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	1.40 (1.55)	3.60	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	2.45	675.96 (25.79)	10.49	58.00	33.20
C.D $_{0.05}$	(0.53)	0.33	(5.18)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.55
Relative Humidity× EFB incidence = -0.01
Rainfall × EFB incidence = 0.18
Colony Strength × EFB incidence = 0.14
Brood Area× EFB incidence = 0.10

The symptoms of European foulbrood diseases were observed in all the months of the study period except December when temperature, rainfall and relative humidity varied from 8.85 to 25.74°C, 5.60 to 225.80 mm and 44.00 to 79.00 per cent, respectively and disease incidence varied from 0.60 to 19.80 per cent. The incidence of European foulbrood disease was significantly maximum in the month of May (19.80%) when average strength and brood area were 5.02 bee frames and 1137.78 cm^2 and temperature, relative humidity and rainfall were 22.61 °C, 44.00 per cent and 21.30 mm, respectively followed by June (13.40%) which was statistically at par with July (11.80%). The European foulbrood incidence in August was 7.20% which was statistically at par with April (5.00%) and September (4.80%) followed by October (3.20%) which was at par with January (1.80%) and November (1.40%). The minimum incidence of European foulbrood was observed in the month of March (0.60%) when temperature, relative humidity and rainfall were low i.e. 13.45°C, 54.00 per cent and 54.60 mm, respectively which was statistically at par with February (1.00%), November (1.40%) and January (1.80%). The disease incidence declined after May till November (1.40 %) and disappeared during December, 2019, when average strength of the colony and brood area was 2.45 bee frame and 675.96 cm^2, respectively. During the year 2019 the European foulbrood disease showed negative correlation with relative humidity (r= -0.01).

b) During 2020

The observations recorded on the seasonal incidence of European foulbrood disease in *A. cerana* are presented in Table 8. The incidence of European foulbrood disease was observed in all the months of the study period except December when external temperature, rainfall and relative humidity varied from 9.1 to 25.05°C, 0.00 to 278.10 mm and 51.00 to 86.00 per cent, respectively. The incidence of European foulbrood disease was significantly maximum in the month of July, 2020 (23.20%) when average colony strength and brood area was 4.24 bee frames and brood area was 727.56 cm^2 and temperature, relative humidity and rainfall were 25.05 °C, 81 per cent and 278.10 mm followed by June (15.20%) which was statistically at par with May (11.80%). The European foulbrood incidence in September was 7.80% which was statistically at par with April (7.20%) and August (5.80%).Whereas, minimum European foulbrood disease incidence was observed in the month of March (1.00 %) which was statistically at par with February (1.20%), January (1.40 %), November (1.60 %) and October (3.20%). The disease incidence was low in January, February, October and November. In the present studies European foulbrood disease had positive significant relationship with mean temperature (r= 0.63).

Table 8. Incidence of European foulbrood disease in *A. cerana* colonies during January to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	1.40 (1.55)*	2.08	245.10 (15.02)*	9.10	68.00	168.30
February	1.20 (1.48)	3.72	1179.06 (33.80)	12.15	57.00	38.50
March	1.00 (1.41)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	7.20 (2.86)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	11.80 (3.58)	4.44	936.54 (30.5)	22.05	53.00	74.80
June	15.20 (4.02)	3.55	839.79 (28.94)	24.10	69.00	58.70
July	23.20 (4.92)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	5.80 (2.61)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	7.80 (2.97)	3.60	957.18 (30.87)	24.10	77.00	6.00
October	3.20 (2.05)	4.09	801.09 (28.20)	20.40	65.00	0.00
November	1.60 (1.61)	2.76	968.79 (30.67)	14.60	68.00	37.70
December	0.00 (1.00)	2.00	509.55 (22.18)	12.20	62.00	23.80
C.D $_{0.05}$	(0.64)	0.42	(5.79)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.63*
Relative Humidity× EFB incidence = 0.31
Rainfall × EFB incidence = 0.55
Colony Strength × EFB incidence = 0.32
Brood Area× EFB incidence = 0.01 (*Significant at 5%)

c) Pooled data (2019-2020)

Pooled European foulbrood incidence of 2019 to 2020 are presented in Table 9 and Fig. 6. During the incidence of the disease the temperature, rainfall and relative humidity varied from 8.98 to 24.92°C, 2.80 to 248.10 mm and 48.50 to 82.50 per cent, respectively and disease incidence varied from 0.80 to 17.50 per cent. The incidence of European foulbrood disease was statistically maximum in the month of July (17.50%) when average colony strength and brood area were 4.11 bee frames and 808.83 cm^2 and temperature, relative

humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm, respectively which was statistically at par with 15.80 per cent in the month of May and June (14.30%) followed by August (6.50%) which was statistically at par with September (6.30%) and April (6.10%). The incidence of European foulbrood disease in the month of October was 3.20% which was statistically at par with January (1.60%) and November (1.50%). Minimum incidence of European foulbrood was observed in the month of March (0.80%) when average colony strength and brood area were 4.48 frames and 1444.80 cm^2 and temperature, relative humidity and rainfall were low i.e. 13.83 °C, 58.00 per cent and 113.20 mm, respectively which was statistically at par with February (1.10%), November (1.50%) and January (1.60%). No incidence of European foulbrood was observed in the month of December in *A. cerana* colonies .The disease incidence disappeared during December, when average strength of the colony and brood area was 2.23 bee frames and 592.75 cm^2, respectively. European foulbrood disease showed significantly positive correlation with mean temperature (r= 0.61).

As the disease increased from April to July, the colony strength and brood area was decreased. When the disease incidence was maximum in the month of July (17.50%) when the temperature and relative humidity were high and rainfall was maximum. In the present studies it was observed that the high incidence of European foulbrood did not show significant reduction in colony strength and brood area. Earlier European foulbrood disease was reported to appear usually in spring and first half of summer season but in recent times its occurrence has not shown a clear dependence on season (Russenova and Parvanov, 2005).

Negi *et al.* (2018) has observed maximum incidence of European foulbrood disease in the month of July, 2016 (23.00 %), when colony strength and healthy brood area were 4.00 bee frames and 904.67cm^2/colony, respectively in *A. cerana* colonies at Nauni, Solan which supports the present findings. In a study conducted by Verma (2005) maximum infection of European foulbrood bacteria (21.24 %) in *A. cerana* was observed in the month of May, 2004 at Nauni, Solan due to high temperature and humidity. Rao (2009) has recorded maximum incidence of European foulbrood (25.33 %) in *A. cerana* in the month of August, 2007 at Katrain, Kullu. No incidence of European foulbrood was observed in the month of December in *A. cerana* colonies. Verma (2005) and Negi *et al.* (2018) found minimum incidence of European foulbrood disease in the same apiary of *A. cerana* during March. The disease disappeared during December which finds support from the findings of Rao (2009) and Negi *et al.* (2018) who also reported *A. cerana* colonies to be free from this brood disease during

winter months. This may be due to less number of available nurse bees resulted in production of less amount of glandular food which caused the diseased larvae to appear starved during winter months and was easily detected and removed by bees (Bailey, 1977). Diwan *et al*. (1971), Rana *et al.* (2004) and Verma (2005) reported similar type of occurrence and severity of disease in *A. cerana* colonies in India.

Table 9. Pooled data on incidence of European foulbrood disease in *A. cerana* colonies during January, 2019 to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	1.60 (1.61)*	2.22	195.43 (14.02)*	8.98	63.50	120.65
February	1.10 (1.45)	4.08	1166.16 (34.16)	11.25	60.00	70.80
March	0.80 (1.34)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	6.10 (2.66)	5.38	1815.00 (42.61)	19.59	50.00	42.25
May	15.80 (4.10)	4.73	1037.16 (32.22)	22.33	48.50	48.05
June	14.30 (3.91)	3.32	880.42 (29.69)	24.92	58.50	78.60
July	17.50 (4.30)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	6.50 (2.74)	3.75	655.96 (25.63)	24.69	82.50	187.20
September	6.30 (2.70)	3.74	957.18 (30.95)	23.79	77.00	78.70
October	3.20 (2.05)	4.55	839.79 (29.00)	19.44	65.00	2.80
November	1.50 (1.58)	3.18	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	2.23	592.75 (24.37)	11.35	60.00	28.50
C.D $_{0.05}$	(0.49)	0.38	(3.51)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.61*
Relative Humidity× EFB incidence = 0.12
Rainfall × EFB incidence = 0.40
Colony Strength × EFB incidence = 0.24
Brood Area× EFB incidence = 0.07 (*Significant at 5%)

4.1.2.3 Incidence of ectoparasitic mite (*Tropilaelaps clareae*)

Incidence of *T. clareae* in *A. cerana* colonies estimated by per cent brood infestation methods (Plate 18). The colonies of *A. cerana* were noticed free from *T. clareae* infestation in

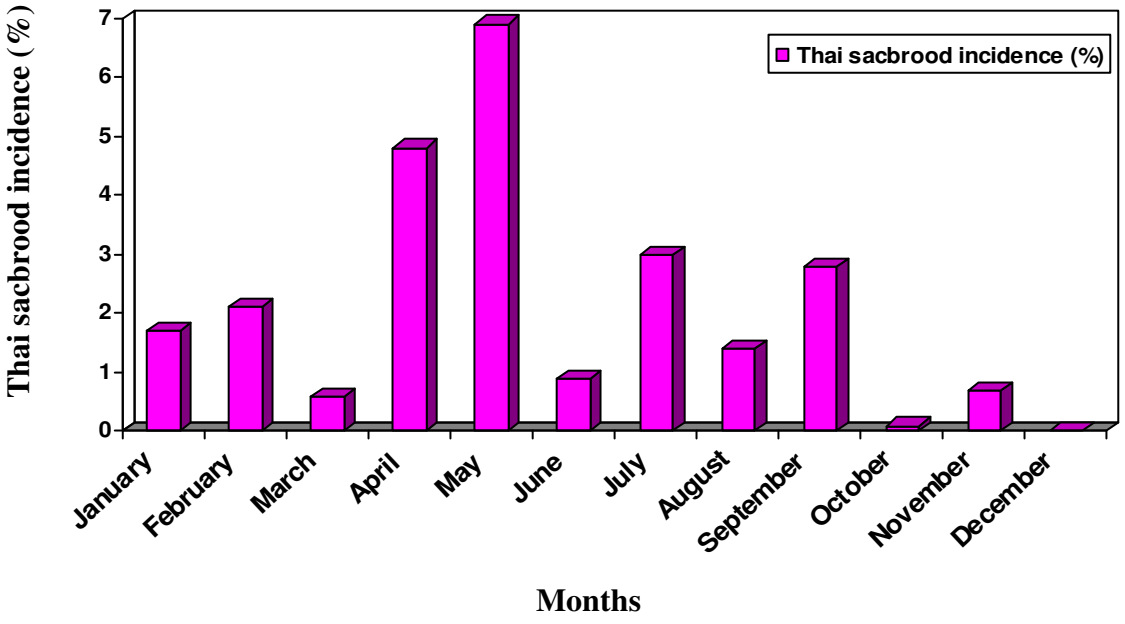


Fig 5. Incidence of Thai sacbrood disease in *A. cerana* (2019-2020)

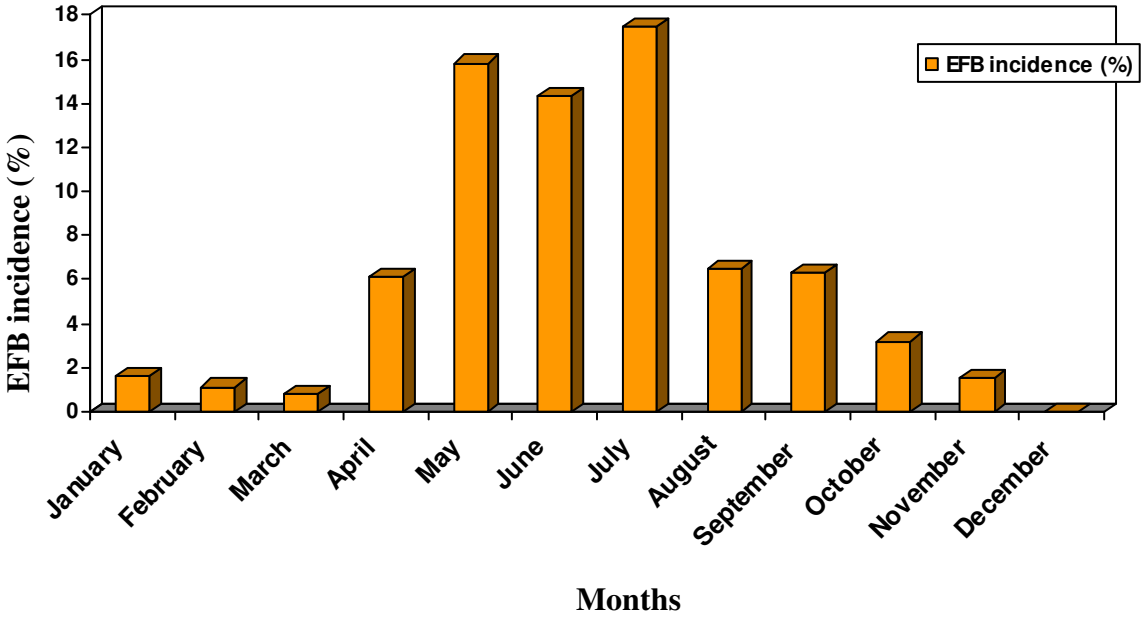


Fig 6. Incidence of European foulbrood disease in *A. cerana* (2019-2020)

January, February, August, September, October, November and December during both the years. No mite population was detected on brood during this period.

a) During 2019

T. clareae incidence in *A. cerana* colonies during January 2019 to December 2019 is presented in Table 10.

Table 10. Incidence of ectoparasitic mite (*T. clareae*) in *A. cerana* colonies during January to December, 2019

Months	Brood infestation# (%)	Colony parameters		Weather parameters		
	T. clareae	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.00 (1.00)*	2.36	145.77 (12.08)*	8.85	59.00	73.00
February	0.00 (1.00)	4.44	1153.26 (33.97)	10.34	63.00	103.10
March	2.60 (1.90)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	2.20 (1.79)	5.24	1849.86 (43.02)	20.03	49.00	36.80
May	3.40 (2.10)	5.02	1137.78 (33.68)	22.61	44.00	21.30
June	7.80 (2.97)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	2.80 (1.95)	3.98	890.10 (29.81)	23.82	79.00	218.10
August	0.00 (1.00)	3.65	647.58 (25.23)	24.43	79.00	225.80
September	0.00 (1.00)	3.88	957.18 (30.89)	23.48	77.00	151.40
October	0.00 (1.00)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	0.00 (1.00)	3.60	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	2.45	675.96 (25.79)	10.49	58.00	33.20
C.D $_{0.05}$	(0.55)	0.33	(5.18)			

*Figures in parentheses are square root (x+1) transformed values, # by visual examination

Pearson correlation Matrix (r) =

Temperature × *T. clareae* incidence = 0.52*
Relative Humidity× *T. clareae* incidence = -0.51
Rainfall × *T. clareae* incidence = 0.01
Colony Strength × *T. clareae* incidence = 0.05
Brood Area× *T. clareae* incidence = 0.29 (*Significant at 5%)

The incidence of *T. clareae* varied from 2.20 per cent to 7.80 per cent. Significantly maximum infestation of mites (7.80%) was observed in the month of June, 2019 when average colony strength and brood area were 3.08 frames and 921.06 cm^2 and temperature, relative humidity and rainfall were 25.74°C, 48 per cent and 98.50 mm respectively. Minimum incidence of *T. clareae* mite was observed in the month of April (2.20%) which was statistically at par with March (2.60%), July (2.80%) and May (3.40%). The colonies were noticed free from *T. clareae* incidence in January, February, August, September, October, November and December. Mite infestation showed negative correlation with relative humidity (r= -0.51).

b) During 2020

Data in Table 11 demonstrated that the infestation of *T. clareae* on brood varied from 1.00 per cent (March) to 6.60 per cent (June) in *A. cerana* colonies when observed through visual observation at Nauni, Solan.

Maximum incidence of *T. clareae* was observed in the month of June (6.60%) when average colony strength and brood area were 3.55 bee frames and 839.79 cm^2 and temperature, relative humidity and rainfall were 24.10°C, 69 per cent and 58.70 mm respectively which was statistically at par with May (6.20%). Minimum incidence was observed in the month of March (1.00%) when average colony strength and brood area were 4.24 bee frames and 1379.00 cm^2 and temperature, relative humidity and rainfall were 14.20°C, 62 per cent and 171.80 mm, respectively which was statistically at par with mite infestation in July (1.20%) and April (2.20%). Incidence of *T. Clareae* showed a negative correlation with relative humidity (r= -0.31) and rainfall (r= -0.02).

Table 11. Incidence of ectoparasitic mite (*T. clareae*) in *A. cerana* colonies during January to December, 2020

Months	Brood infestation# (%)	Colony parameters		Weather parameters		
	T. clareae	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	0.00 (1.00)*	2.08	245.10 (15.02)*	9.10	68.00	168.30
February	0.00 (1.00)	3.72	1179.06 (33.80)	12.15	57.00	38.50
March	1.00 (1.41)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	2.20 (1.79)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	6.20 (2.68)	4.44	936.54 (30.5)	22.05	53.00	74.80
June	6.60 (2.76)	3.55	839.79 (28.94)	24.10	69.00	58.70
July	1.20 (1.48)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	0.00 (1.00)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	0.00 (1.00)	3.60	957.18 (30.87)	24.10	77.00	6.00
October	0.00 (1.00)	4.09	801.09 (28.20)	20.40	65.00	0.00
November	0.00 (1.00)	2.76	968.79 (30.67)	14.60	68.00	37.70
December	0.00 (1.00)	2.00	509.55 (22.18)	12.20	62.00	23.80
C.D $_{0.05}$	(0.46)	0.42	(5.79)			

*Figures in parentheses are square root (x+1) transformed values, # by visual examination

Pearson correlation Matrix (r) =

Temperature × *T. clareae* incidence = 0.42
Relative Humidity× *T. clareae* incidence = -0.31
Rainfall × *T. clareae* incidence = -0.02
Colony Strength × *T. clareae* incidence = 0.23
Brood Area× *T. clareae* incidence = 0.16

c) Pooled data (2019-2020)

Pooled data on the incidence of *T. clareae* in *A. cerana* colonies during January, 2019 to December, 2020 is presented in Table 12 and Fig. 7. The incidence of *T. clareae* varied from 1.80 per cent to 7.20 per cent. Maximum infestation of mites (7.20%) was observed in

the month of June when average colony strength and brood area were 3.32 frames and 880.42 cm^2 and temperature, relative humidity and rainfall were 24.92 °C, 58.50 per cent and 78.60 mm, respectively which was statistically at par with May (4.80%).

Table 12. Pooled data on incidence of ectoparasitic mite (*T. clareae*) in *A. cerana* colonies during January, 2019 to December, 2020

Months	Brood infestation# (%)	Colony parameters		Weather parameters		
	T. clareae	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.00 (1.00)*	2.22	195.43 (14.02)*	8.98	63.50	120.65
February	0.00 (1.00)	4.08	1166.16 (34.16)	11.25	60.00	70.80
March	1.80 (1.67)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	2.20 (1.79)	5.38	1815.00 (42.61)	19.59	50.00	42.25
May	4.80 (2.41)	4.73	1037.16 (32.22)	22.33	48.50	48.05
June	7.20 (2.86)	3.32	880.42 (29.69)	24.92	58.50	78.60
July	2.00 (1.73)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	0.00 (1.00)	3.75	655.96 (25.63)	24.69	82.50	187.20
September	0.00 (1.00)	3.74	957.18 (30.95)	23.79	77.00	78.70
October	0.00 (1.00)	4.55	839.79 (29.00)	19.44	65.00	2.80
November	0.00 (1.00)	3.18	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	2.23	592.75 (24.37)	11.35	60.00	28.50
C.D $_{0.05}$	(0.54)	0.38	(3.51)			

*Figures in parentheses are square root (x+1) transformed values, # by visual examination

Pearson correlation Matrix (r) =

Temperature × *T. clareae* incidence = 0.48
Relative Humidity× *T. clareae* incidence = -0.44
Rainfall × *T. clareae* incidence = 0.01
Colony Strength × *T. clareae* incidence = 0.18
Brood Area× *T. clareae* incidence = 0.24

Minimum incidence of *T. clareae* mite was observed in the month of March (1.80%) when average colony strength and brood area were 4.48 frames and 1444.80 cm^2 and temperature, relative humidity and rainfall were 13.83°C, 58.00 per cent and 113.20 mm, respectively which was statistically at par with July (2.00%) and April (2.20%). The data on correlation of *T. clareae* infestation with colony and weather parameters showed negative correlation with relative humidity (r= -0.44).

Seasonal variation of ectoparasitic mite *T. clareae* varied from 1.80 per cent to 7.20 per cent. Maximum infestation of mites (7.20%) was observed in the month of June when temperature and rainfall was high and minimum incidence of *T. clareae* mite was observed in the month of March (1.80%). These observations coincides with increased brood rearing activity in *A. mellifera* colonies. Our findings are in line with Brar *et al.* (2019) and Camphor *et al*. (2005) who observed maximum incidence of *T. clareae* mite in the month of June and May, respectively when the temperature was high and relative humidity was low in *A. mellifera* colonies. Negi *et al*. (2018) observed *T. clareae* infestation in the same apiary and revealed that maximum infestation was observed in the month of September (10.00%) when the temperature was 23.00℃ and rainfall was 11.20 mm. This may be due to different weather conditions during 2016-2017. No incidence of *T. clarae* mite was observed in the month of January, February, August, September, October, November and December. In earlier studies also minimum incidence of *T. clareae* in *A. cerana* and *A. mellifera* colonies was reported during few months and colonies overwintered in colder areas remained free from mite infestation (Negi, 2018; Brar, 2019 and Woyke, 1987a). They found no incidence in *A. cerana* and *A. mellifera* colonies maintained at university apiary during July to February. Sharma *et al*. (2011) found that *T. clarae* infestation in *A. cerana* have also been found maximum in summer months and was absent in winter months. They further reported that *T. clareae* infestation in *A. cerana* varied greatly from year to year and during different months.

4.1.2.4 Incidence of wax moth

In experimental colonies combs were found infested with larvae, pupae and adults of greater wax moth (Calderone, 2000). Wax moth larvae were also observed feeding on honey, pollen and burrowed in to the pollen store cells and in tunnels in the midrib of the combs. Different wax moth stages were found in the debris at bottom board and in cracks and crevices especially pupae (Plate 19 & 20). Many earlier workers have recorded similar

observations on feeding and various life stages of wax moth (Mandal and Vishwakarma, 2016; Lalita *et al.,* 2018).

a) During 2019

Population of greater wax moth (*G. mellonella*) in *A. cerana* colonies is presented in Table 13 during January 2019 to December 2019. The data revealed that the population of wax moth (larvae, pupae and adults) varied during different months of the year. The incidence of greater wax moth was started in the month of February and March onwards started increasing up to July and was not observed in the month of December.

Table 13. Incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during January to December, 2019

Months	Stages of wax moth				Colony parameters		Weather parameters		
	No. of larvae	No. of pupae	No. of adults	Mean	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.36	145.77 (12.08)*	8.85	59.00	73.00
February	1.60 (1.61)	1.40 (1.55)	0.00 (1.00)	1.00 (1.41)	4.44	1153.26 (33.97)	10.34	63.00	103.10
March	5.20 (2.49)	4.80 (2.41)	0.60 (1.23)	3.53 (2.13)	4.72	1510.59 (38.87)	13.45	54.00	54.60
April	7.00 (2.83)	6.20 (2.68)	1.40 (1.51)	4.87 (2.42)	5.24	1849.86 (43.02)	20.03	49.00	36.80
May	8.80 (3.13)	7.40 (2.90)	1.80 (1.63)	6.00 (2.65)	5.02	1137.78 (33.68)	22.61	44.00	21.30
June	10.00 (3.32)	8.20 (3.03)	3.20 (2.01)	7.13 (2.85)	3.08	921.06 (30.18)	25.74	48.00	98.50
July	13.40 (3.79)	9.00 (3.16)	4.60 (2.36)	9.00 (3.16)	3.98	890.10 (29.81)	23.82	79.00	218.10
August	11.60 (3.55)	8.60 (3.10)	2.40 (1.79)	7.53 (2.92)	3.65	647.58 (25.23)	24.43	79.00	225.80
September	8.20 (3.03)	6.80 (2.79)	0.20 (1.08)	5.07 (2.46)	3.88	957.18 (30.89)	23.48	77.00	151.40
October	5.40 (2.53)	5.00 (2.45)	0.00 (1.00)	3.47 (2.11)	5.00	878.49 (29.44)	18.47	65.00	5.60
November	2.20 (1.79)	2.20 1.79)	0.00 (1.00)	1.46 (1.56)	3.60	906.87 (29.90)	15.50	62.00	32.20
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.45	675.96 (25.79)	10.49	58.00	33.20
Mean	6.12 (2.66)	4.97 (2.44)	1.18 (1.48)						
$CD_{0.05}$	(0.58)	(0.41)	(0.35)		0.33	(5.18)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature × wax moth incidence	=	0.93*	0.93*	0.72*
Relative Humidity× wax moth incidence	=	0.29	0.19	0.15
Rainfall × wax moth incidence	=	0.62*	0.54	0.61*
Colony strength × wax moth incidence	=	0.04	0.15	-0.11
Brood area × wax moth incidence	=	0.21	0.32	0.07

(*Significant at 5%)

Plate 19. Larvae of greater wax moth on comb and on top bars of *A. cerana*

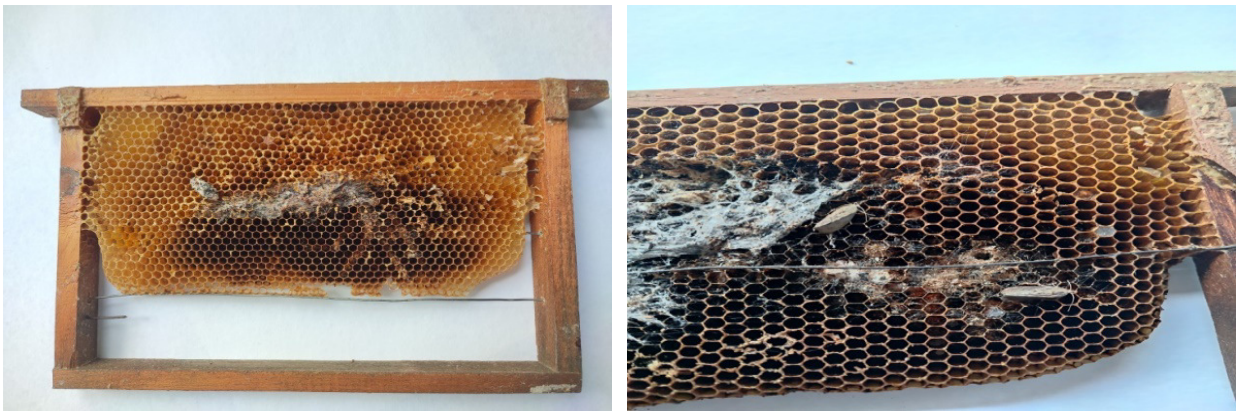


Plate 20. Pupae and adult of greater wax moth (*G. mellonella*) in *A. cerana* colony

The highest average larval population was recorded in the month of July (13.40) when average colony strength and brood area was 3.98 frames and 890.10 cm^2 and the temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm which was statistically at par with August (11.60) and June (10.00) followed by May (8.80) was which was at par with September (8.20) and April (7.00). The larval incidence in the month of October was 5.40 which was at par with March (5.20).While lowest population of larvae was recorded in the month of February (1.60) when the colony strength and brood area was 4.44 frames and 1153.26 cm^2 and temperature, relative humidity and rainfall were, 10.34℃, 63.00 per cent and 103.10 mm which was statistically at par with November (2.20).

The highest number of average pupae were recorded in the month of July (9.00) when average colony strength and brood area was 3.98 frames and 890.10 cm^2 which was statistically at par with August (8.60), June (8.20), May (7.40) and September (6.80). The average number of pupae in the month of April was (6.20) which was statistically at par with October (5.00) and March (4.80). Minimum number of pupae were recorded in the month of February (1.40) which was statistically at par with November (2.20).

The highest number of average adults were also recorded in the month of July (4.60) when average colony strength and brood area were maximum and was statistically at par with June (3.20) followed by August (2.40) which was statistically at par with May (1.80) and April (1.40). Minimum number of population of adults were recorded in the month of September (0.20) when average colony strength and brood area was 3.88 frames and 957.18 cm^2 and the temperature, relative humidity and rainfall were 23.48 °C, 77.00 per cent and 151.40 mm which was statistically at par with March (0.60). No incidence of larvae, pupae and adults were observed in the month of December and January and no adult incidence was observed in the month of January, February, October, November and December.

During the period of study the mean population of wax moth started increasing from March (3.53) to July (9.00). Thereafter August onwards the population start declining till November. Mean population of wax moth was found to be highest (9.00) in the month of July followed by August (7.53), June (7.13), May (6.00), September (5.07), April (4.87), March (3.53), October (3.47) and November (1.46). Minimum number of population of wax moth was observed in the month of February (1.00).

The correlation between number of larvae and weather parameters were found to be positive and significant for temperature (r=0.93) and rainfall (r=0.62). The correlation between number of pupae and weather parameters was also significant for temperature (r=0.93). Adults correlation with weather parameters showed positive and significant relationship with temperature (r=0.72) and rainfall (r=0.61) while, negative correlation with colony strength (r=-0.11).

b) During 2020

Data on seasonal incidence of greater wax moth (*G. mellonella*) during 2020 is presented in Table 14. During the whole incidence period of wax moth the temperature, relative humidity and rainfall varied from 9.10 to 25.05℃, 51 to 86 per cent and 0 to 278.10 mm, respectively. Highest average larvae (12.80) was recorded in the month of July, 2020 when average colony strength and brood area was 4.24 frames and 727.56 cm^2 and the temperature, relative humidity and rainfall were 25.05°C, 81 per cent and 278.10 mm, respectively which was statistically at par with August (10.60) followed by June (9.60) which was statistically at par with May (9.20) and September (9.00). The larval population in April was 6.80 which was statistically at par with March (6.00) and October (5.60). Whereas, lowest population of larvae was recorded in the month of February (0.80) when average colony strength and brood area was 3.72 frames and 1179.06 cm^2, respectively and the temperature, relative humidity and rainfall were 12.15°C, 57 per cent and 38.50 mm, respectively which was statistically at par with November (1.80).

The highest number of average pupae were recorded in the month of August, 2020 (9.40) when average colony strength and brood area was 3.85 frames and 664.35 cm^2 and the temperature, relative humidity and rainfall were 24.95 °C, 86.00 per cent and 148.60 mm which was statistically at par with July (9.20), September (7.40) and June (7.20) followed by May (6.80) which was statistically at par with April (5.20). Average number of pupae in the month of March were 4.00 which was statistically at par with October (3.80) and November (2.80). Significantly minimum number of pupae were recorded in the month of February (1.20).

Table 14. Incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during January to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average No. of larvae	Average No. of pupae	Average No. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.08	245.10 (15.02)*	9.10	68.00	168.30
February	0.80 (1.34)	1.20 (1.48)	0.00 (1.00)	0.66 (1.28)	3.72	1179.06 (33.80)	12.15	57.00	38.50
March	6.00 (2.65)	4.00 (2.24)	1.20 (1.48)	3.73 (2.18)	4.24	1379.00 (37.15)	14.20	62.00	171.80
April	6.80 (2.79)	5.20 (2.49)	1.8 (1.67)	4.60 (2.37)	5.51	1780.20 (42.20)	19.15	51.00	47.70
May	9.20 (3.19)	6.80 (2.79)	2.00 (1.73)	6.00 (2.65)	4.44	936.54 (30.5)	22.05	53.00	74.80
June	9.60 (3.26)	7.20 (2.86)	2.60 (1.90)	6.47 (2.73)	3.55	839.79 (28.94)	24.1	69.00	58.70
July	12.80 (3.71)	9.20 (3.19)	4.80 (2.41)	8.93 (3.15)	4.24	727.56 (26.81)	25.05	81.00	278.10
August	10.60 (3.41)	9.40 (3.22)	3.00 (2.00)	7.67 (2.94)	3.85	664.35 (25.57)	24.95	86.00	148.60
September	9.00 (3.16)	7.40 (2.90)	1.60 (1.61)	6.00 (2.65)	3.60	957.18 (30.87)	24.1	77.00	6.00
October	5.60 (2.57)	3.80 (2.19)	0.00 (1.00)	3.13 (2.03)	4.09	801.09 (28.20)	20.4	65.00	0.00
November	1.80 (1.67)	2.80 (1.95)	0.00 (1.00)	1.53 (1.59)	2.76	968.79 (30.67)	14.6	68.00	37.70
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.00	509.55 (22.18)	12.2	62.00	23.80
Mean	6.02 (2.65)	4.76 (2.39)	1.42 (1.55)						
$CD_{0.05}$	(0.37)	(0.42)	(0.46)		0.42	(5.79)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature× wax moth incidence	=	0.94*	0.93*	0.79*
Relative Humidity× wax moth incidence	=	0.44	0.47	0.49
Rainfall × wax moth incidence	=	0.37	0.32	0.62*
Colony strength × wax moth incidence	=	0.39	0.43	0.41
Brood area × wax moth incidence	=	0.15	0.17	0.05

(*Significant at 5%)

Maximum number of average adults were observed in the month of July, 2020 (4.80) when average colony strength and brood area was 4.24 frames and 727.56 cm^2 and the temperature, relative humidity and rainfall were 25.05 °C, 81 per cent and 278.10 mm which was statistically at par with August (3.00). Least number adults were recorded in the month of March (1.20) which was statistically at par with September (1.60), April (1.80), May (2.00) and June (2.60). There was no incidence of pupae was observed in the month of December and January.

The mean population of wax moth was 0.66 in February and increased from March (3.73) to July (8.93) thereafter, August onwards the population start declining till November. Mean population of all the stages of wax moth was found to be highest (8.93) in the month of July followed by August (7.67), June (6.47), September (6.00), May (6.00), April (4.60), March (3.73), October (3.13) and November (1.53). Lowest population of wax moth larvae, Pupae and adults were found in the month of February (0.66).

The correlation between number of larvae, pupae and weather parameters were found to be positive and significant for temperature (r=0.94 and r=0.93, respectively). Adults correlation with weather parameters showed positive and significant relationship with both temperature (r=0.79) and rainfall (r=0.62).

c) Pooled data (2019-2020)

Pooled data for the year 2019-2020 on incidence of population of greater wax moth (*G. mellonella*) in *A. cerana* colonies is presented in Table 15 and Fig. 8. The data revealed that the population of wax moth (larvae, pupae and adults) varied during different months from January to December. The incidence of greater wax moth was started in the month of February and March onwards started increasing up to July and was not observed in the month of December. The population of wax moth increased from March onwards till July and then the population started decreasing. Significantly highest average larval population (13.10) was recorded in the month of July when average colony strength and brood area was 4.11 frames and 808.83 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm followed by August (11.10) which was statistically at par with June (9.80). The population of larvae in May was (9.00) which was statistically at par with September (8.60) followed by April (6.90) which was at par with March (5.60). No infestation of wax moth larvae was observed in both the years in the month of December and January. While lowest population of larvae were recorded in the month of February (1.20) when average colony strength and brood area was 4.08 frames and 1166.16 cm^2 and the temperature, relative humidity and rainfall were 11.25 °C, 60.00 per cent and 70.80 mm which was statistically at par with November (2.00).

Table 15. Pooled data on incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during January, 2019 to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.22	195.43 (14.02)*	8.98	63.50	120.65
February	1.20 (1.48)	1.30 (1.52)	0.00 (1.00)	0.83 (1.35)	4.08	1166.16 (34.16)	11.25	60.00	70.80
March	5.60 (2.57)	4.40 (2.32)	0.90 (1.38)	3.63 (2.15)	4.48	1444.80 (38.02)	13.83	58.00	113.20
April	6.90 (2.81)	5.70 (2.59)	1.60 (1.61)	4.73 (2.39)	5.38	1815.00 (42.61)	19.59	50.00	42.25
May	9.00 (3.16)	7.10 (2.85)	1.90 (1.70)	6.00 (2.65)	4.73	1037.16 (32.22)	22.33	48.50	48.05
June	9.80 (3.29)	7.70 (2.95)	2.90 (1.97)	6.80 (2.79)	3.32	880.42 (29.69)	24.92	58.50	78.60
July	13.10 (3.75)	9.10 (3.18)	4.70 (2.39)	8.97 (3.16)	4.11	808.83 (28.46)	24.44	80.00	248.10
August	11.10 (3.48)	9.00 (3.16)	2.70 (1.92)	7.60 (2.93)	3.75	655.96 (25.63)	24.69	82.50	187.20
September	8.60 (3.10)	7.10 (2.85)	0.90 (1.38)	5.53 (2.56)	3.74	957.18 (30.95)	23.79	77.00	78.70
October	5.50 (2.55)	4.40 (2.32)	0.00 (1.00)	3.30 (2.07)	4.55	839.79 (29.00)	19.44	65.00	2.80
November	2.00 (1.73)	2.50 (1.87)	0.00 (1.00)	1.50 (1.58)	3.18	937.83 (30.64)	15.05	65.00	34.95
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.23	592.75 (24.37)	11.35	60.00	28.50
Mean	6.07 (2.65)	4.86 (2.42	1.30 (1.51)						
$CD_{0.05}$	(0.25)	(0.26)	(0.31)		0.38	(3.51)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature × wax moth incidence	=	0.94*	0.94*	0.76*
Relative Humidity× wax moth incidence	=	0.38	0.38	0.35
Rainfall × wax moth incidence	=	0.55	0.55	0.73*
Colony strength × wax moth incidence	=	0.25	0.30	0.16
Brood area × wax moth incidence	=	0.19	0.24	0.06

(*Significant at 5%)

The highest number of average pupae were recorded in the month of July (9.10) when average colony strength and brood area was 4.11 frames and 808.83 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm which was statistically at par with August (9.00) and June (7.70) followed by September (7.10) which was statistically same with May (7.10) and was at par with April (5.70). Wax moth pupal population in the month of October was 4.40 which was statistically same with March (4.40). Significantly minimum pupal population was recorded in the month of February (1.30) when

average colony strength and brood area was 4.08 frames and 1166.16 cm^2 and the temperature, relative humidity and rainfall were 11.25 °C, 60.00 per cent and 70.80 mm.

Significantly maximum number of average adults were observed in the month of July (4.70) when average colony strength and brood area was 4.11 frames and 808.83 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm followed by June (2.90) which was statistically at par with August (2.70) and May (1.90). Minimum adult population was recorded in the month of March (0.90) which was statistically same with September (0.90) and was at par with April (1.60). No adult incidence was observed in the month of January, February, October, November and December,.

During the whole two years study, the mean population of all the stages of wax moth was found to be highest (8.97) in the month of July followed by August (7.60), June (6.80), May (6.00), September (5.53), April (4.73), March (3.63), October (3.30) and November (1.50). Minimum number of wax moth (*G. mellonella*) larvae, pupae and adults were found in the month of February (0.83). The correlation between different stages of wax moth and weather parameters showed that number of larvae, pupae and adults had positive and significant correlation with temperature (r=0.94, r=0.94 and r=0.76, respectively). Adults correlation was also positive and significant for rainfall (r=0.73).

Irrespective of different months in *A. cerana* colonies, the larval population was found highest (6.07) followed by pupae (4.86) and adults (1.30). It was observed that larval population was major threat to the honey bee colonies destroying the combs by feeding and covering them with web and fecal matter. Data on the incidence of larvae in the present investigation were comparable to earlier studies carried out in India, indicating that a higher infestation of wax moth occurred during June-July (Bhatnagar *et al.,* 2020; Lalita *et al.,* 2018; Mandal and Vishawkarma, 2016; Gupta, 1987 and Brar *et al.,* 1985). The infestation was first spotted in February and then increased rapidly in the subsequent months till late July during both the years of study. Here a slight difference is noted from the findings of Negi *et al.* (2019) who reported that wax moth population starts building from January, reaching its peak in April and then show decline till October. This may be due to change in weather conditions during period of study. The wax moth incidence was highest in summer months (July, August, June and May) when the temperature was high and lowest incidence was recorded in the months of February and November due to low ambient temperature which find support

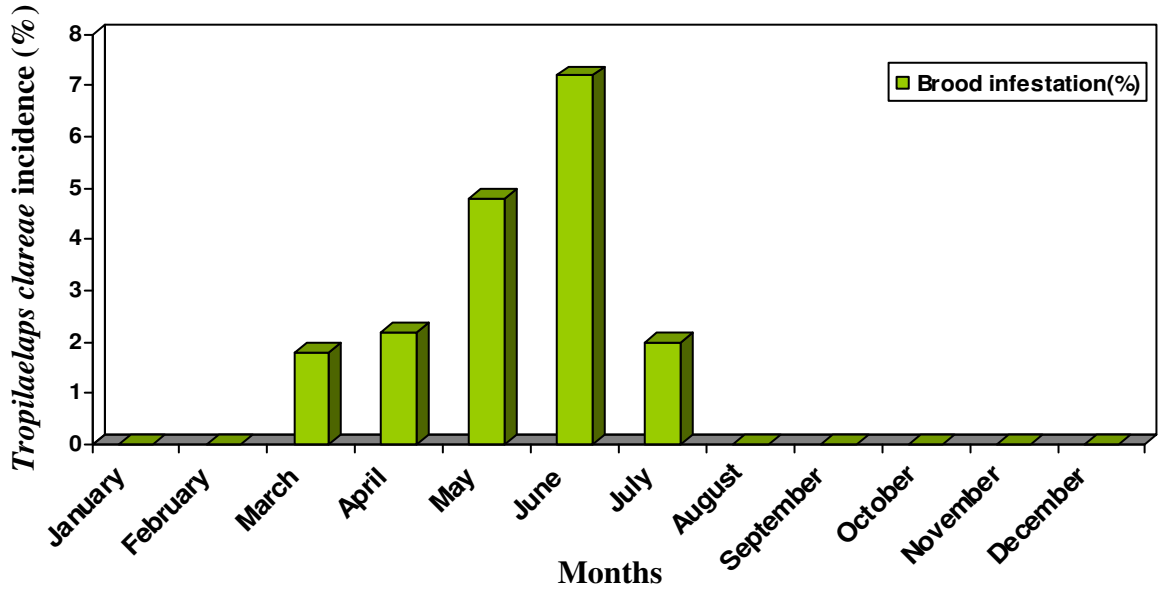


Fig 7. *Tropilaelaps clareae* incidence in *A. cerana* (2019-2020)

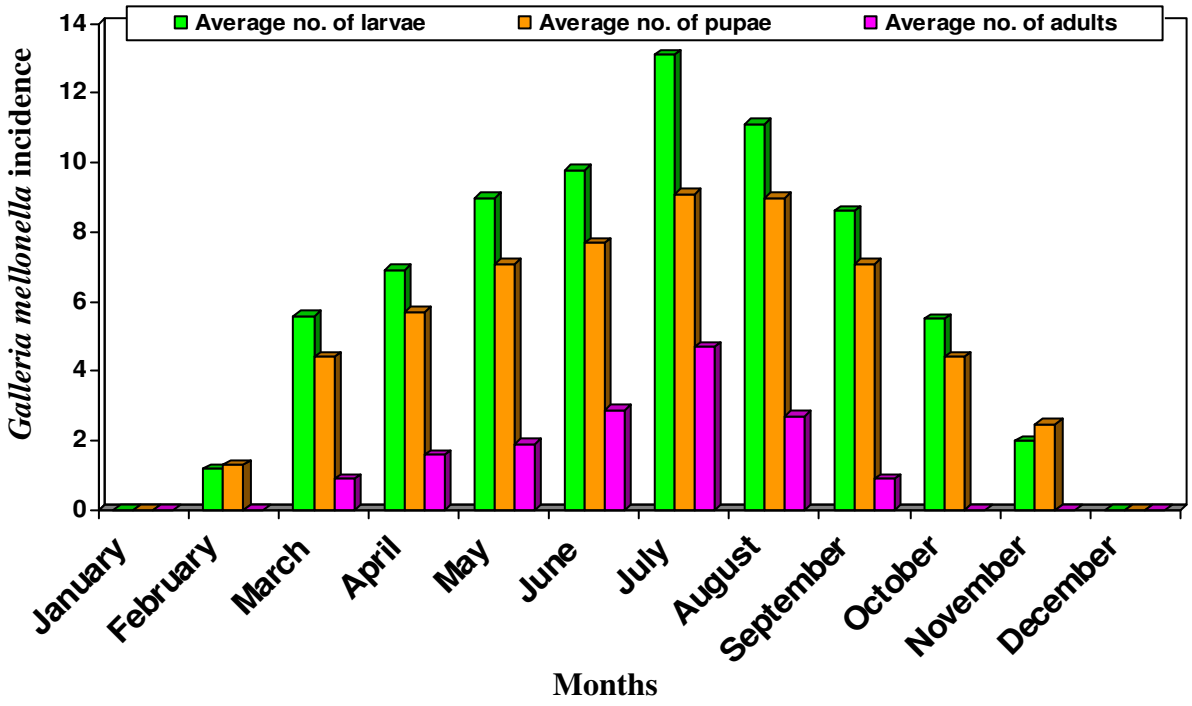


Fig 8. Incidence of greater wax moth (*Galleria mellonella*) in *A. cerana* (2019-2020)

from the results of Swamy *et al.* (2005) who reported seasonal variations of greater wax moth, *G. mellonella* in combs of *A. cerana* during the summer and winter seasons and showed that summer season was more suitable for the development and multiplication of greater wax moth in honey bee colonies. The results of seasonal fluctuations are in agreement with Nagaraja and Rajagopal (2009) who reported that wax moth population fluctuates according to weather conditions. Though the incidence of wax moth infestation is common, however its intensity depended upon prevailing ecological conditions during different seasons at various places. In India, the greater wax moth infestation found throughout the year in both higher and lower altitudes (Nagaraja and Rajagopal, 2009). Brar *et al.* (1985) reported that greater wax moth infestation started in June and acquired peak in September and thereafter decreased up to November in *A. mellifera* colonies in Punjab, similarly in Haryana maximum infestation of wax moth in *A. mellifera* colonies was recorded during July to September and most favourable temperature was 35°C combined with 70% relative humidity (Gupta, 1987). Verma and Raj (2000) observed maximum population of *G. mellonella* during first fortnight of August in Kangra valley of Himachal Pradesh and Negi *et al.* (2019) found more wax moth infestation in summer month (April, May and June) in Nauni, Solan (H.P.). In Jodhpur (Rajsthan) wax moth incidence was first noticed in the month of April which increased in May-June and highest infestation was recorded in the month of July (Bhatnagar *et al.,* 2020).

4.2 SEASONAL INCIDENCE OF BROOD DISEASES (SACBROOD AND EUROPEAN FOULBROOD), MITES (*V. destructor* AND *T. clareae*) AND GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* COLONIES

4.2.1 Colony records

4.2.1.1 During 2019 (stationary conditions Nauni, Solan)

Colony records in *A. mellifera* colonies under stationary (Nauni, Solan) conditions have been recorded at monthly interval during January, 2019 to December, 2019. During the study period five experimental colonies of equal strength of *A. mellifera* in the stationary conditions were selected.

The data on colony records of *A. mellifera* under stationary condition during January, 2019 to December, 2019 is presented in Table16. Maximum average colony strength was recorded in the month of May (5.94 bee frames) which was statistically at par with average colony strength observed in the month of July (5.29 bee frames) followed by April (4.80 bee frames) which was statistically at par with August (4.47 bee frames), June (4.29 bee frames),

September (4.10 bee frames) and March (4.03 bee frames). Average colony strength was minimum in the month of January (2.45 bee frames) which was statistically at par with December (2.60 bee frames), November (2.75 bee frames), February (3.09 bee frames) and October (3.14 bee frames).

The average brood area was maximum during the month of May (3205.65 cm^2) which was statistically at par with average brood area in the month of April (2754.15 cm^2) followed by June (1870.50 cm^2) which was statistically at par with March (1825.35 cm^2) and July (1638.30cm^2). The average brood area in the month of August was 1251.30 cm^2 which was statistically at par with September (1240.48cm^2), October (1225.50 cm^2), February (1154.55cm^2) and November (980.40 cm^2). Significantly minimum average brood area was observed in the month of December (432.15 cm^2).

Table16. Colony records of *A. mellifera* under stationary conditions at Nauni during January to December, 2019

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2019	2.45	715.95 (26.78)*	73.53 (8.63)	93.00 (9.70)
February	3.09	1154.55 (33.99)	95.46 (9.82)	79.00 (8.94)
March	4.03	1825.35 (42.74)	113.52 (10.70)	198.00 (14.11)
April	4.80	2754.15 (52.49)	211.56 (14.58)	234.00 (15.33)
May	5.94	3205.65 (56.63)	156.09 (12.53)	278.00 (16.70)
June	4.29	1870.50 (43.26)	145.77 (12.11)	240.00 (15.52)
July	5.29	1638.30 (40.49)	101.91 (10.14)	364.00 (19.10)
August	4.47	1251.30 (35.39)	63.21 (8.01)	433.00 (20.83)
September	4.10	1240.78 (35.24)	82.56 (9.14)	543.00 (23.32)
October	3.14	1225.50 (35.02)	43.86 (6.70)	256.00 (16.03)
November	2.75	980.40 (31.33)	33.54 (5.88)	198.00 (14.11)
December	2.60	432.15 (20.81)	25.80 (5.18)	145.00 (12.08)
C.D $_{0.05}$	0.82	(4.21)	(1.59)	(3.43)

*Figures in parentheses are square root (x+1) transformed values

Significantly maximum average pollen area was recorded in the month of April (211.56 cm^2) followed by May (156.09 cm^2) which was statistically at par with average pollen area in the month of June (145.77 cm^2). The average pollen area in March was 113.52 cm^2 which was statistically at par with July (101.91 cm^2), February (95.46 cm^2) and September (82.56 cm^2) followed by January (73.53cm^2) which was at par with August (63.21 $cm^{2.}$). Minimum average pollen area (25.80 cm^2) was observed in the month of December which was statistically at par with pollen area in the month of November (33.54 cm^2) and October (43.46cm^2).

Maximum average honey store was observed in the month of September (543.00g) which was statistically at par with August (433.00g) followed by July (364.00 g) which was statistically at par with May (278.00 g) and October (256.00g). The average honey stores in the month of June was (240.00 g) which was statistically at par with April (234.00g), November (198.00g) and March (198.00g). The minimum average honey store (79.00g) in the present study was observed in the month of February which was statistically at par with honey store in the month of January (93.00g) and December (145.00g).

4.2.1.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Colony records in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2019 is presented in Table 17 revealed that when the colonies were shifted to Hisar, average colony strength ranged from 5.18 to 7.03 bee frames in winter months (January, February, November and December) i.e. during the migratory period. Significantly, maximum colony strength was recorded in the month of February (7.03 bee frames) followed by in the month of January (6.59 bee frames) which was statistically at par with July (6.30 bee frames). The colony strength in June was 6.00 bee frames which was statistically at par with the colony strength in the month of August (5.88 bee frames) and May (5.85 bee frames) followed by September (5.56 bee frames) was statistically at par with December (5.43 bee frames) and was further at par with November (5.18 bee frames). Significantly minimum colony strength was recorded in the month of April (3.37 bee frames).

The observations on brood area of *A. mellifera* under migratory conditions further revealed that significantly maximum average brood area (4211.85 cm^2) was observed in the

month of February followed by month of January (2941.20 cm^2) which was statistically at par with brood area in the month of March (2709.00cm^2) and May (2431.65 cm^2). The average brood area in the month of April was (2309.10 cm^2) which was at par with June (2173.65 cm^2) followed by July (1638.30 cm^2) which was statistically at par with December (1631.85 cm^2) and August (1528.65 cm^2). Whereas, statistically minimum brood area (1102.95cm^2) was recorded in the month of November which was statistically at par with October (1393.20 cm^2) and September (1406.10 cm^2).

Table 17. Colony records of *A. mellifera* under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2019**	6.59	2941.20 (54.24)*	425.70 (20.66)	550.00 (23.47)
February**	7.03	4211.85 (64.91)	451.50 (21.27)	1142.00 (33.81)
March	4.51	2709.00 (52.06)	251.55 (15.89)	267.00 (16.37)
April	3.37	2309.10 (48.06)	19.35 (4.51)	408.00 (20.22)
May	5.85	2431.65 (49.32)	96.75 (9.89)	275.00 (16.61)
June	6.00	2173.65 (46.63)	38.70 (6.30)	376.00 (19.42)
July	6.30	1638.30 (40.49)	59.34 (7.77)	394.00 (19.87)
August	5.88	1528.65 (39.11)	70.95 (8.48)	426.00 (20.66)
September	5.56	1406.10 (37.51)	15.48 (4.06)	486.00 (22.07)
October	4.08	1393.20 (37.34)	58.05 (7.68)	106.00 (10.34)
November**	5.18	1102.95 (33.23)	141.90 (11.95)	271.00 (16.49)
December**	5.43	1631.85 (40.41)	374.10 (19.37)	475.00 (21.82)
C.D $_{0.05}$	0.34	(4.98)	(3.59)	(4.75)

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

The observations recorded on average pollen area revealed that maximum pollen area was recorded in the month of February (451.50 cm^2) which was statistically at par with January (425.70 cm^2) and December (374.10 cm^2) followed by March (251.55 cm^2),

November (141.91 cm^2) which was statistically at par with May (96.75 cm^2) and August (70.95 cm^2). The average pollen area in the month of July was 59.34 cm^2 which was statistically at par with October (58.05 cm^2) and June (38.70 cm^2). Minimum pollen area was recorded in the month of September (15.48 cm^2) which was statistically at par with April (19.35cm^2) and June (38.70 cm^2). As evident from Table 17 during the migratory months November, December, January and February high pollen area was recorded i.e. 141.90 cm^2 , 374.10 cm^2, 425.70 cm^2 and 451.50 cm^2, respectively.

The data in table 17 showed that significantly maximum average honey store was found in February (1142.00 g) followed by January (550.00 g) which was statistically at par with average honey stores in the month of September (486.00 g), December (475.00 g), August (426.00 g), April (408.00 g), July (394.00 g)and June (376.00 g). The honey stores in the month of May was (275.00 cm^2) which was statistically at par with November (271.00 g) and March (267.00 cm^2). Significantly minimum honey store was in the month of October (106.00 g).

4.2.1.3 During 2020 (stationary conditions Nauni, Solan)

The data on colony records of *A. mellifera* under stationary conditions during January, 2020 to December, 2020 is presented in Table18. Significantly maximum average colony strength was recorded in the month of May (6.08 bee frames) followed by July 5.28 bee frames, April (4.84 bee frames) and August (4.27 bee frames) which was statistically at par with June (4.06 bee frames). The average colony strength in March was 3.91 bee frames which was statistically at par with September (3.81 bee frames) followed by October (3.47 bee frames) and November (2.49 bee frames) which was statistically at par with February (2.36 bee frames). Minimum average colony strength was observed in the month of January (2.01 bee frames) which was statistically at par with December (2.06 bee frames).

The average brood area was maximum during the month of May (3412.05 cm^2) which was statistically at par with April (2934.75 cm^2) followed by June (1941.45 cm^2) which was statistically at par with March (1922.10 cm^2), July (1870.50 cm^2) and August (1496.40 cm^2). The average brood area in the month of September was 1341.60 cm^2 which was statistically at par with October (1290.00 cm^2), February (1135.20 cm^2) and November (941.70 cm^2). In the year 2020, minimum average brood area was observed in the month of December (574.05

cm^2) which was statistically at par with average brood area in January (606.30 cm^2) and November (941.70 cm^2).

The data on average pollen area in *A. mellifera* colonies revealed that pollen area was maximum in the month of April (187.05 cm^2) which was statistically at par with May (161.25 cm^2), June (129.00 cm^2) and March (122.55 cm^2) followed by average pollen area in the month of July (103.20 cm^2) which was at par with February (90.30 cm^2), September (70.95 cm^2) and January (51.60 cm^2). Least average pollen area was observed in the month of December (19.35 cm^2) which was statistically at par with November (25.80 cm^2), October (38.70 cm^2), August (45.15 cm^2) and January (51.60 cm^2).

Table18. Colony records of *A. mellifera* under stationary conditions at Nauni during January to December, 2020

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2020	2.01	606.30 (24.64)*	51.60 (7.25)	76.00 (8.77)
February	2.36	1135.20 (33.71)	90.30 (9.56)	69.00 (8.37)
March	3.91	1922.10 (43.85)	122.55 (11.12)	102.00 (10.15)
April	4.84	2934.75 (54.18)	187.05 (13.71)	223.00 (14.97)
May	6.08	3412.05 (58.42)	161.25 (12.74)	334.00 (18.30)
June	4.06	1941.45 (44.07)	129.00 (11.40)	267.00 (16.37)
July	5.28	1870.50 (43.26)	103.20 (10.21)	451.00 (21.26)
August	4.27	1496.40 (38.70)	45.15 (6.79)	460.00 (21.47)
September	3.81	1341.60 (36.64)	70.95 (8.48)	493.00 (22.23)
October	3.47	1290.00 (35.93)	38.70 (6.30)	212.00 (14.59)
November	2.49	941.70 (30.70)	25.80 (5.18)	160.00 (12.69)
December	2.06	574.05 (23.98)	19.35 (4.51)	131.00 (11.49)
C.D $_{0.05}$	0.29	(7.42)	(3.35)	(2.79)

*Figures in parentheses are square root (x+1) transformed values

Maximum average honey store was observed in the month of September (493.00 g) which was statistically at par with August (460.00 g) and July (451.00 g) followed by May (334.00 g) which was statistically at par with June (267.00 g). The average honey store in the month of April was 223.00 g which was statistically at par with October (212.00 g) and November (160.00 g). In the month of December the average honey store was 131.00 g which was at par with March (102.00 g) and January (76.00 g). The minimum average honey store (69.00 g) in the present study was observed in the month of February which was statistically at par with January (76.00 g) and March (102.00 g).

4.2.1.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Data presented in Table 19 revealed that when the colonies were shifted to Hisar significantly maximum colony strength was recorded in the month of February (7.62 bee frames) followed by January (6.38 bee frames) which was statistically at par with May (6.25 bee frames). The colony strength in July was 5.61 bee frames which was statistically at par with June (5.50 bee frames) and August (5.44 bee frames).The average colony strength in the month of December was 5.26 bee frames followed by September (4.69 bee frames) and November (4.33 bee frames) which was at par with March (4.27 bee frames). Statistically, minimum colony strength was recorded in the month of April (2.96 bee frames) which was statistically at par with October (3.23 bee frames).

Significantly maximum average brood area (3870.00 cm^2) was recorded in the month of February followed by 2541.30 cm^2 in the month of January which was statistically at par with brood area in the month of March (2412.30 cm^2), May (2212.40 cm^2), April (2122.10 cm^2) and June (1973.70 cm^2). The average brood area in the month of July was 1767.30 cm^2 which was statistically at par with December (1593.20 cm^2), August (1541.60 cm^2), September (1399.70 cm^2) and October (1335.20 cm^2). Whereas, statistically minimum brood area was recorded in the month of November (980.40 cm^2) which was statistically at par with brood area in the month of October and September (1399.70 cm^2).

Average pollen area was maximum in the month of February (412.80 cm^2) which was statistically at par with January (322.50 cm^2) and December (316.18 cm^2) followed by March (199.95 cm^2) which was statistically at par with November (135.45cm^2). The average pollen area in the month of May was 77.40 cm^2 which was at par with August (64.50 cm^2), July

(53.71 cm^2), September (51.60 cm^2) and June (45.15 cm^2). The minimum pollen area 23.22 cm^2 was recorded in the month of October which was statistically at par with April (25.80cm^2), June (45.15 cm^2), September (51.60 cm^2), July (53.71 cm^2) and August (64.50 cm^2).

Table 19. Colony records of *A. mellifera* under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January, 2020**	6.38	2541.30 (50.42)*	322.50 (17.99)	430.00 (20.76)
February**	7.62	3870.00 (62.22)	412.80 (20.34)	1074.00 (32.79)
March	4.27	2412.30 (49.13)	199.95 (14.18)	217.00 (14.76)
April	2.96	2122.05 (46.08)	25.80 (5.18)	464.00 (21.56)
May	6.25	2212.35 (47.05)	77.40 (8.85)	310.00 (17.64)
June	5.50	1973.70 (44.44)	45.15 (6.79)	411.00 (20.30)
July	5.61	1767.30 (42.05)	53.71 (7.40)	453.00 (21.31)
August	5.44	1541.55 (39.28)	64.50 (8.09)	310.00 (17.64)
September	4.69	1399.65 (37.43)	51.60 (7.25)	396.00 (19.92)
October	3.23	1335.15 (36.55)	23.22 (4.92)	119.00 (10.95)
November**	4.33	980.40 (31.33)	135.45 (11.68)	260.00 (16.16)
December**	5.26	1593.15 (39.93)	316.18 (17.81)	390.00 (19.77)
C.D $_{0.05}$	0.31	(6.86)	(3.18)	(3.82)

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Significantly maximum average honey store was found in February (1074.00 g) followed by April (464.00 g) which was statistically at par with average honey stores in the month of July (453.00 g), January (430.00 g), June (411.00 g), September (396.00 g) and

December (390.00 g). The average honey stores in the month of May was 310.00 g which was statistically same with August (310.00 g) and was at par with November (260.00 g) and March (217.00 g). Minimum honey store was observed in the month of October (119.00 g) which was statistically at par with March (217.00 g).

4.2.1.5 Pooled data on colony records under stationary conditions Nauni, Solan (2019-2020)

Pooled data on *A. mellifera* colony records under stationary condition during January, 2019 to December, 2020 is presented in Table 20 and Fig. 9. Significantly maximum average colony strength (was recorded in the month of May (6.01 bee frames) followed by July (5.28 bee frames) which was statistically at par with April (4.82 bee frames). The average colony strength in August was 4.37 bee frames which was statistically at par with June (4.18 bee frames), March (3.96 bee frames) and September (3.95 bee frames). Minimum average colony strength was observed in the month of January (2.23 bee frames) which was statistically at par with December (2.33 bee frames), November (2.62 bee frames) and February (2.73 bee frames).

The average brood area (Fig. 10) was significantly maximum during the month of May (3308.85 cm^2) followed by April (2844.45 cm^2) and June (1905.98 cm^2) which was statistically at par with March (1873.73 cm^2) and July (1754.40 cm^2). The average brood area in the month of August was 1373.85 cm^2 which was statistically at par with September (1291.19 cm^2) and October (1257.75 cm^2) followed by February (1144.88 cm^2), November (961.05 cm^2) and January (661.13 cm^2). Significantly minimum average brood area was observed in the month of December (503.10 cm^2). The data further revealed that the average brood area was significantly low during winter months i.e. December (503.10 cm^2) followed by January (661.13 cm^2) and November (961.05cm^2).Thus, there was decrease in brood area from the month of June (1905.98 cm^2) till December and increase in brood area from January i.e. 661.13 cm^2 to 3308.85 cm^2 in the month of May.

Data further revealed that significantly maximum average pollen area (Fig. 11) was recorded during the month of April (199.31 cm^2) followed by May (158.67cm^2) which was statistically at par with average brood area in the month of June (137.39 cm^2). The average pollen area in the month of March was 118.04 cm^2 which was statistically at par with July (102.56 cm^2) and February (92.88 cm^2).The average pollen area in the month of September

was 76.76 cm^2 which was statistically at par with January (62.67 cm^2) followed by August (54.18 cm^2) which was at par with October (41.28 cm^2). Minimum average pollen area (22.58 cm^2) was recorded in the month of December which was statistically at par with November (29.67 cm^2). There was decline in pollen area from the month of June (137.39 cm^2) till the month of December (22.58 cm^2), and pollen area increased from January (62.57 cm^2) till April (199.31 cm^2).

Maximum average honey store (Fig. 12) was observed in the month of September (518.00 g) which was statistically at par with August (446.50 g) and July (407.50 g) followed by May (306.00 g) which was statistically at par with June (253.50 g), October (234.00 g) and April (228.50 g). The average honey stores in the month of November was (189.00 g) which was at par with March (150.00 g) and December (138.00 g). The minimum average honey store (74.00 g) in the present study was observed in the month of February which was statistically at par with January (84.50 g). There was decrease in brood area in December and January months leading to decline in foraging and nectar collectors which resulted in decreased honey stores.

In present investigation maximum colony strength in *A. mellifera* colonies was recorded in the month of May whereas, minimum was in the month of January statistically at par with December, November and February. Our findings are in close proximity with Negi, 2018 and Brar, 2016 who observed maximum colony strength in July and May, respectively. Spring and summer are most active seasons of brood rearing while autumn and winter are least one (Ismail *et al.,* 2015 and Neupane and Thapa, 2005). In our studies minimum colony strength was observed in the month of January which was at par with December, November and February. Similar type of results were observed by Negi (2018), Brar (2016) and Rana (2003).

Maximum brood area was observed in May followed by April and June which was statistically at par with March and July. Similar results were showed by (Negi, 2018 and Brar, 2016). Spring and summer are most active seasons of brood rearing while autumn and winter are least one (Ismail *et al.,* 2015). Neupane and Thapa (2005) also supported the present studies and reported that number of brood cells (14787.2 brood cells/hive) were highest during spring season and lowest (3811.7 brood cells/ hive) in rainy season. Significantly minimum average brood area was observed in the month of December (503.10 cm^2). Negi

(2018), Brar (2016) and Chaand *et al.* (2017) also found minimum average brood area in the month of December, 2017 (678.33 cm^2/colony), (410.40 cm^2/colony) and (677.94 bee frames/colony), respectively. Whereas, Rana (2003) reported minimum bee brood (283.8 cm^2 brood cells/hive) of *A. mellifera* during the month of January, 2003 in the same apiary at Nauni. In December and January months very low flora was available in Nauni Solan, so very low foraging activity was observed as this is second dearth period. Our findings got support from earlier studies carried out in the same apiary of *A. mellifera* at Nauni (Brar, 2016; Negi, 2018 and Rana, 2003). Camareno and Meza (1973) and Verma (1995) claimed 25℃ a peak foraging temperature for *A. mellifera* bees. According to Mishra and Sharma (1998), worker bees of *A. mellifera* stopped foraging when air temperature dropped down below 14℃ and lost ability to move at temperature below 10℃.

Table 20. Pooled data on colony records of *A. mellifera* under stationary conditions at Nauni during January, 2019 to December, 2020

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January	2.23	661.13 (25.73)*	62.57 (7.97)	84.50 (9.25)
February	2.73	1144.88 (33.85)	92.88 (9.69)	74.00 (8.66)
March	3.96	1873.73 (43.30)	118.04 (10.91)	150.00 (12.29)
April	4.82	2844.45 (53.34)	199.31 (14.15)	228.50 (15.15)
May	6.01	3308.85 (57.53)	158.67 (12.64)	306.00 (17.52)
June	4.18	1905.98 (43.67)	137.39 (11.76)	253.50 (15.95)
July	5.28	1754.40 (41.90)	102.56 (10.18)	407.50 (20.21)
August	4.37	1373.85 (37.08)	54.18 (7.43)	446.50 (21.15)
September	3.95	1291.19 (35.95)	76.76 (8.82)	518.00 (22.78)
October	3.31	1257.75 (35.48)	41.28 (6.50)	234.00 (15.33)
November	2.62	961.05 (31.02)	29.67 (5.54)	189.00 (13.78)
December	2.33	503.10 (22.45)	22.58 (4.86)	138.00 (11.79)
$C.D_{0.05}$	0.54	(2.70)	(1.27)	(3.00)

*Figures in parentheses are square root (x+1) transformed values

In addition, field activities of bees are badly affected by cloudy days, foggy mornings and rains during the months of winter season. Worker bees from super shifts to brood chamber as winter cluster for protecting the broods. Higher numbers of bees are involved to maintain the brood nest temperature during winter months instead of going for foraging pollen and nectar. Therefore, climatic elements are the limiting factors during winter season.

Significantly maximum average pollen area was recorded in the month of April (199.31 cm^2) followed by May (158.67 cm^2) which was statistically at par with June (137.39 cm^2). These results are in close proximity with Negi (2018) and Brar (2016) who observed minimum pollen area in the month of May (142.50 cm^2 /colony) and April (160 $cm^{2/}$colony) in *A. mellifera* colonies in the same apiary. It was mainly due to blooming of abundant pollen flora, which in turn increased the number of pollen foragers. Minimum average pollen area (22.58 cm^2) was recorded in the month of December which was statistically at par with November (29.67 cm^2). Same results were obtained by Negi (2018) and Brar (2016) who observed minimum average pollen area in December (22.16 cm^2/colony) and (24.00 cm^2/colony), respectively. Neupane and Thapa (2005) reported that amount of pollen as beebread (2439.0 gm/hive) was highest during spring season, while the lowest amount of beebread or pollen store (152.5 gm /hive) was recorded in rainy season. Mishra and Sharma, 1998 reported that the winter are very harsh and the worker bees stop foraging when air temperature fall below 14°C.

In present investigation maximum average honey stores were obtained in the month of September which was statistically at par with August and July it could be due to reappearance of bee activity after rainy season and favourable climatic conditions for foraging and the study is supported by Brar (2016) who found maximum honey store (480 g) of *A. mellifera* in the month of September, 2015 at Nauni. The minimum average honey store (74.00g) in the present study was observed in the month of February which was statistically at par with January (84.50g). There was decrease in brood area in December and January months leading to decline in foraging and nectar collectors which resulted in decreased honey stores. In winter months higher numbers of bees are involved to maintain the brood nest temperature during winter months instead of going for foraging pollen and nectar (Neupane and Thapa, 2005). These findings are in line with Negi (2018) and Brar (2016) who observed minimum honey stores in the month of January (100.00 g) and February (75.00 g). Whereas, Gowda *et*

al. (2005) reported minimum honey store (84.81 g) of *A. cerana* in the month of May, 1999 at Karnataka, due to different environmental parameters.

4.2.1.6 Pooled data on colony records under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020)

Pooled data on colony records under stationary and migratory conditions presented in Table 21 and Fig. 9 revealed that when the colonies were shifted to Hisar significantly maximum colony strength was recorded in the month of February (7.33 bee frames) followed by colony strength in the month of January (6.49 bee frames) which was statistically at par with May (6.05 bee frames), July (5.96 bee frames), June (5.75 bee frames) and August (5.66 bee frames). The average colony strength in the month of December was 5.35 bee frames which was at par with September (5.13 bee frames) and November (4.76 bee frames) followed by March (4.39 bee frames). During the migratory period i.e. November, December, January and February the colony strength was much high as compared to stationary beekeeping being highest in the February month throughout the study period. Statistically, minimum colony strength was recorded in the month of April (3.17 bee frames) which was statistically at par with October (3.66 bee frames) followed by March (4.39 bee frames) which was statistically at par with November (4.76 bee frames) and September (5.13 bee frames).

Brood area of *A. mellifera* under migratory conditions (Fig. 10) revealed that statistically maximum average brood area was observed in the month of February (4040.93 cm^2) followed by January (2741.25 cm^2) which was statistically at par with March (2560.65cm^2). The average brood area in the month of May was 2322.00 cm^2 which was statistically at par with April (2215.58 cm^2) and June (2073.68 cm^2) followed by July (1702.80 cm^2) which was at par with December (1612.50 cm^2), August (1535.10cm^2), September (1402.88 cm^2) and October (1364.18 cm^2). Whereas, significantly minimum brood area was recorded in the month of November (1041.68cm^2).

The observations recorded on average pollen area (Fig. 11) revealed that maximum pollen area was recorded in the month of February (432.15 cm^2) which was statistically at par with January (374.10 cm^2), December (345.14 cm^2) and March (225.75 cm^2). Minimum pollen area was recorded in the month of April (22.58 cm^2) which was statistically at par with September (33.54 cm^2), October (40.64 cm^2), June (41.93 cm^2), July (56.53 cm^2), August (67.73 cm^2), and May (87.08 cm^2).

Significantly maximum average honey store (Fig. 12) was observed in the month of February (1108.00 g) followed by January (490.00 g) which was statistically at par with September (441.00 g), April (436.00 g), December (432.50 g), July (423.50 g), June (393.50 g) and August (368.00 g). The average honey store in May was 292.50 g which was statistically at par with November (265.50 g) and March (242.00 g). Significantly minimum honey store was found in the month of October (112.50 g).

Table 21. Pooled data on colony records of *A. mellifera* under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	Colony parameters			
	Average colony strength (Bee frame)	Average brood area (cm^2)	Average pollen area (cm^2)	Average honey store (g)
January**	6.49	2741.25 (52.37)*	374.10 (19.37)	490.00 (22.16)
February**	7.33	4040.93 (63.58)	432.15 (20.81)	1108.00 (33.30)
March	4.39	2560.65 (50.61)	225.75 (15.06)	242.00 (15.59)
April	3.17	2215.58 (47.08)	22.58 (4.86)	436.00 (20.90)
May	6.05	2322.00 (48.20)	87.08 (9.38)	292.50 (17.13)
June	5.75	2073.68 (45.55)	41.93 (6.55)	393.50 (19.86)
July	5.96	1702.80 (41.28)	56.53 (7.58)	423.50 (20.60)
August	5.66	1535.10 (39.19)	67.73 (8.29)	368.00 (19.21)
September	5.13	1402.88 (37.47)	33.54 (5.88)	441.00 (21.02)
October	3.66	1364.18 (36.95)	40.64 (6.45)	112.50 (10.65)
November**	4.76	1041.68 (32.29)	138.68 (11.82)	265.50 (16.32)
December**	5.35	1612.50 (40.17)	345.14 (18.60)	432.50 (20.82)
C.D $_{0.05}$	0.90	(4.00)	(6.41)	(3.28)

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pooled data on colony records under stationary and migratory conditions revealed that when the colonies were shifted to Hisar significantly maximum colony strength was recorded in the month of February followed by January which was at par with May. These studies are

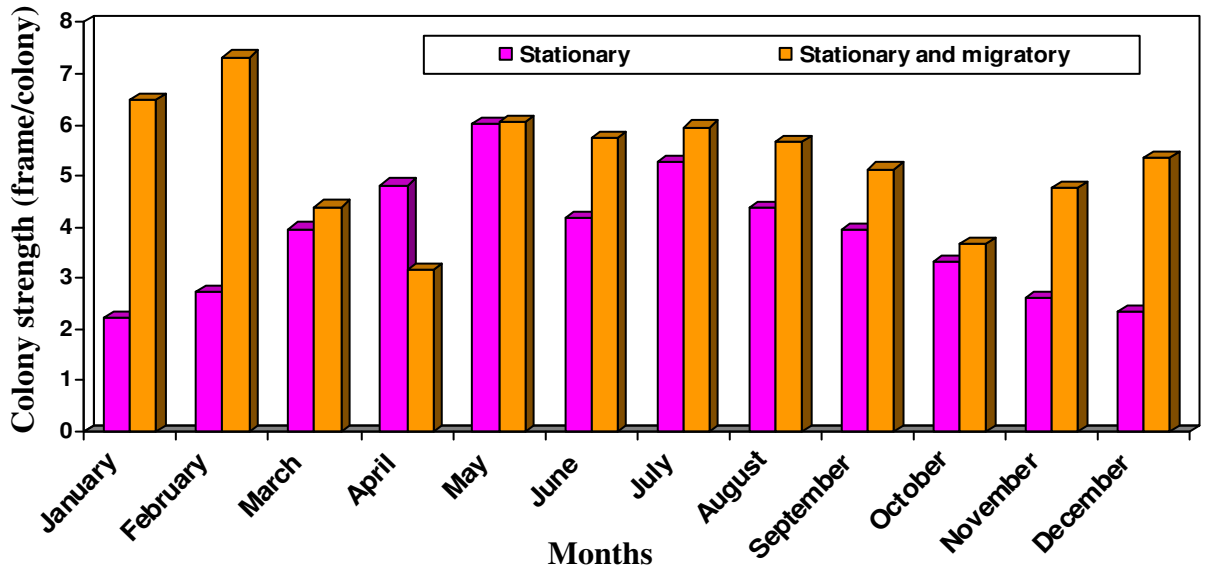


Fig 9. Colony strength of *A. mellifera* under stationary and migratory conditions (2019-2020)

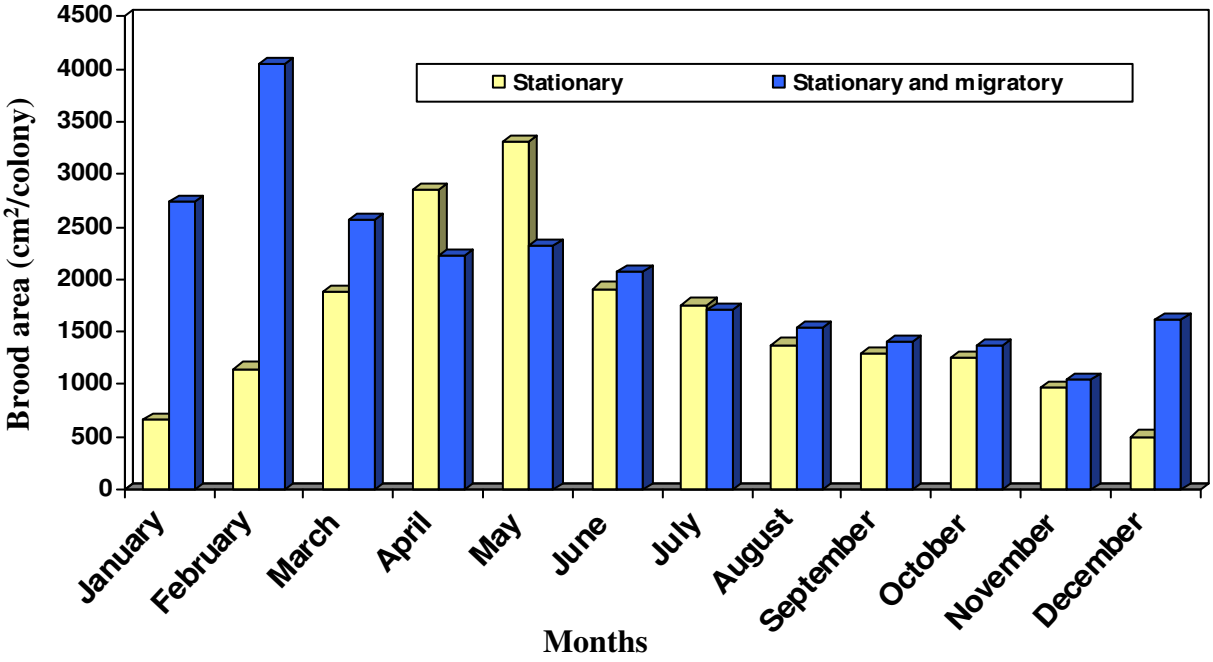


Fig 10. Brood area of *A. mellifera* under stationary and migratory conditions (2019-2020)

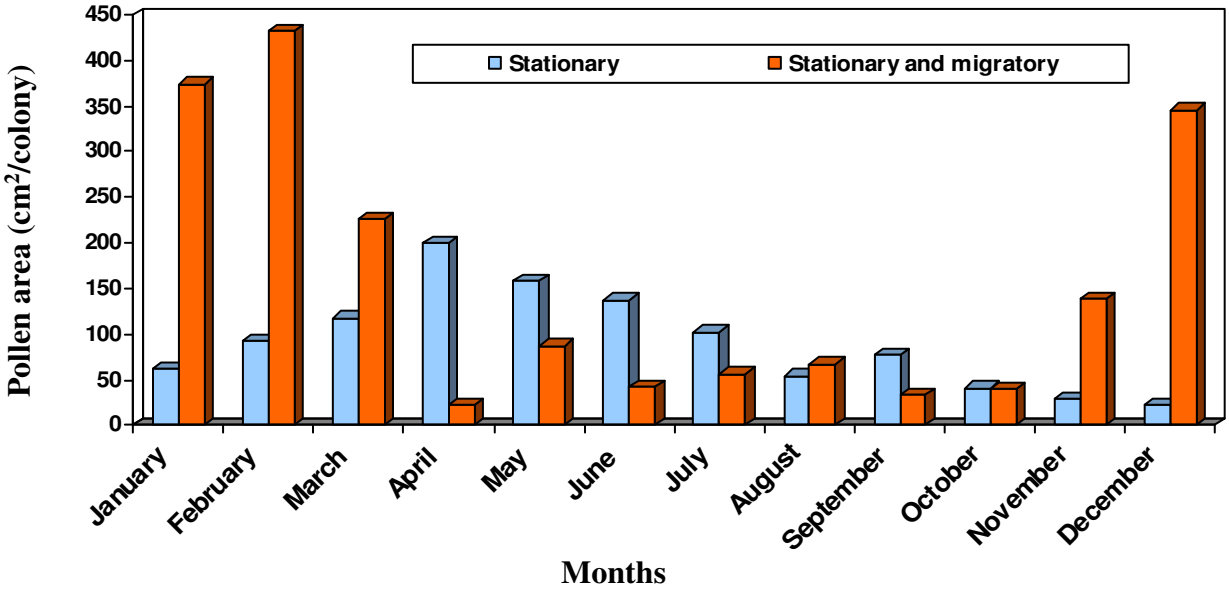


Fig 11. Pollen area of *A. mellifera* under stationary and migratory conditions (2019-2020)

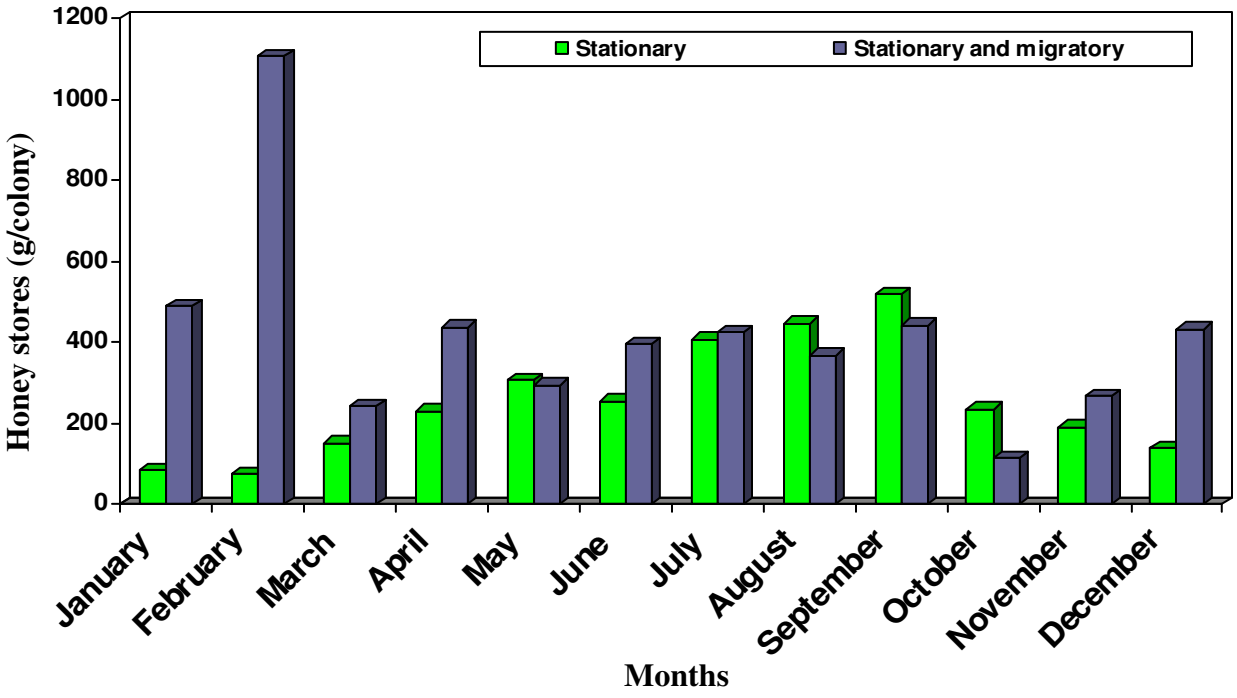


Fig 12. Honey stores of *A. mellifera* under stationary and migratory conditions (2019-2020)

in corroboration with Nadaf *et al.* (2016) who found that maximum colony strength was recorded at its peak from start of December to the end of February when both nectar and pollen were available in abundance. The similar findings were reported by Singh (1997) who recorded more number of active frames (15.13) in February, 1996 in Hisar. The important bee plants that bloom during this period are *B. juncea*, *Eucalyptus* sp., *C. arietinum* and *P. dactylifera*. Our results were also supported by Brar (2016) and Negi (2018) who also observed maximum colony strength in the month of February and March, respectively under migratory conditions of Hisar, Haryana. In our studies minimum colony strength was recorded in the month of October and similar results were obtained by Negi (2018) while, Brar (2016) reported minimum colony strength in the month of April in the same Apiary. During the migratory period i.e. November, December, January and February the colony strength was much high as compared to stationary beekeeping, being highest in the February month throughout the study period.

Brood area under migratory conditions was maximum in the month of February followed by January and was at par with March. Similar observations were recorded by Negi (2018) and Brar (2016) who found maximum brood area in the month of February and March. Nadaf *et al.* (2016) also recorded maximum brood area in the month of February (7301 cm^2) in Hisar Haryana. The similar results were reported by Singh (1997) who recorded more brood area (7155.00 cm^2) in February, 1996 from 6621.25 cm^2 in mid-December, 1995, respectively in Hisar because important bee plants bloom during this period viz., *B. juncea*, *Eucalyptus* sp., *C. arietinum* and *P. dactylifera*. In our studies minimum brood area was observed in the month of November same results were obtained by Negi (2018) while Brar (2016) obtained minimum brood area in the month of May which may be due to different weather conditions during his studies because in our studies in the month of May the rainfall was very low. Thus in the present study, there was increase in the brood area from 1041.68 cm^2 to 4040.93 cm^2 from the month of November to February as compared to the stationary conditions.

In the present investigation maximum pollen stores were observed in the month of February which was at par with January, December and March while minimum pollen area was recorded in the month of April which was at par with September, October, June, July, August and May. Pollen area was maximum in migratory months January, February,

November and December in Hisar Haryana these observations were supported by Nadaf *et al.* (2016) who reported maximum pollen area in the month of February (3045.30 cm^2) and Singh (1997) who recorded maximum pollen store (2957.50 cm^2) in February, 1996. While minimum pollen stores were observed in the month of April which was at par with September, October, June, July, August and May. Negi (2018) also reported similar results in the same apiary.

Maximum honey stores in our studies were recorded in the month of February (1108.00g) and significantly minimum honey stores were observed in the month of October. These observations are in corroboration with Negi (2018) and Brar (2016) who also observed maximum honey stores in the month of February in migratory conditions of Hisar Haryana and minimum in the month of October. A study from Hisar, Haryana also showed maximum honey stores in the month of February due to bloom of *B. juncea*, *Eucalyptus* sp., *C. arietinum* and *P. Dactylifera* (Nadaf *et al.,* (2016) and Singh (1997). The main honey plants in Hisar were *C. sinensis* and *T. Alexandrinum* and other honey plants were *A. indica*, *A. marmelos*, *C. sativum*, *P. glabra* and *P. dactylifera*.

4.2.2 Incidence of European Foulbrood

In *A. mellifera*, European foulbrood disease was recorded for the first time from U.K. in 1885) (Cheshire and Cheyne, 1885) and from USA in 1907 (White, 1907). It affected 4.3 to 7.2 per cent colonies in Colorado (Moffett, 1952), 7.0 per cent in Italy (Giavarini, 1956), 26.0 per cent in Hungry (Buza and Kovacs, 1969) and 25- 40 per cent colonies in India (Anonymous, 1998; Viraktamath, 1998). The brood was observed and symptoms found were; colour of the infected larvae (brood) was pale yellow and slightly displaced upward or downward direction from their actual position in the comb cells. The infected rotten larvae emitted sour or vinegar like smell. Infected brood died when they were 3-5 days old age. Finally left brood in the form of soft scale was noticed (Plate 21).

4.2.2.1 During 2019 (stationary conditions Nauni, Solan))

Seasonal incidence of European foulbrood disease under stationary conditions in *A. mellifera* colonies from January, 2019 to December, 2019 are presented in Table 22. The symptoms of European foulbrood disease were observed in all the months of the study period when temperature, rainfall and relative humidity varied from 8.85 to 25.74°C, 5.60 to 225.80

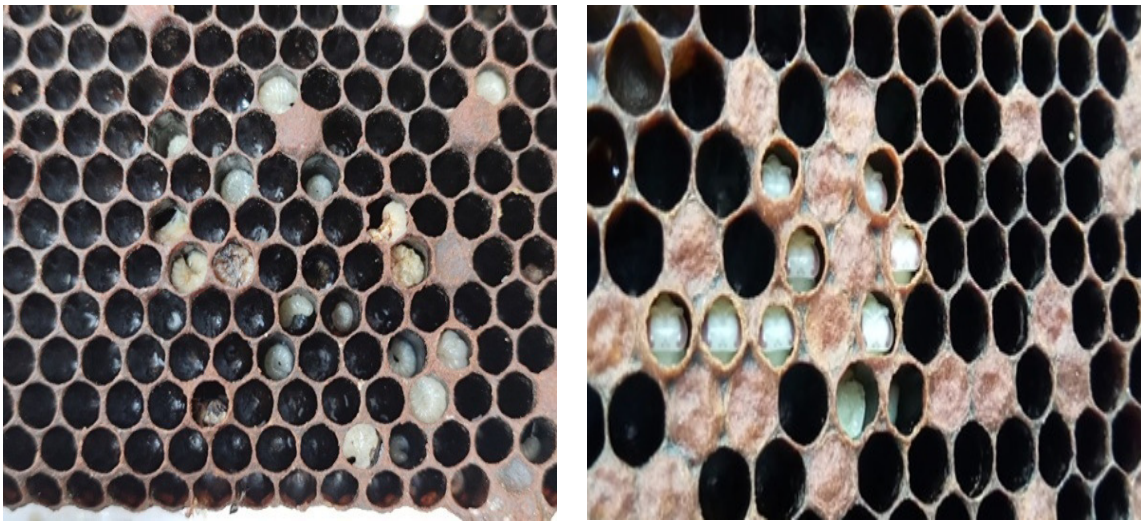


Plate 21. European foulbrood infected larvae and prepupae in *A. mellifera* colonies

Plate 22. Sacbrood infected larvae with tongue like projection and sac like structure in *A. mellifera*

mm and 44.00 to79.00 per cent, respectively and disease incidence varied from 1.60 to 35.80 per cent. The incidence of European foulbrood disease was maximum in the month of September (35.80%) when average strength and brood area were 4.10 bee frames and 1240.78 cm^2 and temperature, relative humidity and rainfall were 23.48°C, 77.00 per cent and 151.40 mm, respectively which was statistically at par with European foulbrood incidence in the month of June (32.60%) and May (23.60%).

Table 22. Incidence of European foulbrood disease in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2019

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	3.20 (2.05)*	2.45	715.95 (26.78)	8.85	59.00	73.00
February	5.40 (2.53)	3.09	1154.55 (33.99)	10.34	63.00	103.10
March	15.80 (4.10)	4.03	1825.35 (42.74)	13.45	54.00	54.60
April	17.20 (4.27)	4.80	2754.15 (52.49)	20.03	49.00	36.80
May	23.60 (4.96)	5.94	3205.65 (56.63)	22.61	44.00	21.30
June	32.60 (5.80)	4.29	1870.50 (43.26)	25.74	48.00	98.50
July	17.40 (4.29)	5.29	1638.30 (40.49)	23.82	79.00	218.10
August	13.60 (3.82)	4.47	1251.30 (35.39)	24.43	79.00	225.80
September	35.80 (6.07)	4.10	1240.78 (35.24)	23.48	77.00	151.40
October	9.60 (3.26)	3.14	1225.50 (35.02)	18.47	65.00	5.60
November	0.00 (1.00)	2.75	980.40 (31.33)	15.50	62.00	32.20
December	1.60 (1.61)	2.60	432.15 (20.81)	10.49	58.00	33.20
C.D $_{0.05}$	(1.17)	0.82	(4.21)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.81*
Relative Humidity× EFB incidence =- 0.03
Rainfall × EFB incidence = 0.28
Colony Strength × EFB incidence = 0.69*
Brood Area× EFB incidence = 0.55 (*Significant at 5%)

The European foulbrood incidence in the month of July was 17.40% which was statistically at par with April (17.20%), March (15.80%), August (13.60%) and October (9.60 %). Significantly minimum incidence of European foulbrood was observed in the month of December (1.60%) when temperature, relative humidity and rainfall were low i.e. 10.49°C, 58.00 per cent and 33.20 mm, respectively which was statistically at par with January (3.20%) and February (5.40%). European foulbrood indicated positive correlation (significant) with temperature (r= 0.81) and colony strength (r= 0.69).

4.2.2.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Seasonal incidence of European foulbrood in Table 23 revealed that the disease was observed in all the months of the study period when temperature, rainfall and relative humidity varied from 11.15 to 25.74, 4.5 to 225.80 and 44.00 to 97.00 per cent.

The disease incidence varied from 1.40 to 41.60 per cent. The incidence of European foulbrood disease was maximum in the month of July (41.60%) when temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm, respectively which was statistically at par with March (35.80%) and August (30.20%) followed by September was 26.20% which was statistically at par with June (24.20%) and May (18.60%) which was further at par with April (13.40%) and October (9.80%). The minimum incidence of European foulbrood was observed in the month of December (1.40%) when temperature and rainfall were low i.e. 11.40°C and 4.50 mm, respectively which was statistically at par with January (2.40%), February (2.60%) and November (3.80%). EFB in *A. mellifera* colonies showed positive correlation (significant) with rainfall (r= 0.81) and negative correlation with colony strength (r=-0.02) and relative humidity (r= -0.44).

Table 23. Incidence of European foulbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019**	2.40 (1.84)*	6.59	2941.20 (54.24)	11.15	97.00	10.40
February**	2.60 (1.90)	7.03	4211.85 (64.91)	14.45	93.00	10.90
March	35.80 (6.07)	4.51	2709.00 (52.06)	13.45	54.00	54.60
April	13.40 (3.79)	3.37	2309.10 (48.06)	20.03	49.00	36.80
May	18.60 (4.43)	5.85	2431.65 (49.32)	22.61	44.00	21.30
June	24.20 (5.02)	6.00	2173.65 (46.63)	25.74	48.00	98.50
July	41.60 (6.53)	6.30	1638.30 (40.49)	23.82	79.00	218.10
August	30.20 (5.59)	5.88	1528.65 (39.11)	24.43	79.00	225.80
September	26.20 (5.22)	5.56	1406.10 (37.51)	23.48	77.00	151.40
October	9.80 (3.29)	4.08	1393.20 (37.34)	18.47	65.00	5.60
November**	3.80 (2.19)	5.18	1102.95 (33.23)	19.90	89.00	12.30
December**	1.40 (1.55)	5.43	1631.85 (40.41)	11.40	94.00	4.50
C.D $_{0.05}$	(1.26)	0.34	(4.98)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.58
Relative Humidity× EFB incidence = -0.44
Rainfall × EFB incidence = 0.81*
Colony Strength × EFB incidence = -0.02
Brood Area× EFB incidence = 0.23

(*Significant at 5%)

4.2.2.3 During 2020 (stationary conditions Nauni, Solan)

The observations recorded on the seasonal incidence of European foulbrood disease in *A. mellifera* are presented in Table 24.

Table 24. Incidence of European foulbrood disease in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	4.60 (2.37)*	2.01	606.30 (24.64)	9.10	68.00	168.30
February	8.60 (3.10)	2.36	1135.20 (33.71)	12.15	57.00	38.50
March	10.60 (3.41)	3.91	1922.10 (43.85)	14.20	62.00	171.80
April	19.20 (4.49)	4.84	2934.75 (54.18)	19.15	51.00	47.70
May	21.00 (4.69)	6.08	3412.05 (58.42)	22.05	53.00	74.80
June	24.60 (5.06)	4.06	1941.45 (44.07)	24.10	69.00	58.70
July	13.80 (3.85)	5.28	1870.50 (43.26)	25.05	81.00	278.10
August	15.60 (4.07)	4.27	1496.40 (38.70)	24.95	86.00	148.60
September	38.40 (6.28)	3.81	1341.60 (36.64)	24.10	77.00	6.00
October	11.80 (3.58)	3.47	1290.00 (35.93)	20.40	65.00	0.00
November	7.00 (2.82)	2.49	941.70 (30.70)	14.60	68.00	37.70
December	2.20 (1.79)	2.06	574.05 (23.98)	12.2	62.00	23.80
C.D $_{0.05}$	(0.99)	0.29	(7.42)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.70*
Relative Humidity× EFB incidence = 0.22
Rainfall × EFB incidence = -0.15
Colony Strength × EFB incidence = 0.64
Brood Area× EFB incidence = 0.52 (*Significant at 5%)

The incidence of European foulbrood disease was observed in all the months of the study period when external temperature, rainfall and relative humidity varied from 9.1 to 25.05°C, 0.00 to 278.10 mm and 51.00 to 86.00 per cent, respectively. The incidence of European foulbrood disease was significantly maximum in the month of September (38.40%) when average colony strength and brood area were 3.81 bee frames and 1341.60 cm^2, respectively and temperature, relative humidity and rainfall were 24.10 °C, 77.00 per cent and 6.00 mm followed by 24.60 per cent in the month of June which was statistically at par with May (21.00%), April (19.20%) and August (15.60%).The incidence of European foulbrood in the month of July was 13.80 per cent which was at par with October (11.80%), March (10.60%) and February (8.60%). Significantly minimum incidence of European foulbrood was observed in the month of December (2.20%) when temperature, relative humidity and rainfall were 12.20°C, 62.00 per cent and 23.80 mm, respectively which was at par with January (4.60%). Correlation of European foulbrood disease with colony and weather parameters was positive (significant) with temperature (r= 0.70) and negative (non-significant) with rainfall (r=- 0.15).

4.2.2.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

The observations recorded on the seasonal incidence of European foulbrood disease in *A. mellifera* colonies under stationary (Nauni) and migratory (Hisar) conditions is presented Table 25. The incidence of European foulbrood disease was observed in all the months of the study period when external temperature, rainfall and relative humidity varied from 11.70 to 25.05°C, 0.00 to 278.10 mm and 51.00 to 98.00 per cent, respectively. The incidence of European foulbrood disease was maximum in the month of July (35.00%) when average colony strength and brood area was 5.61 bee frames and brood area was 1767.30 cm^2 and temperature, relative humidity and rainfall were 25.05°C, 81.00 per cent and 278.10 mm which was statistically at par with March (33.80%) and August (29.80%).

The European foulbrood incidence in September was 20.02% which was statistically at par with June (19.00%), May (18.00%), April (12.40%) and October (10.60%). Least incidence of European foulbrood was observed in the month of January, 2020 (1.00%) when temperature, relative humidity and rainfall were 11.70°C, 98.00 per cent and 8.90 mm, respectively which was statistically at par with February (2.20%), November (2.40%) and

December (3.20%). The data on correlation of European foulbrood with colony and weather parameters in *A. mellifera* colonies under migratory conditions indicated positive correlation (significant) with temperature (r=0.57) and negative correlation with colony strength (r=-0.14), brood area (r=- 0.16) and relative humidity (r= -0.41).

Table 25 Incidence of European foulbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020**	1.00 (1.41)*	6.38	2541.30 (50.42)	11.70	98.00	8.90
February**	2.20 (1.79)	7.62	3870.00 (62.22)	16.90	97.00	8.70
March	33.80 (5.90)	4.27	2412.30 (49.13)	14.20	62.00	171.80
April	12.40 (3.66)	2.96	2122.05 (46.08)	19.15	51.00	47.70
May	18.00 (4.36)	6.25	2212.35 (47.05)	22.05	53.00	74.80
June	19.00 (4.47)	5.50	1973.70 (44.44)	24.10	69.00	58.70
July	35.00 (6.00)	5.61	1767.30 (42.05)	25.05	81.00	278.10
August	29.80 (5.55)	5.44	1541.55 (39.28)	24.95	86.00	148.60
September	20.20 (4.60)	4.69	1399.65 (37.43)	24.10	77.00	6.00
October	10.60 (3.41)	3.23	1335.15 (36.55)	20.40	65.00	0.00
November**	2.40 (1.84)	4.33	980.40 (31.33)	18.00	89.00	19.90
December**	3.20 (2.05)	5.26	1593.15 (39.93)	13.30	93.00	0.00
C.D $_{0.05}$	(1.38)	0.31	(6.86)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =
Temperature × EFB incidence = 0.57*
Relative Humidity× EFB incidence = -0.41
Rainfall × EFB incidence = 0.86
Colony Strength × EFB incidence = -0.14
Brood Area× EFB incidence = -0.16 (*Significant at 5%)

4.2.2.5 Pooled data under stationary conditions Nauni, Solan (2019-2020)

Pooled data on European foulbrood incidence from January, 2019 to December, 2020 is presented in Table 26 and Fig. 13. The symptoms of European foulbrood disease were observed in all the months of the study period when temperature, rainfall and relative humidity varied from 8.98 to 24.92°C, 2.80 to 248.10 mm and 48.50 to 82.50 per cent, respectively and disease incidence varied from 1.90 to 37.10 per cent. The incidence of European foulbrood disease was significantly maximum in the month of September (37.10%) when average colony strength and brood area were 3.96 bee frames and 1291.19 cm^2 and temperature, relative humidity and rainfall were 23.79°C, 77.00 per cent and 78.70 mm, respectively followed by June (28.60%) which was statistically at par with May (22.30%). The European foulbrood incidence in April was 18.20% which was statistically at par with July (15.60%), August (14.6%) and March (13.20%) which was further at par with October (10.70%). Minimum incidence was recorded in the month of December (1.90%) when the average colony strength and brood area was 2.33 bee frames and 503.10 cm^2 which was statistically at par with November (3.50%) and January (3.90%). The EFB disease showed positive (significant) correlation with temperature (r= 0.81) and colony strength (r=0.66).

European foulbrood disease firstly appeared in March and found maximum in the month of September (37.10%) when the colony strength and brood area was low. During maximum incidence of European foulbrood disease the temperature was high and relative humidity and rainfall were moderate. After September the disease was decreased and become minimum in the month of December (1.90%). High incidence of European foulbrood disease showed significant reduction in brood area and colony strength. Earlier European foulbrood disease was reported to appear usually in spring and first half of summer season but in recent times its occurrence has not shown a clear dependence on season (Russenova and Parvanov, 2005). Negi (2018) reported maximum incidence of European foulbrood disease in September, 2017 (30.16%) when colony strength and brood area were low and temperature was high while relative humidity was moderate which supports present findings. In a study conducted by Brar *et al.*(2019) maximum incidence of European foulbrood disease (29.00 %) was observed in the month of September which was statistically at par with July (26 %). Minimum incidence was observed in the month of December (1.90%), the disease incidence was low in winter month. Generally during winter months, less number of available nurse

bees resulted in production of less amount of glandular food which caused the diseased larvae to appear starved and was easily detected and removed by bees. (Bailey, 1977).

Table 26. Pooled data on incidence of European foulbrood disease in *A. mellifera* colonies under stationary conditions at Nauni during January, 2019 to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	3.90 (2.21)*	2.23	661.13 (25.73)	8.98	63.50	120.65
February	7.00 (2.82)	2.73	1144.88 (33.85)	11.25	60.00	70.80
March	13.20 (3.77)	3.96	1873.73 (43.30)	13.83	58.00	113.20
April	18.20 (4.38)	4.82	2844.45 (53.34)	19.59	50.00	42.25
May	22.30 (4.83)	6.01	3308.85 (57.53)	22.33	48.50	48.05
June	28.60 (5.44)	4.18	1905.98 (43.67)	24.92	58.50	78.60
July	15.60 (4.07)	5.28	1754.40 (41.90)	24.44	80.00	248.10
August	14.60 (3.95)	4.37	1373.85 (37.08)	24.69	82.50	187.20
September	37.10 (6.17)	3.96	1291.19 (35.95)	23.79	77.00	78.70
October	10.70 (3.42)	3.31	1257.75 (35.48)	19.44	65.00	2.80
November	3.50 (2.12)	2.62	961.05 (31.02)	15.05	65.00	34.95
December	1.90 (1.70)	2.33	503.10 (22.45)	11.35	60.00	28.50
C.D $_{0.05}$	(0.68)	0.54	(2.70)			

*Figures in parentheses are square root (x+1) transformed value

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.81*
Relative Humidity× EFB incidence = 0.12
Rainfall × EFB incidence = 0.08
Colony Strength × EFB incidence = 0.66*
Brood Area× EFB incidence = 0.53 (*Significant at 5%)

Negi (2018) and Brar *et al.* (2019) also reported similar type severity of disease in *A. mellifera* colonies. Pooled data on correlation of European foulbrood with colony and

weather parameters in *A. mellifera* colonies indicated positive correlation (significant) with temperature (r= 0.81) and colony strength (0.66).

4.2.2.6 Pooled data under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020)

Data on pooled incidence of European foulbrood disease under stationary (Nauni, Solan) and migratory (Hisar, Haryana) conditions from January, 2019 to December, 2020 is presented in Table 27 and Fig. 13.The symptoms of European foulbrood diseases were observed in all the months of the study period when temperature, rainfall and relative humidity varied from 11.43 to 24.92°C, 2.25 to 248.10 mm and 8.00 to 97.50 per cent, respectively and disease incidence varied from 1.70 to 38.30 per cent. The incidence of European foulbrood disease was statistically maximum in the month of July (38.30%) when average colony strength and brood area were 5.96 bee frames and 1702.80 cm^2, respectively and temperature, relative humidity and rainfall were 24.44°C, 8.00 per cent and 248.10 mm, respectively which was statistically at par with March (34.80%) followed by August (30.00%) and September (23.20%) which was statistically at par with June (21.60%) and May (18.30%). The disease incidence in the month of April was 12.90% which was statistically at par with October (10.20%). Minimum incidence of European foulbrood was observed in the month of January (1.70%) which was statistically at par with December (2.30%), February (2.40%) and November (3.10%). Pooled data on correlation of European foulbrood with colony and weather parameters in *A. mellifera* colonies under migratory conditions indicated positive correlation (significant) with temperature (r=0.58) and rainfall (r= 0.89) while, negative correlation with colony strength (r=-0.09), brood area (r=- 0.20) and relative humidity (r= -0.71).

In our investigations maximum incidence of European foulbrood disease under stationary and migratory conditions was observed in the month of July (38.30%) when temperature was high and relative humidity was low. Negi (2018) supports the present findings and observed that the incidence of European foulbrood disease was maximum in the month of July, 2017 (33.22%) when temperature, relative humidity and rainfall were 24.00 °C, 81.00 per cent and 162.30 mm. Brar *et al.* (2019) also found maximum incidence of European foulbrood disease (38.00 %) in the month of July which are in line with our results. In our studies very low European foulbrood incidence was observed in winter months.

Naveen and Yadav (2021) also observed very less incidence of European foulbrood in August, September, November and February while, maximum incidence of European foulbrood in the month of October (16.00%) and minimum in second fortnight of February (2.00%) in Madhya Pradesh.

Table 27 Pooled data on incidence of European foulbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	EFB incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January**	1.70 (1.64)*	6.49	2741.25 (52.37)	11.43	97.50	9.65
February**	2.40 (1.84)	7.33	4040.93 (63.58)	15.68	95.00	9.80
March	34.80 (5.98)	4.39	2560.65 (50.61)	13.83	58.00	113.20
April	12.90 (3.73)	3.17	2215.58 (47.08)	19.59	50.00	42.25
May	18.30 (4.39)	6.05	2322.00 (48.20)	22.33	48.50	48.05
June	21.60 (4.75)	5.75	2073.68 (45.55)	24.92	58.50	78.60
July	38.30 (6.27)	5.96	1702.80 (41.28)	24.44	8.00	248.10
August	30.00 (5.57)	5.66	1535.10 (39.19)	24.69	82.50	187.20
September	23.20 (4.92)	5.13	1402.88 (37.47)	23.79	77.00	78.70
October	10.20 (3.35)	3.66	1364.18 (36.95)	19.44	65.00	2.80
November**	3.10 (2.02)	4.76	1041.68 (32.29)	18.95	89.00	16.10
December**	2.30 (1.82)	5.35	1612.50 (40.17)	12.35	93.50	2.25
C.D $_{0.05}$	(0.56)	0.90	(4.00)			

*Figures in parentheses are square root (x+1) transformed value

Pearson correlation Matrix (r) =

Temperature × EFB incidence = 0.58*
Relative Humidity× EFB incidence =- 0.71
Rainfall × EFB incidence = 0.89*
Colony Strength × EFB incidence = -0.09
Brood Area× EFB incidence = -0.20 (*Significant at 5%)

Minimum incidence of European foulbrood was observed in the month of January (1.70%) which was statistically at par with December (2.30%), February (1.84%) and November (3.10%). Negi (2018) and Brar *et al.* (2019) reported similar type of results which supports the present results. Rao (2009) have recorded maximum incidence of European foulbrood in September (18.52%) in *A. mellifera* colonies at Nauni. These results are not same as we observed in the same apiary, this may be due to different weather conditions. He also observed that *A. mellifera* colonies during winter months were free from European foulbrood infestation. Similar extents of damage have also been reported in *A. mellifera* colonies (Moffett, 1952; Giavarini, 1956; Buza and Kovacs, 1969) in other countries by different workers in different parts of world.

4.2.3 Incidence of sacbrood disease

For the first time sacbrood disease of *A. mellifera* Linn. Was reported from USA in 1913 (White, 1913, 1917). Its extent of damage was from a few cells to 90 per cent in brood and 0 to 100 per cent colony infection per apiary in different countries (Shimanuki *et al.,* 1992). The brood was observed and symptoms found were; scattered pattern of brood area, tongue-like projection, change in colour of the brood, and sac-like structure of brood (Plate 22) (Devaneson and Jacob, 2001; Srinivasan *et al.,* 2014).

4.2.3.1 During 2019 (stationary conditions Nauni, Solan)

The data on incidence of sacbrood disease observed in *A. mellifera* colonies is presented in Table 28. The incidence varied from 0.20 to 7.00 per cent. Maximum incidence was observed in the month of May (7.00%) when average colony strength and brood area were 5.94 bee frames and 3205.65 cm^2 which was statistically at par with June (4.80%) followed by sacbood incidence in April (3.40%) which was statistically at par with August (3.00%), July (2.00%) and March (1.60%). During the month of May when the incidence of sacbrood virus was maximum the temperature, relative humidity and rainfall were 22.61°C, 44 per cent and 21.30 mm, respectively. The temperature, relative humidity and rainfall during the whole disease incidence period varied from 8.85 to25.74°C, 44 to 79 per cent and 5.6 to225.80 per cent. Whereas, minimum incidence was recorded in the month of October (0.20%) when temperature, relative humidity and rainfall were 18.47°C, 65.00 per cent and 5.60 mm, respectively which was statistically at par with January (0.40%) and September (0.60%), February (1.20%) and March (1.60%). No incidence of sacbrood disease was

observed in the month of November and December. Correlation of sacbrood with colony and weather parameters in *A. mellifera* colonies demonstrated positive correlation (significant) with temperature (r= 0.59) and negative correlation with relative humidity (r= -0.49).

Table 28. Incidence of sacbrood disease in *A. mellifera* under stationary conditions at Nauni during January to December, 2019

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.40 (1.18)*	2.45	715.95 (26.78)	8.85	59.00	73.00
February	1.20 (1.48)	3.09	1154.55 (33.99)	10.34	63.00	103.10
March	1.60 (1.61)	4.03	1825.35 (42.74)	13.45	54.00	54.60
April	3.40 (2.10)	4.80	2754.15 (52.49)	20.03	49.00	36.80
May	7.00 (2.83)	5.94	3205.65 (56.63)	22.61	44.00	21.30
June	4.80 (2.41)	4.29	1870.50 (43.26)	25.74	48.00	98.50
July	2.00 (1.73)	5.29	1638.30 (40.49)	23.82	79.00	218.10
August	3.00 (2.00)	4.47	1251.30 (35.39)	24.43	79.00	225.80
September	0.60 (1.26)	4.10	1240.78 (35.24)	23.48	77.00	151.40
October	0.20 (1.10)	3.14	1225.50 (35.02)	18.47	65.00	5.60
November	0.00 (1.00)	2.75	980.40 (31.33)	15.50	62.00	32.20
December	0.00 (1.00)	2.60	432.15 (20.81)	10.49	58.00	33.20
C.D $_{0.05}$	(0.56)	0.82	(4.21)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.59*
Relative Humidity× Sacbrood incidence = -0.49
Rainfall × Sacbrood incidence = 0.03
Colony Strength × Sacbrood incidence = 0.82
Brood Area× Sacbrood incidence = 0.85 (*Significant at 5%)

4.2.3.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

The data on incidence of sacbrood disease under stationary (Nauni) and Migratory (Hisar) conditions during 2019 was observed in *A. mellifera* colonies is presented in Table 29. The incidence varied from 0.40 to 6.00 per cent.

Table 29. Incidence of sacbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019**	1.40 (1.55)*	6.59	2941.20 (54.24)	11.15	97.00	10.40
February**	3.00 (2.00)	7.03	4211.85 (64.91)	14.45	93.00	10.90
March	2.60 (1.90)	4.51	2709.00 (52.06)	13.45	54.00	54.60
April	3.80 (2.19)	3.37	2309.10 (48.06)	20.03	49.00	36.80
May	6.00 (2.65)	5.85	2431.65 (49.32)	22.61	44.00	21.30
June	4.40 (2.32)	6.00	2173.65 (46.63)	25.74	48.00	98.50
July	1.80 (1.67)	6.30	1638.30 (40.49)	23.82	79.00	218.10
August	4.20 (2.28)	5.88	1528.65 (39.11)	24.43	79.00	225.80
September	2.20 (1.79)	5.56	1406.10 (37.51)	23.48	77.00	151.40
October	1.00 (1.41)	4.08	1393.20 (37.34)	18.47	65.00	5.60
November**	0.80 (1.34)	5.18	1102.95 (33.23)	19.90	89.00	12.30
December**	0.40 (1.18)	5.43	1631.85 (40.41)	11.40	94.00	4.50
C.D $_{0.05}$	(0.67)	0.34	(4.98)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.53
Relative Humidity× Sacbrood incidence = -0.68
Rainfall × Sacbrood incidence = 0.22
Colony Strength × Sacbrood incidence = 0.09
Brood Area× Sacbrood incidence = 0.31

Maximum incidence was observed in the month of May (6.00%) when average colony strength and brood area were 5.85 bee frames and 2431.65 cm^2, respectively which was statistically at par with June (4.40%), August (4.20%) April (3.80%) and February (3.00%). During maximum incidence of sacbrood virus the temperature, relative humidity and rainfall were 22.61°C, 44 per cent and 21.30 mm, respectively. The temperature, relative humidity and rainfall during the whole disease incidence period varied from 11.15 to 25.74°C, 44 to 97 per cent and 4.5 to 225.80 per cent. Minimum incidence was recorded in the month of December (0.40%) when temperature, relative humidity and rainfall were 11.40°C, 94.00 per cent and 4.50 mm respectively, which was statistically at par with November (0.80%), October (1.00%), January (1.40%), July (1.80%) and September (2.20%). Sacbrood disease showed negative correlation with relative humidity (r= -0.68).

4.2.3.3 During 2020 (stationary conditions Nauni, Solan)

In the present study (Table 30) maximum incidence was observed in the month of May (6.20%) when temperature, rainfall and relative humidity were high i.e. 22.05°C , 53.00 mm and 74.80 per cent, respectively and average colony strength and brood area were 6.08 bee frames and 3412.05 cm^2, respectively which was statistically at par with June (5.80%) and April (3.20%).

Whereas, minimum incidence was recorded in the month of October (0.60%), when average bee strength and brood area was 3.47 bee frame and 1290.00 cm^2, respectively and temperature, rainfall and relative humidity were 20.40°C, 0.00 mm and 65.00 per cent, respectively which was statistically at par with January (1.00%), September (1.20%), February (1.80%), March (2.20%) and July (2.60%). There was no incidence of sacbrood was observed in the month of November and December. The correlation of the disease with colony and weather parameters in *A. mellifera* colonies indicated negative correlation (non-significant) with relative humidity (r=- 0.17).

Table 30. Incidence of sacbrood disease in *A. mellifera* colonies under stationary conditions at Nauni during January to December 2020

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	1.00 (1.41)*	2.01	606.30 (24.64)	9.10	68.00	168.30
February	1.80 (1.67)	2.36	1135.20 (33.71)	12.15	57.00	38.50
March	2.20 (1.79)	3.91	1922.10 (43.85)	14.20	62.00	171.80
April	3.20 (2.05)	4.84	2934.75 (54.18)	19.15	51.00	47.70
May	6.20 (2.68)	6.08	3412.05 (58.42)	22.05	53.00	74.80
June	5.80 (2.61)	4.06	1941.45 (44.07)	24.10	69.00	58.70
July	2.60 (1.90)	5.28	1870.50 (43.26)	25.05	81.00	278.10
August	2.80 (1.95)	4.27	1496.40 (38.70)	24.95	86.00	148.60
September	1.20 (1.48)	3.81	1341.60 (36.64)	24.10	77.00	6.00
October	0.60 (1.26)	3.47	1290.00 (35.93)	20.40	65.00	0.00
November	0.00 (1.00)	2.49	941.70 (30.70)	14.60	68.00	37.70
December	0.00 (1.00)	2.06	574.05 (23.98)	12.2	62.00	23.80
C.D $_{0.05}$	(0.65)	0.29	(7.42)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.54
Relative Humidity× Sacbrood incidence = -0.17
Rainfall × Sacbrood incidence = 0.17
Colony Strength × Sacbrood incidence = 0.75
Brood Area× Sacbrood incidence = 0.81

4.2.3.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Data in Table 31 revealed that maximum incidence of sacbrood disease was observed in the month of May (5.60%) when temperature, rainfall and relative humidity were high i.e. 22.05°C, 53.00 mm and 74.80 per cent, respectively and average colony strength and brood area were 6.25 bee frames and 2212.35 cm^2 which was statistically at par with June (5.00%), August (4.60%), April (3.40%), March (3.00%) and February (2.80%).Whereas, minimum

incidence was recorded in the month of December (0.60%), when average bee strength and brood area was 5.26 bee frame and 1593.15 $cm^2$and temperature, rainfall and relative humidity were 13.30℃, 0.00 mm and 93.00 per cent, respectively which was statistically at par with November (1.20%), July (1.40), January (1.60%), October (1.80%), September (2.40%) and February (2.80%). The data on correlation of sacbrood disease with colony and weather parameters in *A. mellifera* colonies showed negative correlation with relative humidity (r= -0.55).

Table 31. Incidence of sacbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January**, 2020	1.60 (1.61)*	6.38	2541.30 (50.42)	11.70	98.00	8.90
February**	2.80 (1.95)	7.62	3870.00 (62.22)	16.90	97.00	8.70
March	3.00 (2.00)	4.27	2412.30 (49.13)	14.20	62.00	171.80
April	3.40 (2.10)	2.96	2122.05 (46.08)	19.15	51.00	47.70
May	5.60 (2.57)	6.25	2212.35 (47.05)	22.05	53.00	74.80
June	5.00 (2.45)	5.50	1973.70 (44.44)	24.10	69.00	58.70
July	1.40 (1.55)	5.61	1767.30 (42.05)	25.05	81.00	278.10
August	4.60 (2.37)	5.44	1541.55 (39.28)	24.95	86.00	148.60
September	2.40 (1.84)	4.69	1399.65 (37.43)	24.10	77.00	6.00
October	1.80 (1.67)	3.23	1335.15 (36.55)	20.40	65.00	0.00
November**	1.20 (1.48)	4.33	980.40 (31.33)	18.00	89.00	19.90
December**	0.60 (1.26)	5.26	1593.15 (39.93)	13.30	93.00	0.00
C.D $_{0.05}$	(0.71)	0.31	(6.86)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.49
Relative Humidity× Sacbrood incidence = -0.55
Rainfall × Sacbrood incidence = 0.16
Colony Strength × Sacbrood incidence = 0.17
Brood Area× Sacbrood incidence = 0.22

4.2.3.5 Pooled data under stationary conditions Nauni, Solan (2019-2020)

Sacbrood disease in *A. mellifera* colonies is presented in Table 32 and Fig. 14. Pooled data on the incidence of sacbrood varied from 0.40 to 6.60 per cent. Maximum incidence was observed in the month of May (6.60%) when average colony strength and brood area were 6.01 bee frames and 3308.85 cm^2, respectively and the temperature, relative humidity and rainfall were 22.33°C, 48.50 per cent and 48.05mm, respectively which was statistically at par with June (5.30%) followed by April (3.30%) which was statistically at par with August (2.90%) and July (2.30%). The sacbrood incidence in the month of March was 1.90% which was statistically at par with February (1.50%). Whereas, minimum incidence was recorded in the month of October (0.40%) when average colony strength and brood area were 3.31 bee frames and 1257.75 cm^2, respectively and the temperature, relative humidity and rainfall were 19.44°C, 65.00 per cent and 2.80 mm, respectively which was statistically at par with January (0.70%) and September (0.90%). No incidence of Thai sacbrood disease was observed in the month of November and December. The pooled data on correlation of sacbrood with colony and weather parameters in *A. mellifera* colonies indicated negative correlation (non-significant) with relative humidity (r=- 0.37).

Studies on incidence of sacbrood disease in *A. mellifera* in stationary conditions found that colonies were affected with this disease from January to October and free from this disease during November and December 2019 and 2020. Disease firstly appeared in January with 0.70 per cent infection and maximum incidence was observed in May (6.60%) which was statistically at par with June. During the present studies when the disease incidence was maximum the brood area and colony strength was also maximum. These findings were supported by Aruna *et al*. (2016) who reported that prevalence of the disease during winter (October to January) season which prolonged to spring (Late January to March) season and was influenced by brood rearing. The occurrence of viral disease in *A. mellifera* in build- up period is due to the existence of greater proportions of susceptible young adults and larvae in colonies (Bailey, 1968). Our findings are in corroboration with Rana (2003) & Negi (2018) who reported maximum incidence of sac brood (5.20 %) in *A. mellifera* at Nauni during the month of May and June when the bee strength and brood area in the colonies were at peak.

Table 32. Pooled data on incidence of sacbrood disease in *A. mellifera* colonies under stationary conditions at Nauni during January, 2019 to December 2020

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.70 (1.30)*	2.23	661.13 (25.73)	8.98	63.50	120.65
February	1.50 (1.58)	2.73	1144.88 (33.85)	11.25	60.00	70.80
March	1.90 (1.70)	3.96	1873.73 (43.30)	13.83	58.00	113.20
April	3.30 (2.07)	4.82	2844.45 (53.34)	19.59	50.00	42.25
May	6.60 (2.76)	6.01	3308.85 (57.53)	22.33	48.50	48.05
June	5.30 (2.51)	4.18	1905.98 (43.67)	24.92	58.50	78.60
July	2.30 (1.82)	5.28	1754.40 (41.90)	24.44	80.00	248.10
August	2.90 (1.97)	4.37	1373.85 (37.08)	24.69	82.50	187.20
September	0.90 (1.38)	3.96	1291.19 (35.95)	23.79	77.00	78.70
October	0.40 (1.18)	3.31	1257.75 (35.48)	19.44	65.00	2.80
November	0.00 (1.00)	2.62	961.05 (31.02)	15.05	65.00	34.95
December	0.00 (1.00)	2.33	503.10 (22.45)	11.35	60.00	28.50
C.D $_{0.05}$	(0.29)	0.54	(2.70)			

*Figures in parentheses are square root (x+1) transformed values

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.57
Relative Humidity× Sacbrood incidence = -0.37
Rainfall × Sacbrood incidence = 0.12
Colony Strength × Sacbrood incidence = 0.79
Brood Area× Sacbrood incidence = 0.84

In present study maximum disease occurrence was found during the months from spring to summer, the occurrence of brood disease during spring to summer has also been reported by different workers (Hornitzky and Anderson, 2003; Rana and Rana, 2005 and Kshrisagar *et al*., 1982). Minimum incidence of sacbrood disease was observed in the month of October (0.40%) when the brood area and colony strength were low. Negi (2018) and

Rana (2003) has found minimum incidence of sacbrood disease in October and November in *A. mellifera* colonies in the same apiary at Nauni, Solan.

4.2.3.6 Pooled data under migratory [Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020]

Pooled data on incidence of sacbrood disease was observed in *A. mellifera* colonies under stationary (Nauni, Solan)) and migratory (Hisar, Haryana) conditions from January, 2019 to December 2020 is presented in Table 33 and Fig. 14. During the whole incidence period the temperature, relative humidity and rainfall varied from 11.43 to 24.92℃, 8 to 97.50 per cent and 2.25 to 248.10 mm. Pooled data on the incidence of sacbrood varied from 0.50 to 5.80 per cent. Maximum incidence was observed in the month of May (5.80%) when average colony strength and brood area were 6.05 bee frames and 2322.00 cm^2, respectively which was statistically at par with June (4.70%) followed by August (4.40%) which was statistically at par with April (3.60%). The sacbrood incidence in the month of February was 2.90 per cent which was at par with March (2.80%) and September (2.30%) followed by July (1.60%) which was statistically at par with January (1.50%) and October (1.40%). During migratory months, sacbrood incidence was observed but it was low as compared to stationary conditions. Whereas, minimum incidence was recorded in the month of December (0.50%) when average colony strength and brood area were 5.35 bee frames and 1612.50 cm^2, respectively which was statistically at par with November (1.00%). Pooled data on correlation of sacbrood with colony and weather parameters showed negative correlation with relative humidity (r= -0.27).

During the present investigation maximum incidence under stationary and migratory conditions were observed in the month of May (5.80%) when temperature was high and relative humidity and rainfall were moderate. However during migratory months January (1.50%), February (2.90%), November (1.00%) and December (0.50%) sacbrood incidence was observed than under stationary conditions. These investigations were supported by Negi (2018) and Rana (2003) who reported maximum incidence of sacbrood disease under stationary and migratory conditions in July and May when temperature, relative humidity and rainfall were high and brood area in the colonies were at peak. Minimum incidence was observed in the month of December (0.50%). These results are in close proximity with Negi (2018) who observed minimum incidence of sacbrood disease in the month of November which was at par with February, November and December.

Table 33. Pooled data on incidence of sacbrood disease in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	Sacbrood incidence (%)	Colony parameters		Weather parameters		
		Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January**	1.50 (1.58)*	6.49	2741.25 (52.37)	11.43	97.50	9.65
February**	2.90 (1.97)	7.33	4040.93 (63.58)	15.68	95.00	9.80
March	2.80 (1.95)	4.39	2560.65 (50.61)	13.83	58.00	113.20
April	3.60 (2.14)	3.17	2215.58 (47.08)	19.59	50.00	42.25
May	5.80 (2.61)	6.05	2322.00 (48.20)	22.33	48.50	48.05
June	4.70 (2.39)	5.75	2073.68 (45.55)	24.92	58.50	78.60
July	1.60 (1.61)	5.96	1702.80 (41.28)	24.44	8.00	248.10
August	4.40 (2.32)	5.66	1535.10 (39.19)	24.69	82.50	187.20
September	2.30 (1.82)	5.13	1402.88 (37.47)	23.79	77.00	78.70
October	1.40 (1.55)	3.66	1364.18 (36.95)	19.44	65.00	2.80
November**	1.00 (1.41)	4.76	1041.68 (32.29)	18.95	89.00	16.10
December**	0.50 (1.22)	5.35	1612.50 (40.17)	12.35	93.50	2.25
C.D $_{0.05}$	(0.23)	0.90	(4.00)			

*Figures in parentheses are square root (x+1) transformed values

**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

Temperature × Sacbrood incidence = 0.14
Relative Humidity× Sacbrood incidence = -0.27
Rainfall × Sacbrood incidence = 0.57
Colony Strength × Sacbrood incidence = 0.37
Brood Area× Sacbrood incidence = 0.12

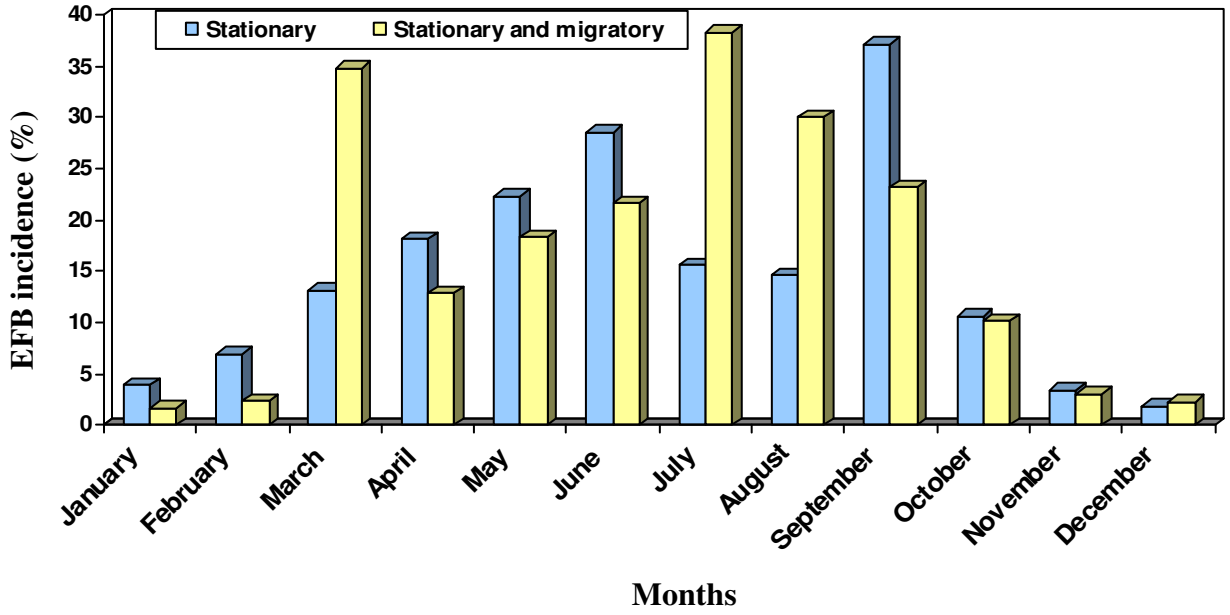


Fig 13. Incidence of EFB in *A. mellifera* under stationary and migratory conditions (2019-2020)

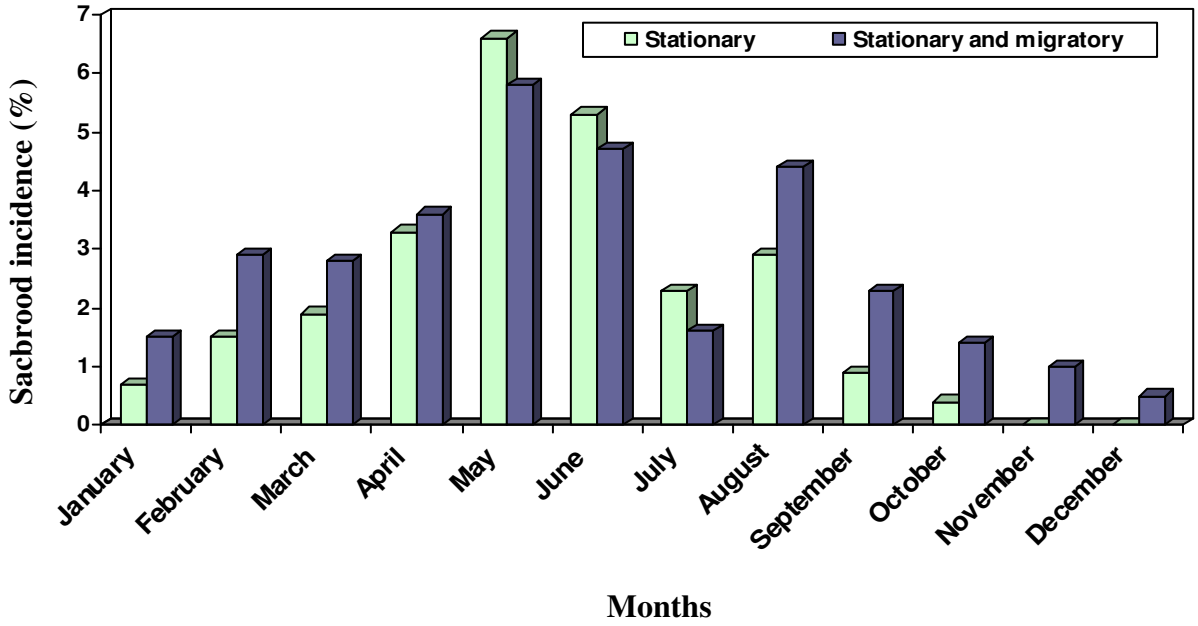


Fig 14. Incidence of sacbrood in *A. mellifera* under stationary and migratory conditions (2019-2020)

Brar *et al.* (2019) found *A. mellifera* colonies to be free from sac brood disease during July, 2015 to June, 2016 in the same apiary at Nauni, Solan. Rana (2003) reported minimum incidence of sac brood (0.08 %) in *A. mellifera* at Nauni during the month of November due to low temperature and less availability of brood. Naveen and Yadav (2021) found sac brood disease incidence in *A. mellifera* colonies with 5% and 2% brood infestation in the first and second fortnight of July, 2019. Brood was also found infested in the first and second fortnight of February 2020 with 5% and 7.30% brood infestation, respectively. These observations were not same as we observed this may be due to different environmental conditions in Rajsthan.

4.2.4 Incidence of *Tropilaelaps clareae*

Incidence of *T. clareae* was estimated by per 100 bees and visual examination of 100 brood cells for the presence of *T. clareae* mite (Plate 23).

4.2.4.1 During 2019 (stationary conditions Nauni, Solan)

The data on incidence of *T. clareae* in *A. mellifera* colonies under stationary conditions presented in Table 34 revealed that in per hundred bees method maximum mite infestation was found in the month of June (11.40 mites/colony) which was statistically at par with May (7.80 mites/ colony) followed by April (7.00 mites/ colony) which was statistically at par with March (6.60 mites/colony). Minimum mite infestation was found in the month of February (3.20 mites /colony) which was statistically at par with August (3.40 mites/colony). Data on incidence of *T. clareae* through visual examination of brood cells also showed that maximum incidence was recorded in the month of June (7.80 %) which was statistically at par with May (6.60 %) and April (4.80%). In both the methods of estimation of mite population, maximum infestation was observed in the month of June when average colony strength and brood area were 4.29 bee frames and 1870.50 cm^2, respectively and the temperature, relative humidity and rainfall were 25.74°C, 48.00 per cent and 98.50mm, respectively. Experimental colonies were found free from *T. clareae* in January, September, October, November and December. The data on correlation of incidence of *T. clareae* with colony and weather parameters showed significant positive correlation with colony strength {(r= 0.76 (per 100 bees), 0.75 (visual examination)} and negative correlation (non-significant) with relative humidity {(r= -0.50 (per 100 bees), (r=-0.42 (visual examination)}.

Table 34. Incidence of *T. clareae* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2019

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.00 (1.00)*	0.00 (1.00)	2.45	715.95 (26.78)	8.85	59.00	73.00
February	3.20 (2.05)	1.80 (1.67)	3.09	1154.55 (33.99)	10.34	63.00	103.10
March	6.60 (2.76)	2.20 (1.79)	4.03	1825.35 (42.74)	13.45	54.00	54.60
April	7.00 (2.83)	4.80 (2.41)	4.80	2754.15 (52.49)	20.03	49.00	36.80
May	7.80 (2.97)	6.60 (2.76)	5.94	3205.65 (56.63)	22.61	44.00	21.30
June	11.40 (3.52)	7.80 (2.97)	4.29	1870.50 (43.26)	25.74	48.00	98.50
July	4.00 (2.24)	3.20 (2.05)	5.29	1638.30 (40.49)	23.82	79.00	218.10
August	3.40 (2.10)	2.40 (1.84)	4.47	1251.30 (35.39)	24.43	79.00	225.80
September	0.00 (1.00)	0.00 (1.00)	4.10	1240.78 (35.24)	23.48	77.00	151.40
October	0.00 (1.00)	0.00 (1.00)	3.14	1225.50 (35.02)	18.47	65.00	5.60
November	0.00 (1.00)	0.00 (1.00)	2.75	980.40 (31.33)	15.50	62.00	32.20
December	0.00 (1.00)	0.00 (1.00)	2.60	432.15 (20.81)	10.49	58.00	33.20
$CD_{0.05}$	(0.63)	(0.59)	0.82	(4.21)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.42	0.50
Relative humidity × *T. clareae* incidence	=	-0.50	-0.42
Rainfall × *T. clareae* incidence	=	0.05	0.10
Colony strength × *T. clareae* incidence	=	0.76*	0.75*
Brood area × *T. clareae* incidence	=	0.05	0.50

(*Significant at 5%)

4.2.4.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

T. clareae incidence in *A. mellifera* colonies under stationary (Nauni, Solan) and migratory conditions (Hisar, Haryana) during January, 2019 to December, 2019 is presented

Plate 23. Per hundred bees method and ectoparasitic mites *(Tropilaelaps clareae*) and *(Varroa destructor)* on *A. mellifera* larva

in Table 35. Maximum mites (14.60 mites/ colony) were found in the month of June when average colony strength and brood area were 6.00 bee frames and 2173.65 cm^2, respectively and the temperature, relative humidity and rainfall were 25.74°C, 48.00 per cent and 98.50mm, respectively in per 100 bees method which was statistically at par with May (10.00 mites/ colony).

Table 35. Incidence of *T. clareae* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019**	0.00 (1.00)*	0.00 (1.00)	6.59	2941.20 (54.24)	11.15	97.00	10.40
February**	1.20 (1.48)	1.00 (1.41)	7.03	4211.85 (64.91)	14.45	93.00	10.90
March	2.00 (1.73)	1.60 (1.61)	4.51	2709.00 (52.06)	13.45	54.00	54.60
April	3.20 (2.05)	2.40 (1.84)	3.37	2309.10 (48.06)	20.03	49.00	36.80
May	10.00 (3.32)	4.60 (2.37)	5.85	2431.65 (49.32)	22.61	44.00	21.30
June	14.60 (3.95)	7.40 (2.90)	6.00	2173.65 (46.63)	25.74	48.00	98.50
July	2.60 (1.90)	3.80 (2.19)	6.30	1638.30 (40.49)	23.82	79.00	218.10
August	2.40 (1.84)	0.00 (1.00)	5.88	1528.65 (39.11)	24.43	79.00	225.80
September	0.00 (1.00)	0.00 (1.00)	5.56	1406.10 (37.51)	23.48	77.00	151.40
October	0.00 (1.00)	0.00 (1.00)	4.08	1393.20 (37.34)	18.47	65.00	5.60
November**	0.00 (1.00)	0.00 (1.00)	5.18	1102.95 (33.23)	19.90	89.00	12.30
December**	0.00 (1.00)	0.00 (1.00)	5.43	1631.85 (40.41)	11.40	94.00	4.50
$CD_{0.05}$	(0.71)	(0.67)	0.34	(4.98)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.54	0.54
Relative humidity× *T. clareae* incidence	=	-0.69	-0.70
Rainfall× *T. clareae* incidence	=	0.11	0.19
Colony strength× *T. clareae* incidence	=	0.14	0.07
Brood area× *T. clareae* incidence	=	0.12	0.06

(*Significant at 5%)

Minimum infestation was found in the month of February (1.20 mites/colony) which was statistically at par with March (2.00 mites/colony), August (2.40 mites/colony), July (2.60 mites/colony) and April (3.20 mites/ colony). Brood infestation in migratory colonies was maximum in the month of June (7.40 %) which was statistically at par with May (4.60%) and July (3.80%). Minimum mite infestation was found in the month of February (1.00%) which was statistically at par with March (1.60%) and April (2.40%). The incidence of *T. clareae* mite showed negative significant correlation with relative humidity {(r= -0.69) (per 100 bees), (r=-0.70) (visual examination)}.

4.2.4.3 During 2020 (stationary conditions Nauni, Solan)

T. clareae infestation in *A. mellifera* colonies under stationary conditions in Table 36 showed that significantly maximum population of *T. clareae* was recorded in the month of June (9.40mites/colony) followed by May (6.40 mites/colony) which was statistically at par with April (5.80 mites/colony) observed in per 100 bees method. The mite infestation in March was 3.20 mites/colony which was at par with July (3.00 mites/colony). Significantly minimum (2.40 mites/colony) population of *T. clareae* was observed in *A. mellifera* colonies in the month of February. Further data revealed that through visual examination of brood cells brood infestation was significantly maximum in the month of June (6.80 %) followed by May (3.60 %) which was statistically at par with April (3.40%) and July (3.20%). Minimum infestation was found in the month of March (1.60%) which was statistically at par with February (2.00%). In both the methods of estimation of mite population, maximum population of mites was observed in the month of June when average colony strength and brood area were 4.06 bee frames and 1941.45 cm^2, respectively and the temperature, relative humidity and rainfall were 24.10°C, 69.00 per cent and 58.70 mm, respectively. Experimental colonies were found free from *T. clareae* in January, September, October, November and December in both the methods of estimation of mites. Incidence of *T. clareae* with colony and weather parameters showed significant positive correlation with brood area {(r= 0.74 (per 100 bees) (r= 0.64 (visual examination) and negative correlation with relative humidity {(r= -0.39 (per 100 bees), -0.25 (visual examination)}.

Table 36. Incidence of *T. clareae* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2020

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	0.00 (1.00)*	0.00 (1.00)	2.01	606.30 (24.64)	9.10	68.00	168.30
February	2.40 (1.84)	2.00 (1.73)	2.36	1135.20 (33.71)	12.15	57.00	38.50
March	3.20 (2.05)	1.60 (1.61)	3.91	1922.10 (43.85)	14.20	62.00	171.80
April	5.80 (2.61)	3.40 (2.10)	4.84	2934.75 (54.18)	19.15	51.00	47.70
May	6.40 (2.72)	3.60 (2.14)	6.08	3412.05 (58.42)	22.05	53.00	74.80
June	9.40 (3.22)	6.80 (2.79)	4.06	1941.45 (44.07)	24.10	69.00	58.70
July	3.00 (2.00)	3.20 (2.05)	5.28	1870.50 (43.26)	25.05	81.00	278.10
August	0.00 (1.00)	0.00 (1.00)	4.27	1496.40 (38.70)	24.95	86.00	148.60
September	0.00 (1.00)	0.00 (1.00)	3.81	1341.60 (36.64)	24.10	77.00	6.00
October	0.00 (1.00)	0.00 (1.00)	3.47	1290.00 (35.93)	20.40	65.00	0.00
November	0.00 (1.00)	0.00 (1.00)	2.49	941.70 (30.70)	14.60	68.00	37.70
December	0.00 (1.00)	0.00 (1.00)	2.06	574.05 (23.98)	12.2	62.00	23.80
$CD_{0.05}$	(0.40)	(0.61)	0.29	(7.42)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.34	0.39
Relative humidity× *T. clareae* incidence	=	-0.39	-0.25
Rainfall× *T. clareae* incidence	=	0.04	0.13
Colony strength× *T. clareae* incidence	=	0.58	0.54
Brood area× *T. clareae* incidence	=	0.74*	0.64*

(*Significant at 5%)

4.2.4.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

The data recorded on mite infestation by per 100 bees method and brood infestation method is presented in Table 37 in *A. mellifera* colonies showed that in per hundred bees method significantly maximum *T. clareae* mite infestation was observed in the months of June (12.80 mites/ colony) when the average colony strength and brood area were 5.50 bee frames and 1973.70 cm^2, respectively and the temperature, relative humidity and rainfall

were 24.10°C, 53.00 per cent and 74.80 mm, respectively followed by May (6.00 mites/ colony) was statistically at par with April (4.60 mites/colony). Minimum mite incidence was recorded in February (0.60 mites/colony) which was statistically at par with March (1.40 mites/colony) and July (1.60 mites/colony).

Table 37. Incidence of *T. clareae* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020**	0.00 (1.00)*	0.00 (1.00)	6.38	2541.30 (50.42)	11.70	98.00	8.90
February**	0.60 (1.26)	1.40 (1.55)	7.62	3870.00 (62.22)	16.90	97.00	8.70
March	1.40 (1.55)	3.00 (2.00)	4.27	2412.30 (49.13)	14.20	62.00	171.80
April	4.60 (2.37)	4.20 (2.28)	2.96	2122.05 (46.08)	19.15	51.00	47.70
May	6.00 (2.65)	6.60 (2.76)	6.25	2212.35 (47.05)	22.05	53.00	74.80
June	12.80 (3.71)	9.20 (3.19)	5.50	1973.70 (44.44)	24.10	69.00	58.70
July	1.60 (1.61)	2.60 (1.90)	5.61	1767.30 (42.05)	25.05	81.00	278.10
August	0.00 (1.00)	1.80 (1.67)	5.44	1541.55 (39.28)	24.95	86.00	148.60
September	0.00 (1.00)	0.00 (1.00)	4.69	1399.65 (37.43)	24.10	77.00	6.00
October	0.00 (1.00)	0.00 (1.00)	3.23	1335.15 (36.55)	20.40	65.00	0.00
November**	0.00 (1.00)	0.00 (1.00)	4.33	980.40 (31.33)	18.00	89.00	19.90
December**	0.00 (1.00)	0.00 (1.00)	5.26	1593.15 (39.93)	13.30	93.00	0.00
$CD_{0.05}$	(0.75)	(0.53)	0.31	(6.86)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.36	0.40
Relative humidity× *T. clareae* incidence	=	-0.51*	-0.59*
Rainfall× *T. clareae* incidence	=	0.07	0.27
Colony strength× *T. clareae* incidence	=	0.04	0.10
Brood area× *T. clareae* incidence	=	0.10	0.19

(*Significant at 5%)

In the remaining months of present study (January, February and August to December) no mite population was detected on adult bees. *T. clareae* infestation estimated by examining visually also showed the similar trend of mite infestation. Maximum infestation in brood was observed in June (9.20%) which was statistically at par with May (6.60%). Least mite population was noticed in February (1.40%) which was statistically at par with August (1.80%), July (2.60%) and March (3.00%). During maximum infestation of mite the average colony strength and brood area were 5.50 bee frames and 1973.70 cm^2, respectively and the temperature, relative humidity and rainfall were 24.10°C, 69.00 per cent and 58.70 mm, respectively. The data on correlation of incidence of *T. clareae* with colony and weather parameters demonstrated negative correlation (significant) with relative humidity (r= -0.51 (per 100 bees), -0.59 (visual examination)}.

4.2.4.5 Pooled data under stationary conditions Nauni, Solan (2019-2020)

Pooled data on incidence of *T. clareae* in *A. mellifera* colonies under stationary conditions presented in Table 38 and Fig. 15 revealed that in per hundred bees method maximum population of *T. clareae* was recorded in the month of June (10.40mites/colony) when the average colony strength and brood area were 4.18 bee frames and 1905.98 cm^2 , respectively and the temperature, relative humidity and rainfall were 24.92°C, 58.50 per cent and 78.60 mm, respectively which was statistically at par with May (7.10 mites/colony) followed by April (6.40 mites/colony) which was statistically at par with March (4.90 mites/colony). Minimum 1.70 mites/colony was observed in *A. mellifera* colonies in the month of August which was statistically at par with February (2.80 mites / colony) and July (3.50 mites / colony).

Brood infestation was maximum in the month of June (7.30 %) which was statistically at par with May (5.10 %) followed by April (4.10%) which was statistically at par with July (3.20%). Significantly minimum mite infestation was found in the month of August (1.20%) which was statistically at par with February (1.80 %) and March (1.90 %). In both the methods of estimation of mite population, maximum population was observed in the month of June. Experimental colonies were found free from *T. clareae* in January, September, October, November and December. Pooled data on correlation of incidence of *T. clareae* with colony and weather parameters showed highly significant positive correlation with brood area {(r= 0.76 (per 100 bees))} and r= 0.75 (visual examination)}, colony strength {(r= 0.63 (per 100 bees) and r= 0.67 (visual examination)} and negative with relative humidity {(r= -0.50 (per 100 bees), -0.42 (visual examination)}.

Table 38. Pooled data on incidence of *T. clareae* in *A. mellifera* colonies under stationary conditions at Nauni during January, 2019 to December, 2020

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.00 (1.00)*	0.00 (1.00)	2.23	661.13 (25.73)	8.98	63.50	120.65
February	2.80 (1.95)	1.80 (1.67)	2.73	1144.88 (33.85)	11.25	60.00	70.80
March	4.90 (2.43)	1.90 (1.70)	3.96	1873.73 (43.30)	13.83	58.00	113.20
April	6.40 (2.72)	4.10 (2.26)	4.82	2844.45 (53.34)	19.59	50.00	42.25
May	7.10 (2.85)	5.10 (2.47)	6.01	3308.85 (57.53)	22.33	48.50	48.05
June	10.40 (3.38)	7.30 (2.88)	4.18	1905.98 (43.67)	24.92	58.50	78.60
July	3.50 (2.12)	3.20 (2.05)	5.28	1754.40 (41.90)	24.44	80.00	248.10
August	1.70 (1.64)	1.20 (1.48)	4.37	1373.85 (37.08)	24.69	82.50	187.20
September	0.00 (1.00)	0.00 (1.00)	3.96	1291.19 (35.95)	23.79	77.00	78.70
October	0.00 (1.00)	0.00 (1.00)	3.31	1257.75 (35.48)	19.44	65.00	2.80
November	0.00 (1.00)	0.00 (1.00)	2.62	961.05 (31.02)	15.05	65.00	34.95
December	0.00 (1.00)	0.00 (1.00)	2.33	503.10 (22.45)	11.35	60.00	28.50
$CD_{0.05}$	(0.58)	(0.53)	0.54	(2.70)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.42	0.51
Relative humidity× *T. clareae* incidence	=	-0.50	-0.42
Rainfall× *T. clareae* incidence	=	0.05	0.10
Colony strength× *T. clareae* incidence	=	0.63*	0.67*
Brood area× *T. clareae* incidence	=	0.76*	0.75*

(*Significant at 5%)

Seasonal variations of ectoparasitic mite, *T. clareae* showed that in per hundred bees method and per cent brood infestation method, maximum population of *T. clareae* was recorded in the month of June (10.40 mites/ colony and 7.30%, respectively) when the

temperature was high and relative humidity was low (Table 38). These observations coincides with increased brood rearing activity in *A. mellifera* colonies. These findings were comparable with earlier work of Chahal *et al.* (1986) who observed two peak periods of *T. clareae* infestation in *A. mellifera* colonies i.e. February to May (33.7-51.7%) and September to November (26.8-42.0%), coincided with peak of brood rearing activity in Ludhiana, Punjab. Our findings are also in line with those of Brar *et al.* (2019) who reported maximum incidence of *T. clareae* in the month of June and May, respectively when the temperature was high and relative humidity was low. The incidence of *T. clareae* in *A. mellifera* have also been reported to vary greatly from year to year and during different months. The reason for the variations of mite infestation in *A. mellifera* colonies during different months of the year may be attributed to fluctuation of the temperature, rainfall and relative humidity (Camphor and Martin, 2009). In Palampur district of Himachal Pradesh *T. clareae* infestation was observed in the months of September (28.80±9.21) and October (25.70±8.52) than other months of the year (Mattu and Sharma, 2016) may be due to different environmental conditions prevailing in that area.

4.2.4.6 Pooled data under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020)

Pooled data on *T. clareae* incidence in *A. mellifera* colonies under stationary (Nauni, Solan) and migratory (Hisar, Haryana) conditions during January, 2019 to December, 2020 is presented in Table 39 and Fig. 15. In per hundred bees method the incidence of *T. clareae* mite was significantly maximum in the month of June (13.70 mites/colony) when the average colony strength and brood area were 4.29 bee frames and 1870.50 cm^2 and the temperature, relative humidity and rainfall were 25.74°C, 48.00 per cent and 98.50 mm, respectively followed by May (8.00 mites/colony) and April (3.90 mites/colony) which was statistically at par with July (2.10 mites/colony). Significantly minimum mite infestation was found in August (1.20 mites/colony).

Per cent brood infestation was also maximum in the month of June (8.30%) which was statistically at par with May (5.60%) followed by April (3.30%) which was statistically at par with July (3.20%). Minimum incidence was observed in the month of August (0.90%) which was statistically at par with February (1.20%).

Table 39. Pooled data on incidence of *T. clareae* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	Incidence of *Tropilaelaps clareae*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January**	0.00 (1.00)*	0.00 (1.00)	6.49	2741.25 (52.37)	11.43	97.50	9.65
February**	0.90 (1.00)	1.20 (1.48)	7.33	4040.93 (63.58)	15.68	95.00	9.80
March	1.70 (1.64)	2.30 (1.82)	4.39	2560.65 (50.61)	13.83	58.00	113.20
April	3.90 (2.21)	3.30 (2.07)	3.17	2215.58 (47.08)	19.59	50.00	42.25
May	8.00 (3.00)	5.60 (2.57)	6.05	2322.00 (48.20)	22.33	48.50	48.05
June	13.70 (3.83)	8.30 (3.05)	5.75	2073.68 (45.55)	24.92	58.50	78.60
July	2.10 (1.76)	3.20 (2.05)	5.96	1702.80 (41.28)	24.44	8.00	248.10
August	1.20 (1.48)	0.90 (1.38)	5.66	1535.10 (39.19)	24.69	82.50	187.20
September	0.00 (1.00)	0.00 (1.00)	5.13	1402.88 (37.47)	23.79	77.00	78.70
October	0.00 (1.00)	0.00 (1.00)	3.66	1364.18 (36.95)	19.44	65.00	2.80
November**	0.00 (1.00)	0.00 (1.00)	4.76	1041.68 (32.29)	18.95	89.00	16.10
December**	0.00 (1.00)	0.00 (1.00)	5.35	1612.50 (40.17)	12.35	93.50	2.25
$CD_{0.05}$	(0.56)	(0.54)	0.90	(4.00)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *T. clareae* incidence	=	0.47	0.46
Relative humidity× *T. clareae* incidence	=	-0.39	-0.56
Rainfall× *T. clareae* incidence	=	0.10	0.24
Colony strength× *T. clareae* incidence	=	0.10	0.11
Brood area× *T. clareae* incidence	=	0.12	0.18

(*Significant at 5%)

In June when mite population was maximum the average colony strength and brood area were 5.75 bee frames and 2073.68 cm^2, respectively and the temperature, relative

humidity and rainfall were 24.92°C, 58.50 per cent and 78.60 mm, respectively. Experimental colonies were found free from *T. clareae* in January, September, October, November and December. Pooled data on correlation of incidence of *T. clareae* with colony and weather parameters showed negative correlation with relative humidity (r= -0.39 (per 100 bees), -0.56 (visual examination)} though non- significant.

Under migratory conditions, in per hundred bees method and per cent brood infestation method maximum infestation was found in the month of June thereafter incidence decreased. Experimental colonies were found free from *T. clareae* in January, September, October, November and December while, minimum infestation was found in the month of February in both the methods of estimation. The present studies are in close proximity with the finding of Brar *et al.* (2019) who also reported maximum *T. clarae* mite infestation in the month of June in per hundred method (11.00 mites /colony) and brood examination method (5.00%) while minimum infestation was in the month of April (2.00 mites/ colony). The mite incidence was found maximum when the brood rearing was high. Similar observations were reported earlier by Singh *et al*. (2011), Gatoria *et al.* (1995) and Padhi and Rath (2012). Thakur *et al*. (2009) observed two peaks in mite population in May-June and September-October. The difference in peak population from the present findings could be due to the different climatic conditions in study locations which affected the brood rearing in *A. mellifera* in H.P. In the present finding *T. clareae* mite was absent during migratory period from November, December and January and minimum incidence in the month of February could be attributed to the prevailing high humidity and low temperature conditions at Hisar. These studies are in conformity to the observations of earlier workers (Aggarwal and Kapil, 2013) who have reported no mite population during winter. The present studies are also supported by findings of Chahal *et al.* (1986), who have found positive correlation of *T. clareae* incidence with temperature and rainfall.

4.2.5 Incidence of *Varroa destructor*

Incidence of *V. destructor* was estimated by two different methods viz. per 100 bees and visual examination of 100 brood cells (Plate 23).

4.2.5.1 During 2019 (stationary conditions Nauni, Solan)

Incidence of *V. destructor* in *A. mellifera* colonies under stationary conditions during January, 2019 to December, 2019 is presented in Table 40.

Table 40. Incidence of *V. destructor* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2019

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation[#] (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.00 (1.00)*	0.00 (1.00)	2.45	715.95 (26.78)	8.85	59.00	73.00
February	3.80 (2.19)	1.20 (1.48)	3.09	1154.55 (33.99)	10.34	63.00	103.10
March	8.00 (3.00)	4.80 (2.41)	4.03	1825.35 (42.74)	13.45	54.00	54.60
April	9.40 (3.22)	5.00 (2.45)	4.80	2754.15 (52.49)	20.03	49.00	36.80
May	11.20 (3.49)	7.20 (2.86)	5.94	3205.65 (56.63)	22.61	44.00	21.30
June	13.00 (3.74)	9.00 (3.16)	4.29	1870.50 (43.26)	25.74	48.00	98.50
July	5.20 (2.49)	2.80 (1.95)	5.29	1638.30 (40.49)	23.82	79.00	218.10
August	0.00 (1.00)	0.00 (1.00)	4.47	1251.30 (35.39)	24.43	79.00	225.80
September	0.00 (1.00)	0.00 (1.00)	4.10	1240.78 (35.24)	23.48	77.00	151.40
October	0.00 (1.00)	0.00 (1.00)	3.14	1225.50 (35.02)	18.47	65.00	5.60
November	0.00 (1.00)	0.00 (1.00)	2.75	980.40 (31.33)	15.50	62.00	32.20
December	0.00 (1.00)	0.00 (1.00)	2.60	432.15 (20.81)	10.49	58.00	33.20
$CD_{0.05}$	(0.58)	(0.47)	0.82	(4.21)	8.85	59.00	73.00

*Figures in parentheses are square root (x+1) transformed values, [#]by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.38	0.43
Relative humidity× *V. destructor* incidence	=	0.67*	0.68*
Rainfall× *V. destructor* incidence	=	-0.14	-0.15
Colony strength× *V. destructor* incidence	=	0.66*	0.64*
Brood area× *V. destructor* incidence	=	0.82*	0.79*

(*Significant at 5%)

The data on mite population recorded by per 100 bees method revealed that *A. mellifera* colonies showed maximum infestation in June (13.00 mites/ colony) which was statistically at par with May (11.20 mites/colony) and April (9.40 mites/colony). Minimum mite population was recorded in the month of February (3.8 mites/ colony) which was statistically at par with mite infestation in July (5.20 mites/ colony). In remaining months of study (January and August to December, 2019), no mite incidence was observed on adult bees. *Varroa* infestation estimated by examining visually in brood cells showed similar trend of infestation. Significantly maximum infestation in brood was observed in June (9.00%) which was statistically at par with May (7.20%) followed by April (5.00%) which was at par with March (4.80%). Minimum infestation was found in the month of February (1.20 %) which was statistically at par with July (2.80 %). Maximum infestation was observed in the month of June when average colony strength and brood area were 4.29 bee frames and 1870.50 cm^2, respectively and the temperature, relative humidity and rainfall were 25.74°C, 48.00 per cent and 98.50mm, respectively. Experimental colonies were found free from *V. destructor* in January, August, September, October, November and December. Data on correlation of incidence of *V. destructor* with colony and weather parameters showed positive significant correlation with colony strength {(r= 0.66 (per 100 bees), 0.64 (visual examination)} and brood area {(r= 0.82 (per 100 bees), 0.79 (visual examination)}while, negative correlation with relative humidity (significant) and rainfall (non- significant) {(r= -0.67,-0.14 (per 100 bees) and -0.68, -0.15 (visual examination)}.

4.2.5.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

V. destructor incidence in *A. mellifera* colonies under stationary (Nauni, Solan) and migratory (Hisar, Haryana) conditions during January, 2019 to December, 2019 is presented in Table 41. The incidence of *Varroa* mite was detected in the month of March (1.20 mites/ colony) and was found significantly maximum (18.00mites/ colony) in the month of June in per 100 bees method when average colony strength and brood area were 6.00 bee frames and 2173.65 cm^2 , respectively and the temperature, relative humidity and rainfall were 25.74°C, 48.00 per cent and 98.50mm, respectively followed by May (9.60 mites/ colony) and July (4.40 mites/colony) which was statistically at par with April (4.20 mites/colony).

Table 41. Incidence of *V. destructor* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019**	0.00 (1.00)*	0.00 (1.00)	6.59	2941.20 (54.24)	11.15	97.00	10.40
February**	0.00 (1.00)	0.00 (1.00)	7.03	4211.85 (64.91)	14.45	93.00	10.90
March	1.20 (1.48)	1.80 (1.67)	4.51	2709.00 (52.06)	13.45	54.00	54.60
April	4.20 (2.28)	5.20 (2.49)	3.37	2309.10 (48.06)	20.03	49.00	36.80
May	9.60 (3.26)	7.80 (2.97)	5.85	2431.65 (49.32)	22.61	44.00	21.30
June	18.00 (4.35)	9.00 (3.16)	6.00	2173.65 (46.63)	25.74	48.00	98.50
July	4.40 (2.32)	3.20 (2.05)	6.30	1638.30 (40.49)	23.82	79.00	218.10
August	1.60 (1.61)	0.00 (1.00)	5.88	1528.65 (39.11)	24.43	79.00	225.80
September	0.00 (1.00)	0.00 (1.00)	5.56	1406.10 (37.51)	23.48	77.00	151.40
October	0.00 (1.00)	0.00 (1.00)	4.08	1393.20 (37.34)	18.47	65.00	5.60
November**	0.00 (1.00)	0.00 (1.00)	5.18	1102.95 (33.23)	19.90	89.00	12.30
December**	0.00 (1.00)	0.00 (1.00)	5.43	1631.85 (40.41)	11.40	94.00	4.50
$CD_{0.05}$	(0.69)	(0.44)	0.34	(4.98)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.57*	0.51*
Relative humidity× *V. destructor* incidence	=	-0.68*	-0.80*
Rainfall× *V. destructor* incidence	=	-0.15	-0.04
Colony strength× *V. destructor* incidence	=	0.10	0.07
Brood area× *V. destructor* incidence	=	0.04	0.10

(*Significant at 5%)

Minimum infestation was found in the month of March (1.20 mites/colony) which was statistically at par with August (1.60 mites/colony). Brood infestation in migratory colonies was noticed maximum in the month of June (9.00 mites/ colony) which was statistically at par with May (7.80 mites/ colony). Minimum *Varroa* mite incidence was observed in the month of March (1.80 %) which was statistically at par with July (3.20%). *V. destructor* correlation with colony and weather parameters showed negative correlation with relative humidity (significant) and rainfall (non-significant) (r= -0.68,-0.15 (per 100 bees) and -0.80, -0.04 (visual examination)} while, positive significant correlation with temperature, {(r= 0.57 (per 100 bees), 0.51 (visual examination, respectively)}.

4.2.5.3 During 2020 (stationary conditions Nauni, Solan)

Data recorded on incidence of *V. destructor* is presented in Table 42. *A. mellifera* colonies showed maximum *Varroa* mites in the month of June (16.80 mites/ colony) when average colony strength and brood area were 4.06 bee frames and 1941.45 cm^2, respectively and the temperature, relative humidity and rainfall were 24.10°C, 69.00 per cent and 58.70 mm, respectively which was statistically at par with May (13.60 mites/ colony) followed by April (8.20 mites/colony) which was further at par with March (7.60 mites/colony). Minimum mite incidence was recorded in February (2.80 mites/ colony) which was statistically at par with July (3.40 mites/colony). *Varroa* infestation estimated by examining visually in brood cells showed maximum infestation in brood in June (11.40%) which was statistically at par with May (8.60%) which was further at par with April (6.40%). The mite incidence in the month of March was 2.20% which was at par with July (1.80 %). Significantly minimum mite population was noticed in February with 0.60 per cent infestation, in remaining months of study no mite population was detected on adult bees. Correlation of incidence of *V. destructor* with colony and weather parameters demonstrated positive significant correlation with colony strength {(r= 0.59 (per 100 bees), 0.60 (visual examination)}, brood area {(r= 0.75 (per 100 bees), 0.76 (visual examination)} and negative correlation with relative humidity and rainfall (r= -0.41, -0.02 (per 100 bees) and -0.38, -0.06 (visual examination)} though non-significant.

Table 42. Incidence of *V. destructor* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2020

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	0.00 (1.00)*	0.00 (1.00)	2.01	606.30 (24.64)	9.10	68.00	168.30
February	2.80 (1.95)	0.60 (1.26)	2.36	1135.20 (33.71)	12.15	57.00	38.50
March	7.60 (2.93)	2.20 (1.79)	3.91	1922.10 (43.85)	14.20	62.00	171.80
April	8.20 (3.03)	6.40 (2.72)	4.84	2934.75 (54.18)	19.15	51.00	47.70
May	13.60 (3.82)	8.60 (3.10)	6.08	3412.05 (58.42)	22.05	53.00	74.80
June	16.80 (4.22)	11.40 (3.52)	4.06	1941.45 (44.07)	24.10	69.00	58.70
July	3.40 (2.10)	1.80 (1.67)	5.28	1870.50 (43.26)	25.05	81.00	278.10
August	0.00 (1.00)	0.00 (1.00)	4.27	1496.40 (38.70)	24.95	86.00	148.60
September	0.00 (1.00)	0.00 (1.00)	3.81	1341.60 (36.64)	24.10	77.00	6.00
October	0.00 (1.00)	0.00 (1.00)	3.47	1290.00 (35.93)	20.40	65.00	0.00
November	0.00 (1.00)	0.00 (1.00)	2.49	941.70 (30.70)	14.60	68.00	37.70
December	0.00 (1.00)	0.00 (1.00)	2.06	574.05 (23.98)	12.2	62.00	23.80
$CD_{0.05}$	(0.62)	(0.59)	0.29	(7.42)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.32	0.38
Relative humidity× *V. destructor* incidence	=	-0.41	-0.38
Rainfall× *V. destructor* incidence	=	-0.02	-0.06
Colony strength× *V. destructor* incidence	=	0.59*	0.60*
Brood area× *V. destructor* incidence	=	0.75*	0.76*

(*Significant at 5%)

4.2.5.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

The data recorded on mite incidence under stationary (Nauni, Solan) and migratory (Hisar, Haryana) conditions is presented in Table 43 revealed that *A. mellifera* colonies showed

Table 43. Incidence of *V. destructor* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020**	0.00 (1.00)*	0.00 (1.00)	6.38	2541.30 (50.42)	11.70	98.00	8.90
February**	0.00 (1.00)	0.00 (1.00)	7.62	3870.00 (62.22)	16.90	97.00	8.70
March	2.20 (1.79)	0.80 (1.34)	4.27	2412.30 (49.13)	14.20	62.00	171.80
April	5.80 (2.61)	4.40 (2.32)	2.96	2122.05 (46.08)	19.15	51.00	47.70
May	8.40 (3.07)	8.20 (3.03)	6.25	2212.35 (47.05)	22.05	53.00	74.80
June	14.60 (3.95)	11.40 (3.52)	5.50	1973.70 (44.44)	24.10	69.00	58.70
July	5.20 (2.49)	1.40 (1.55)	5.61	1767.30 (42.05)	25.05	81.00	278.10
August	0.00 (1.00)	0.00 (1.00)	5.44	1541.55 (39.28)	24.95	86.00	148.60
September	0.00 (1.00)	0.00 (1.00)	4.69	1399.65 (37.43)	24.10	77.00	6.00
October	0.00 (1.00)	0.00 (1.00)	3.23	1335.15 (36.55)	20.40	65.00	0.00
November**	0.00 (1.00)	0.00 (1.00)	4.33	980.40 (31.33)	18.00	89.00	19.90
December**	0.00 (1.00)	0.00 (1.00)	5.26	1593.15 (39.93)	13.30	93.00	0.00
$CD_{0.05}$	(0.43)	(0.35)	0.31	(6.86)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.43	0.37
Relative humidity× *V. destructor* incidence	=	-0.56	-0.57
Rainfall× *V. destructor* incidence	=	0.24	0.06
Colony strength× *V. destructor* incidence	=	0.04	0.07
Brood area× *V. destructor* incidence	=	0.07	0.08

(*Significant at 5%)

significantly maximum *Varroa* mite infestation in the months of June (14.60 mites/ colony) when average colony strength and brood area were 5.50 bee frames and 1973.70 cm^2, respectively and the temperature, relative humidity and rainfall were 24.10°C, 69.00 per cent and 58.70 mm, respectively followed by May (8.40 mites/ colony), April (5.80 mites/colony) which was statistically at par with July (5.20) in per 100 bees method. Significantly minimum mite incidence was recorded in March (2.20 mites/ colony). In remaining months of study (January, February, August, September, October, November and December) no mite population was detected. *Varroa* infestation by visual examination showed that significantly maximum infestation in brood was observed in June (11.40%) followed by May (8.20%) and April (4.40%). Minimum mite population was recorded in March (0.80 %) which was statistically at par with July (1.40 %). The data on correlation of incidence of *V. destructor* with colony and weather parameters showed negative non-significant correlation with relative humidity (r= -0.56 (per 100 bees) and -0.57 (visual examination)}.

4.2.5.5 Pooled data under stationary conditions Nauni, Solan (2019-2020)

Incidence of *V. destructor* in *A. mellifera* colonies under stationary conditions during January, 2019 to December, 2020 is presented in Table 44 and Fig. 16. The pooled data on mite population observed by per hundred bees method revealed that in both the methods of estimation of mite population (per 100 bees method and visual examination), maximum infestation was observed in the month of June when average colony strength and brood area were 4.18 bee frames and 1905.98 cm^2, respectively and the temperature, relative humidity and rainfall were 24.92°C, 58.50 per cent and 78.60 mm, respectively. In remaining months of study (January, August, September, October, November and December) no mite population was detected.

In per 100 bees method *A. mellifera* colonies showed maximum *Varroa* mite infestation in June (14.90 mites/ colony) which was statistically at par with May (12.40 mites/colony) followed by April (8.80 mites/colony) which was statistically at par with July (4.30 mites/colony) and March (7.80 mites/colony). Significantly minimum number of *Varroa* mites were observed in the month of February (3.30 mites/colony).

Table 44. Pooled data on incidence of *V. destructor* in *A. mellifera* colonies under stationary (Nauni, Solan) conditions at Nauni during January, 2019 to December, 2020

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.00 (1.00)*	0.00 (1.00)	2.23	661.13 (25.73)	8.98	63.50	120.65
February	3.30 (2.07)	0.90 (1.38)	2.73	1144.88 (33.85)	11.25	60.00	70.80
March	7.80 (2.97)	3.50 (2.12)	3.96	1873.73 (43.30)	13.83	58.00	113.20
April	8.80 (3.13)	5.70 (2.59)	4.82	2844.45 (53.34)	19.59	50.00	42.25
May	12.40 (3.66)	7.90 (2.98)	6.01	3308.85 (57.53)	22.33	48.50	48.05
June	14.90 (3.99)	10.20 (3.35)	4.18	1905.98 (43.67)	24.92	58.50	78.60
July	4.30 (2.30)	2.30 (1.82)	5.28	1754.40 (41.90)	24.44	80.00	248.10
August	0.00 (1.00)	0.00 (1.00)	4.37	1373.85 (37.08)	24.69	82.50	187.20
September	0.00 (1.00)	0.00 (1.00)	3.96	1291.19 (35.95)	23.79	77.00	78.70
October	0.00 (1.00)	0.00 (1.00)	3.31	1257.75 (35.48)	19.44	65.00	2.80
November	0.00 (1.00)	0.00 (1.00)	2.62	961.05 (31.02)	15.05	65.00	34.95
December	0.00 (1.00)	0.00 (1.00)	2.33	503.10 (22.45)	11.35	60.00	28.50
$CD_{0.05}$	(0.44)	(0.50)	0.54	(2.70)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.36	0.42
Relative humidity× *V. destructor* incidence	=	-0.59*	-0.57*
Rainfall× *V. destructor* incidence	=	-0.04	-0.08
Colony strength× *V. destructor* incidence	=	0.63*	0.62*
Brood area× *V. destructor* incidence	=	0.79*	0.77*

(*Significant at 5%)

Open and sealed brood cells were also examined for the presence of *Varroa* mite showed that maximum *varroa* infestation on brood was observed in June (10.20%) which was statistically at par with May (7.90%) followed by April (5.70%) which was statistically at par with March (3.50%). Minimum number of mites were noticed in February with 0.90 per cent infestation which were statistically at par with July (2.30 %). Pooled data on correlation of incidence of *V. destructor* with colony and weather parameters showed positive significant correlation of incidence of *V. destructor* with colony strength {(r= 0.63 (per 100 bees), 0.62 (visual examination)}, brood area {(r= 0.79 (per 100 bees), 0.77 (visual examination)}and negative correlation with relative humidity (significant) and rainfall (non-significant) (r= -0.59, -0.04 (per 100 bees) and -0.57, -0.08 (visual examination)}.

Seasonal variation in population of ectoparasitic mite, *V. destructor* showed that maximum incidence was in June (14.90 mites/ colony and 10.20%, respectively) in both the methods of estimation (per hundred bees method and per cent brood infestation) when the temperature was high and relative humidity and rainfall was moderate. Whereas, minimum incidence of mite was observed in the month of March (3.30 mites/ colony and 0.90%, respectively). These observations coincides with increased brood rearing activity in *A. mellifera* colonies. Our findings are in line with those of Brar *et al.* (2019) who observed maximum incidence of *Varroa* mite in the month of June when the temperature was high and relative humidity was low. No *Varroa* incidence was recorded from August to December during the period of present investigation due to considerable increase in relative humidity in these months. The present finding got support from the observations of different research workers who have found possitive correlation of *Varroa* incidence with temperature (Deosi and Chhuneja, 2012), Asha *et al.* (2013) and Poonia *et al.* (2014) who have also observed positive correlation of *Varroa* incidence with temperature and rainfall. According to Basavarajappa *et al.* (2010) in general, the infestation was high during post monsoon both in 2008 and 2009 followed by monsoon season. However, during winter the per cent mite's infestation was reported low. In our studies, *Varroa* mite was present from March onwards become maximum in June and was absent in winter months. Our findings also got support from earlier studies by Kamath (2001) and Anonymous (2007) who reported that perhaps, the prevailed moderate weather fluctuations throughout year might have influenced the per cent incidence of mite's infestation that varied significantly during different months.

4.2.5.6 Pooled data under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020)

Pooled data on incidence of *V. destructor* in *A. mellifera* colonies under stationary (Nauni, Solan) and migratory (Hisar, Haryana) conditions during January, 2019 to December, 2020 is presented in Table 45 and Fig. 16. In per hundred bees method, the incidence of *Varroa* mite was detected significantly maximum (16.30 mites/colony) during June when average colony strength and brood area were 5.75 bee frames and 2073.68 cm^2, respectively and the temperature, relative humidity and rainfall were 24.92°C, 58.50 per cent and 78.60 mm, respectively followed by May (9.00 mites/colony), April (5.00 mites/colony) which was statistically at par with July (4.80 mites/colony). Significantly minimum infestation was found in the month of August (0.80 mites/colony). Experimental colonies were found free from *V. destructor* in January, September, October, November and December in visual examination of the brood.

Brood infestation in migratory colonies was also found to be maximum in the month of June (10.20%) which was statistically at par with May (8.00%) followed by April (4.80%). Minimum *Varroa* mite infestation was found in the month of March (1.30 %) which was statistically at par with July (2.30%). Experimental colonies were found free from *V. destructor* in January, August, September, October, November and December in visual examination of the brood. Pooled data on correlation of incidence of *V. destructor* with colony and weather parameters showed negative correlation (non-significant) with relative humidity (r= -0.51(per 100 bees) and -0.49(visual examination)}.

Experimental colonies were found free from *V. destructor* in January, February August, October, November and December. Maximum incidence was observed in the month of June in both the methods of estimation. Brar *et al.* (2019) also reported maximum *V. destructor* infestation in the month of June in per hundred method (16.00 mites /colony) and in brood examination method (7.00%). Minimum infestation was recorded in the month of April (4.00 mites/colony) in per hundred method and May (3.00%) in brood examination method under migratory conditions. In present finding the absence of *Varroa* mite in bee colonies during migratory period from November to February could be attributed to the prevailing high humidity and low temperature conditions at Hisar.

Table 45. Pooled data on incidence of *V. destructor* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	Incidence of *Varroa destructor*		Colony parameters		Weather parameters		
	Per 100 bees (no.)	Brood infestation# (%)	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020**	0.00 (1.00)*	0.00 (1.00)	6.49	2741.25 (52.37)	11.43	97.50	9.65
February**	0.00 (1.00)	0.00 (1.00)	7.33	4040.93 (63.58)	15.68	95.00	9.80
March	1.70 (1.64)	1.30 (1.52)	4.39	2560.65 (50.61)	13.83	58.00	113.20
April	5.00 (2.45)	4.80 (2.41)	3.17	2215.58 (47.08)	19.59	50.00	42.25
May	9.00 (3.16)	8.00 (3.00)	6.05	2322.00 (48.20)	22.33	48.50	48.05
June	16.30 (4.16)	10.20 (3.35)	5.75	2073.68 (45.55)	24.92	58.50	78.60
July	4.80 (2.41)	2.30 (1.82)	5.96	1702.80 (41.28)	24.44	8.00	248.10
August	0.80 (1.34)	0.00 (1.00)	5.66	1535.10 (39.19)	24.69	82.50	187.20
September	0.00 (1.00)	0.00 (1.00)	5.13	1402.88 (37.47)	23.79	77.00	78.70
October	0.00 (1.00)	0.00 (1.00)	3.66	1364.18 (36.95)	19.44	65.00	2.80
November**	0.00 (1.00)	0.00 (1.00)	4.76	1041.68 (32.29)	18.95	89.00	16.10
December**	0.00 (1.00)	0.00 (1.00)	5.35	1612.50 (40.17)	12.35	93.50	2.25
$CD_{0.05}$	(0.50)	(0.38)	0.90	(4.00)			

*Figures in parentheses are square root (x+1) transformed values, #by visual examination

**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		Per 100 bees	Brood infestation
Temperature× *V. destructor* incidence	=	0.51	0.45
Relative humidity× *V. destructor* incidence	=	-0.51	-0.49
Rainfall× *V. destructor* incidence	=	0.19	0.08
Colony strength× *V. destructor* incidence	=	0.07	0.01
Brood area× *V. destructor* incidence	=	0.05	0.09

(*Significant at 5%)

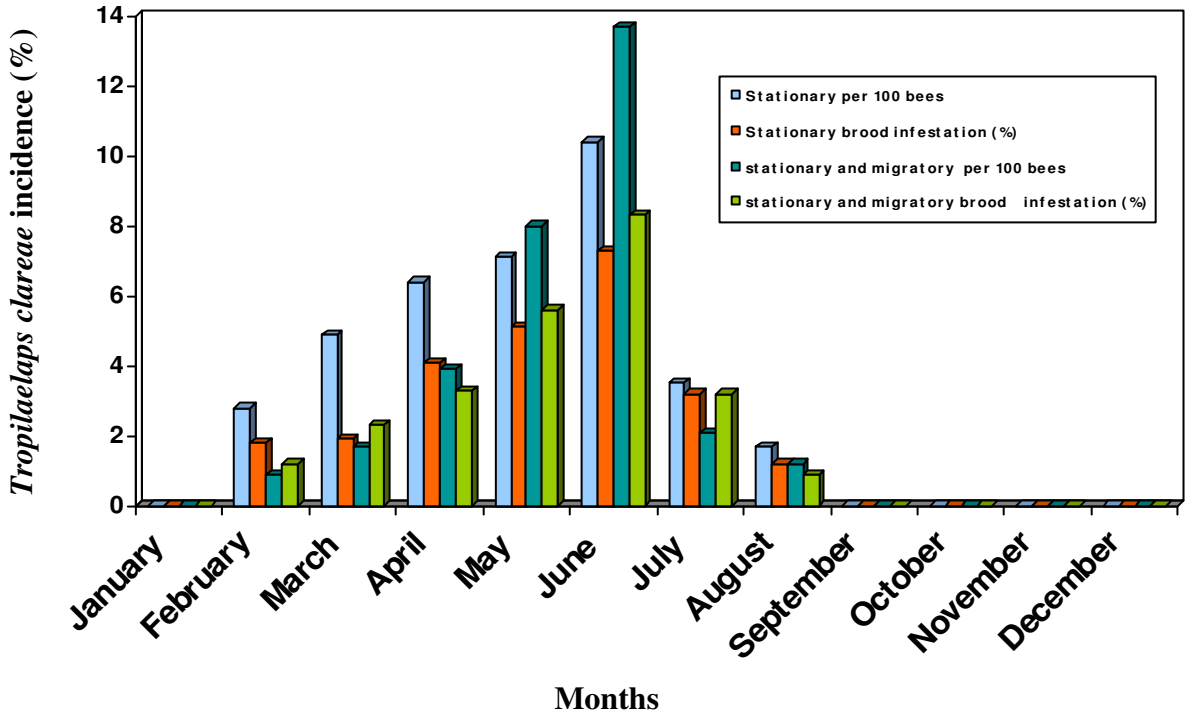


Fig 15. Incidence of *Tropilaelaps clareae* in *A. mellifera* under stationary and migratory conditions (2019-2020)

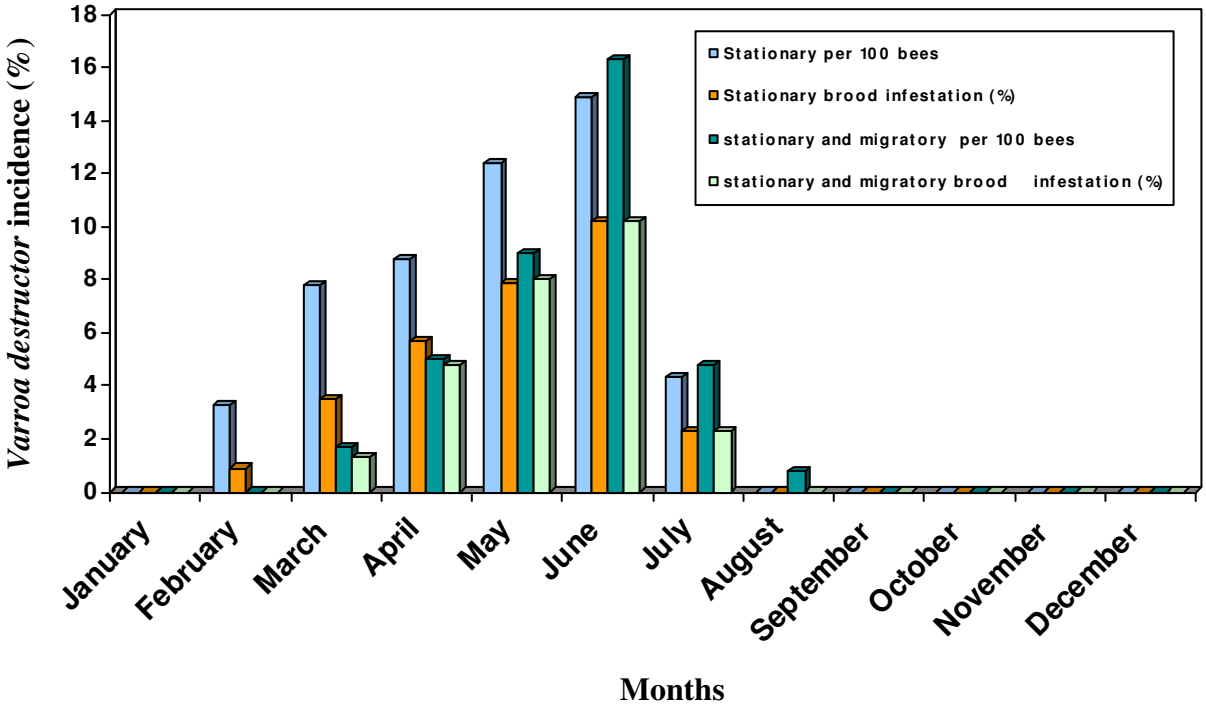


Fig 16. Incidence of *Varroa destructor* in *A. mellifera* under stationary and migratory conditions (2019-2020)

These studies are in conformity to the observations of earlier workers (Poonia *et al.,* 2014) who have reported no mite population during winters in Hisar, Haryana. They have also found the positive correlation of *Varroa* incidence with temperature and rainfall. Similar results were also reported by Asha *et al.* (2013), Deosi and Chhuneja (2012), De jong *et al.*(1982), De jong (1990) and Kokkinis and Liakos (2004).

4.2.6 Incidence of Greater wax moth (*G. mellonella*)

In experimental colonies combs were found infested with larvae, pupae and adults of greater wax moth (Calderone, 2000). Wax moth larvae were also observed feeding on honey, pollen and burrowed in to the pollen store cells and in tunnels in the midrib of the combs. Different wax moth stages were found in the debris at bottom board and in cracks and crevices especially pupae (Plate 24 & 25). Many earlier workers have recorded similar observations on feeding and various life stages of wax moth (Mandal and Vishwakarma, 2016 and Lalita *et al.,* 2018).

4.2.6.1 During 2019 (stationary conditions Nauni, Solan)

Seasonal incidence of greater wax moth (*G. mellonella*) in *A. mellifera* colonies was presented in Table 46 during January 2019 to December 2020. The data revealed that the population of wax moth (larvae, pupae and adults) varied during different months of the year. The incidence of greater wax moth was started in the month of February and March onwards started increasing up to July and was not observed in the month of December. The highest larval population (12.20) was recorded in the month of July when average colony strength and brood area was 5.29 bee frames and 1638.30 cm^2, respectively and the temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm, respectively which was statistically at par with August (10.40) and June (8.80) followed by May (7.60) which was statistically at par with September (7.00) and April (5.80). While lowest population of larvae was recorded in the month of February (1.20) when the colony strength and brood area was 3.09 frames and 1154.55 cm^2, respectively and temperature, relative humidity and rainfall, 10.34℃, 63.00 per cent and 103.10 mm, respectively which was statistically at par with November (1.60) and March (4.00).

The highest number of pupae were recorded in the month of July (7.80) when average colony strength and brood area was 5.29 bee frames and 1638.30 cm^2, respectively which

was statistically at par with August (7.40), June (7.00), May (6.20), September (5.60) and April (5.00). The pupal incidence in the month of October was (3.80) which was statistically at par with March (3.60) and November (1.80). Minimum number of pupae were recorded in the month of February (0.80) which was statistically at par with November (1.80). No wax moth larval and pupal incidence was recorded in the month of December and January.

Table 46. Incidence of *G. mellonella* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2019

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.45	715.95 (26.78)	8.85	59.00	73.00
February	1.20 (1.43)	0.80 (1.28)	0.00 (1.00)	0.67 (1.24)	3.09	1154.55 (33.99)	10.34	63.00	103.10
March	4.00 (2.06)	3.60 (2.05)	0.60 (1.23)	2.73 (1.78)	4.03	1825.35 (42.74)	13.45	54.00	54.60
April	5.80 (2.59)	5.00 (2.35)	1.20 (1.43)	4.00 (2.12)	4.80	2754.15 (52.49)	20.03	49.00	36.80
May	7.60 (2.91)	6.20 (2.64)	1.60 (1.58)	5.13 (2.38)	5.94	3205.65 (56.63)	22.61	44.00	21.30
June	8.80 (3.11)	7.00 (2.82)	2.80 (1.88)	6.20 (2.60)	4.29	1870.50 (43.26)	25.74	48.00	98.50
July	12.20 (3.62)	7.80 (2.94)	3.40 (2.08)	7.80 (2.88)	5.29	1638.30 (40.49)	23.82	79.00	218.10
August	10.40 (3.36)	7.40 (2.89)	1.80 (1.59)	6.53 (2.61)	4.47	1251.30 (35.39)	24.43	79.00	225.80
September	7.00 (2.78)	5.60 (2.55)	0.40 (1.17)	4.33 (2.17)	4.10	1240.78 (35.24)	23.48	77.00	151.40
October	4.20 (2.14)	3.80 (2.11)	0.00 (1.00)	2.67 (1.75)	3.14	1225.50 (35.02)	18.47	65.00	5.60
November	1.60 (1.52)	1.80 (1.63)	0.00 (1.00)	1.13 (1.38)	2.75	980.40 (31.33)	15.50	62.00	32.20
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.60	432.15 (20.81)	10.49	58.00	33.20
Mean	5.23 (2.29)	4.08 (2.10)	0.98 (1.33)						
$CD_{0.05}$	(0.67)	(0.61)	(0.41)		0.82	(4.21)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature× wax moth incidence	=	0.92*	0.96*	0.76*
Relative Humidity× wax moth incidence	=	0.32	0.21	0.08
Rainfall × wax moth incidence	=	0.66*	0.54	0.57
Colony strength × wax moth incidence	=	0.84	0.86	0.76
Brood area × wax moth incidence	=	0.50	0.59	0.48

(*Significant at 5%)

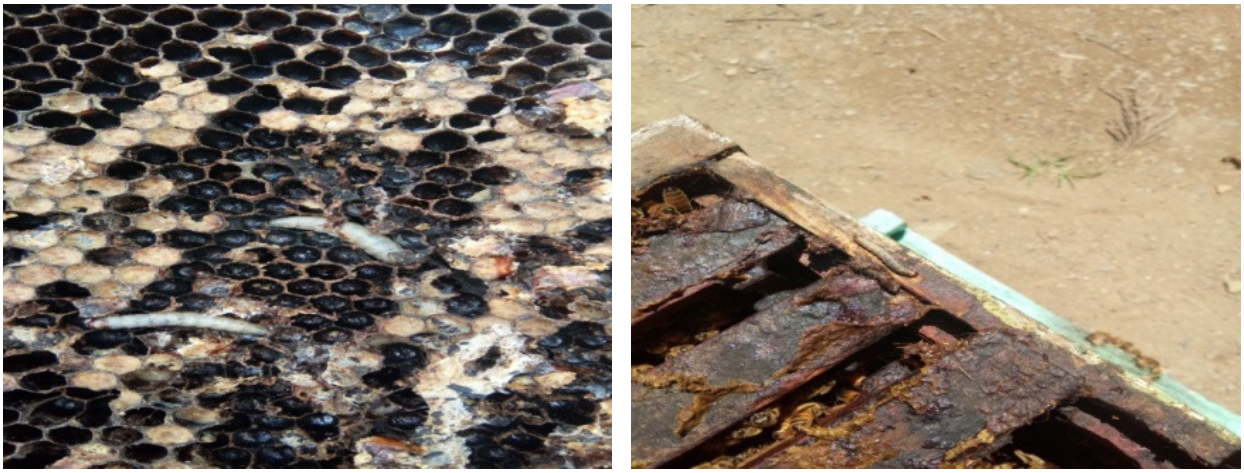


Plate 24. Larvae of greater wax moth on comb and on top bars of *A. mellifera*

Plate 25. Pupae and adult of greater wax moth (*G. mellonella*) in *A. mellifera* colony

The highest number of Adults were recorded in the month of July (3.40) when average colony strength and brood area was 5.29 bee frames and 1638.30 cm^2 which was statistically at par with June (2.80). Minimum number of population of adults was recorded in the month of September (0.40) when average colony strength and brood area was 4.10 frames and 1240.78 cm^2, respectively and the temperature, relative humidity and rainfall were 23.48°C, 77.00 per cent and 151.40 mm, respectively which was statistically at par with March (0.60), April (1.20) and May (1.60). No adults were observed in the month of January, February, October, November and December.

During the period of study, overall mean population of wax moth was found to be highest (7.80) in the month of July followed by August (6.53), June (6.20), May (5.13), September (4.33), April (4.00), March (2.73), October (2.67) and November (1.13). Lowest number of larval, pupal and adult population was found in the month of February (0.67). Correlation between wax moth developmental stages and weather parameters revealed that correlation between number of larvae and weather parameters were found to be positive and significant for temperature (r=0.92) and rainfall (r=0.66). The correlation between number of pupae, adults and weather parameters was also significant for temperature [(r=0.96) and (r=0.76), respectively].

4.2.6.2 During 2019 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Greater wax moth *(G. mellonella*) incidence in *A. mellifera* colonies was presented in Table 47 during January 2019 to December, 2019 under stationary (Nauni) and migratory conditions in (Hisar). The data revealed that highest larval population (9.80) was recorded in the month of July when average colony strength and brood area was 6.30 bee frames and 1638.30cm^2, respectively and the temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm, respectively which was statistically at par with August (8.60), September (7.40) and June (7.00) followed by May (5.80) which was statistically at par with April (4.00). The larval population in the month of March was 2.60 which was statistically at par with October (2.20), February (1.80) and November (1.60). Minimum number of larvae were recorded in the month of December (0.60) when the colony strength and brood area was 5.43 bee frames and 1631.85 cm^2 and temperature, relative humidity and rainfall were 11.40°C, 94.00 per cent and 4.50 mm , respectively which was statistically at par with

January (0.80), November (1.60) and February (1.80). Under stationary conditions during December and January no larval incidence was recorded but in migratory conditions larval incidence was recorded and varied from 0.6-1.8 larvae per hive and maximum larvae were found in the month of February (1.80) during migratory conditions in Hisar.

Table 47. Incidence of *G. mellonella* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2019

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2019**	0.80 (1.34)*	0.40 (1.18)	0.00 (1.00)	0.40 (1.83)	6.59	2941.20 (54.24)	11.15	97.00	10.40
February	1.80 (1.67)	1.20 (1.48)	0.60 (1.26)	1.20 (1.48	7.03	4211.85 (64.91)	14.45	93.00	10.90
March	2.60 (1.90)	1.80 (1.67)	1.20 (1.48)	1.87 (1.69)	4.51	2709.00 (52.06)	13.45	54.00	54.60
April	4.00 (2.24)	3.20 (2.05)	1.80 (1.67)	3.00 (2.00)	3.37	2309.10 (48.06)	20.03	49.00	36.80
May	5.80 (2.61)	4.40 (2.32)	2.00 (1.73)	4.07 (2.25)	5.85	2431.65 (49.32)	22.61	44.00	21.30
June	7.00 (2.83)	5.20 (2.49)	2.20 (1.79)	4.80 (2.40	6.00	2173.65 (46.63)	25.74	48.00	98.50
July	9.80 (3.29)	6.00 (2.65)	3.00 (2.00)	6.27 (2.69	6.30	1638.30 (40.49)	23.82	79.00	218.10
August	8.60 (3.10)	5.60 (2.57)	2.80 (1.95)	5.67 (2.58)	5.88	1528.65 (39.11)	24.43	79.00	225.80
September	7.40 (2.90)	3.40 (2.10)	0.80 (1.34)	3.87 (2.20)	5.56	1406.10 (37.51)	23.48	77.00	151.40
October	2.20 (1.79)	2.20 (1.79)	0.40 (1.18)	1.60 (1.61)	4.08	1393.20 (37.34)	18.47	65.00	5.60
November **	1.60 (1.61)	1.00 (1.41)	0.20 (1.10)	0.93 (1.39)	5.18	1102.95 (33.23)	19.90	89.00	12.30
December**	0.60 (1.26)	0.20 (1.10)	0.00 (1.00)	0.27 (1.13)	5.43	1631.85 (40.41)	11.40	94.00	4.50
Mean	4.35 (2.31)	2.88 (1.97)	1.25 (1.50)						
$CD_{0.05}$	(0.52)	(0.60)	(0.45)		0.34	(4.98)			

*Figures in parentheses are square root (x+1) transformed values.
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature × wax moth incidence	=	0.86*	0.88*	0.74*
Relative Humidity× wax moth incidence	=	-0.34	-0.51	-0.51
Rainfall × wax moth incidence	=	0.90*	0.79*	0.75*
Colony strength × wax moth incidence	=	0.18	0.08	0.06
Brood area × wax moth incidence	=	-0.31	-0.26	-0.11

(*Significant at 5%)

Highest number of pupae were also recorded in the month of July (6.00) when average colony strength and brood area was 6.30 bee frames and 1638.30 cm^2 and the temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm, respectively which was statistically at par with August (5.60), June (5.20), May (4.40), September (3.40) and April (3.20). Minimum number of pupae were recorded in the month of December (0.20) which was statistically at par with January (0.40), November (1.00), February (1.20) and March (1.80). Under migratory conditions the pupal population vary from 0.2-1.2 pupae/hive and maximum population was found in the month of February (1.20).

The highest number of Adults were recorded in the month of July (3.00) when the temperature, relative humidity and rainfall were 23.82°C, 79.00 per cent and 218.10 mm, respectively which was statistically at par with August (2.80) followed by June (2.20) which was statistically at par with May (2.00) and April (1.80).The adult population in the month of March was 1.20 which was statistically at par with September (0.80). Minimum number of adults were recorded in the month of November (0.20) when average colony strength and brood area was 5.18 bee frames and 1102.95 cm^2 , respectively and the temperature, relative humidity and rainfall were 11.90°C, 89.00 per cent and 12.30 mm, respectively which was statistically at par with October (0.40) and February (0.60). No adult incidence was observed in the month of January and February. During migratory period the population was very low and observed only in the month of February (0.60).

The mean population of all stages of wax moth during stationary (Nauni) and migratory (Hisar) conditions was found highest (6.27) in the month of July followed by August (5.67), June (4.80), May (4.07), September (3.87), April (3.00), March (1.87), October (1.60), February (1.20), November (0.93) and January (0.40). Lowest number of larval, pupal and adult population was found in the month of December (0.27). During migratory period maximum number of larval, pupal and adult population was found in the month of February (1.20) and minimum in the month of December (0.27). The correlation between number of larvae, pupae and adults and weather parameters were found to be positive and significant for temperature [(r=0.86), (r=0.88) and (r=0.74 respectively)] and rainfall [(r=0.90), (r=079)and (r=0.75)] while, negative for brood area [(r=-0.31), (r=-0.26) (r=-0.11)] and relative humidity [(r=-0.34), (r=-0.51) and (r=-0.51)].

4.2.6.3 During 2020 (stationary conditions Nauni, Solan)

Data in Table 48 revealed that during the whole incidence period of wax moth (*G. mellonella*) during 2020 the temperature relative humidity and rainfall varies from 9.10 to 25.05, 51 per cent to 86 per cent and 0 to 278.10 mm, respectively.

Table 48. Incidence of *G. mellonella* in *A. mellifera* colonies under stationary conditions at Nauni during January to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.01	606.30 (24.64)	9.10	68.00	168.30
February	0.60 (1.23)	0.40 (1.15)	0.00 (1.00)	0.33 (1.13)	2.36	1135.20 (33.71)	12.15	57.00	38.50
March	3.40 (1.94)	3.20 (2.01)	0.80 (1.28)	2.46 (1.75)	3.91	1922.10 (43.85)	14.20	62.00	171.80
April	4.60 (2.23)	5.80 (2.60)	1.00 (1.35)	3.80 (2.06)	4.84	2934.75 (54.18)	19.15	51.00	47.70
May	6.80 (2.78)	5.40 (2.51)	1.20 (1.39)	4.46 (2.23)	6.08	3412.05 (58.42)	22.05	53.00	74.80
June	9.20 (3.18)	6.00 (2.52)	3.20 (1.97)	6.13 (2.56)	4.06	1941.45 (44.07)	24.1	69.00	58.70
July	11.00 (3.44)	6.40 (2.59)	4.00 (2.22)	7.13 (2.75)	5.28	1870.50 (43.26)	25.05	81.00	278.10
August	10.60 (3.40)	7.60 (2.92)	2.20 (1.73)	6.80 (2.68)	4.27	1496.40 (38.70)	24.95	86.00	148.60
September	5.80 (2.49)	5.00 (2.43)	0.20 (1.08)	3.66 (2.00)	3.81	1341.60 (36.64)	24.1	77.00	6.00
October	2.60 (1.78)	3.20 (1.97)	0.00 (1.00)	1.93 (1.58)	3.47	1290.00 (35.93)	20.4	65.00	0.00
November	1.20 (1.37)	1.60 (1.49)	0.00 (1.00)	0.93 (1.29)	2.49	941.70 (30.70)	14.6	68.00	37.70
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.06	574.05 (23.98)	12.2	62.00	23.80
Mean	4.65 (2.15)	3.72 (2.02)	1.05 (1.35						
$CD_{0.05}$	(0.73)	(0.66)	(0.45)		0.29	(7.42)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature× wax moth incidence	=	0.91*	0.93*	0.69*
Relative Humidity× wax moth incidence	=	0.54	0.38	0.45
Rainfall× wax moth incidence	=	0.45	0.27	0.61*
Colony strength× wax moth incidence	=	0.78	0.85	0.64
Brood area× wax moth incidence	=	0.52	0.66	0.41

(*Significant at 5%)

Highest number of larvae (11.00) were recorded in the month of July when average colony strength and brood area was 5.28 bee frames and 1870.50 cm^2, respectively and the temperature, relative humidity and rainfall were 25.05°C, 81 per cent and 278.10 mm,

respectively which was statistically at par with August (10.60), June (9.20) and May (6.80) which was further at par with September (5.80) and April (4.60). Minimum number of larvae were recorded in the month of February (0.60) when average colony strength and brood area were 2.36 frames and 1135.20 cm^2 and the temperature, relative humidity and rainfall were 12.15°C, 57 per cent and 38.50mm, respectively which was statistically at par with November (1.20), October (2.60) and March (3.40).

Maximum number of pupae were recorded in the month of August (7.60) when average colony strength and brood area was 4.27 bee frames and 1496.40 cm^2 and the temperature, relative humidity and rainfall were 24.95°C, 86.00 per cent and 148.60 mm, respectively which was statistically at par with July (6.40) , June (6.00), April (5.80), May (5.40) and September (5.00). The wax moth pupal incidence in the month of October was 3.20 which was statistically at par with March (3.20) and November (1.60). Minimum number of pupae were recorded in the month of February (0.40) when average colony strength and brood area was 2.36 frames and 1135.20 cm^2 and the temperature, relative humidity and rainfall were 12.15°C, 57 per cent and 38.50 mm, respectively which was statistically at par with November (1.60).

Maximum number of adults were observed in the month of July (4.00) when average colony strength and brood area was 5.28 bee frames and 1870.50 cm^2 and the temperature, relative humidity and rainfall were 25.05°C, 81 per cent and 278.10 mm, respectively which was statistically at par with June (3.20). Whereas, minimum number of adults were recorded in the month of September (0.20) which was statistically at par with March (0.80), April (1.00) and May (1.20). There was no incidence of adults was observed in the month of January, February October, November and December.

Mean population of wax moth was found highest (7.13) in the month of July followed by August (6.80), June (6.13), May (4.46), April (3.80), September (3.66), March (2.46), October (1.93) and November (0.93). Lowest population of wax moth larvae, pupae and adults were found in the month of February (0.33). The correlation between number of larvae, pupae and weather parameters were found to be positive and highly significant for temperature [(r=0.91) and (r=0.93), respectively]. For Adults incidence the correlation was positive and significant with temperature (r=0.69) and rainfall (r=0.61).

4.2.6.4 During 2020 [migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions]

Incidence of greater wax moth in *A. mellifera* colonies from January, 2020 to December, 2020 under stationary (Nauni, Solan) and migratory conditions (Hisar, Haryana) is presented in Table 49. The data revealed that the highest average larvae (10.80) were recorded in the month of July when average colony strength and brood area was 5.61 bee frames and 1767.30 cm^2 and the temperature, relative humidity and rainfall were 25.05°C, 81.00 per cent and 278.10 mm, respectively which was statistically at par with August (9.20), September (7.80) and June (7.40) followed by May (5.60) which was statistically at par with April (3.20) and March (3.00). While lowest population of larvae were recorded in the month of December (0.80) when the colony strength and brood area was 5.26 bee frames and 1593.15 cm^2 and temperature, relative humidity and rainfall, 13.30°C, 93.00 per cent and 0.00 mm, respectively which was statistically at par with January (1.00), November (1.20) and February (1.40) and October (2.00). The wax moth larval incidence during migratory conditions vary from 0.8-1.4 larvae per hive and maximum was found in the month of February (1.40) during migratory conditions in Hisar.

The highest number of pupae were recorded in the month of July (6.20) when average colony strength and brood area was 5.61 bee frames and 1767.30 cm^2 and the temperature, relative humidity and rainfall were 25.05°C, 81.00 per cent and 278.10 mm, respectively which was statistically at par with June (6.00), August (5.00) and May (3.80) and was further at par with September (3.60), April (3.00), October (2.60) and March (2.20). Minimum number of pupae were recorded in the month of December (0.40) which was statistically at par with January (0.60), November (1.40) and February (1.60). Under migratory conditions the pupal population varied from 0.40-1.60 pupae/hive and maximum population was found in the month of February (1.60).

The highest number of adults were recorded in the month of July (3.40) when the temperature, relative humidity and rainfall were 25.05°C, 81.00 per cent and 278.10 mm, respectively which was statistically at par with June (3.00), May (2.60) and April (2.00). Minimum number of population of adults were recorded in the month of November (0.40) when average colony strength and brood area was 4.33 bee frames and 980.40 cm^2 and the temperature, relative humidity and rainfall were 18.00°C, 89.00 per cent and 19.90 mm, respectively which was statistically at par with October (0.60) and February (0.80),

September (1.00) and March (1.40). No adult incidence was observed in the month of January and December during stationary conditions. Adult population in the colonies was low during migratory period and maximum was in the month of February (0.80).

Table 49. Incidence of *G. mellonella* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January, 2020**	1.00 (1.41)*	0.60 (1.26)	0.00 (1.00)	0.53 (1.24	6.38	2541.30 (50.42)	11.70	98.00	8.90
February	1.40 (1.55)	1.60 (1.61)	0.80 (1.34)	1.27 (1.51)	7.62	3870.00 (62.22)	16.90	97.00	8.70
March	3.00 (2.00)	2.20 (1.79)	1.40 (1.55)	2.20 (1.79)	4.27	2412.30 (49.13)	14.20	62.00	171.80
April	3.20 (2.05)	3.00 (2.00)	2.00 (1.73)	2.73 (1.93	2.96	2122.05 (46.08)	19.15	51.00	47.70
May	5.60 (2.57)	3.80 (2.19)	2.60 (1.90)	4.00 (2.24)	6.25	2212.35 (47.05)	22.05	53.00	74.80
June	7.40 (2.90)	6.00 (2.65)	3.00 (2.00)	5.47 (2.54)	5.50	1973.70 (44.44)	24.10	69.00	58.70
July	10.80 (3.44)	6.20 (2.68)	3.40 (2.10)	6.80 (2.79)	5.61	1767.30 (42.05)	25.05	81.00	278.10
August	9.20 (3.19)	5.00 (2.45)	1.80 (1.67)	5.33 (2.52)	5.44	1541.55 (39.28)	24.95	86.00	148.60
September	7.80 (2.97)	3.60 (2.14)	1.00 (1.41)	4.13 (2.27)	4.69	1399.65 (37.43)	24.10	77.00	6.00
October	2.00 (1.73)	2.60 (1.90)	0.60 (1.26)	1.73 (1.65)	3.23	1335.15 (36.55)	20.40	65.00	0.00
November**	1.20 (1.48)	1.40 (1.55)	0.40 (1.18)	1.00 (1.41)	4.33	980.40 (31.33)	18.00	89.00	19.90
December**	0.80 (1.34)	0.40 (1.18)	0.00 (1.00)	0.40 (1.18)	5.26	1593.15 (39.93)	13.30	93.00	0.00
Mean	4.45 (2.33)	3.03 (2.00)	1.41 (1.55)						
$CD_{0.05}$	(0.62)	(0.53)	(0.47)		0.31	(6.86)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature × wax moth incidence	=	0.87*	0.90*	0.73*
Relative Humidity× wax moth incidence	=	-0.20	-0.38	-0.53
Rainfall × wax moth incidence	=	0.67*	0.63*	0.69*
Colony strength × wax moth incidence	=	0.08	-0.01	0.04
Brood area × wax moth incidence	=	-0.25	-0.19	0.01

(*Significant at 5%)

Mean population of all stages of wax moth was found highest (6.80) in the month of July followed by June (5.47), August (5.33), September (4.13), May (4.00), April (2.73), March (2.20), October (1.73), February (1.27) and November (1.00). Minimum number of wax moth population was found in the month of December (0.40). The correlation between different stages of wax moth and weather parameters showed that number of larvae had

positive and significant relation with temperature (r=0.87) and rainfall (r=0.67) while, negative correlation with brood area (r=-0.25) and relative humidity (r=-0.20) though non-significant. The correlation between number of pupae and weather parameters were found to be positive and significant for temperature (r=0.90) and rainfall (r=0.63) and negative correlated (non-significant) for brood area (r=-0.19) and relative humidity (r=-0.38). Number of adults showed positive and significant correlation with temperature (r=0.73) and rainfall (r=0.69) and negative correlateion with relative humidity (r=-0.53).

4.2.6.5 Pooled data under stationary conditions Nauni, Solan (2019-2020)

Pooled data on incidence of greater wax moth (*G. mellonella*) in *A. mellifera* colonies during the year 2019 to 2020 under stationary conditions is presented in table 50 and Fig. 17. The data revealed that the population of wax moth varied during different months. In the months of January and December no incidence of larvae was observed.

The highest larval population (11.60) was recorded in the month of July when average colony strength and brood area was 5.28 bee frames and 1754.40 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm, respectively which was statistically at par with August (10.50) followed by June (9.00) and May (7.20) which was statistically at par with September (6.40). The larval incidence in the month of April was 5.20 followed by March (3.70) which was statistically at par with October (3.40). In the present study, no infestation of wax moth was observed in the month of December and January when temperature, relative humidity and rainfall were in the range of 8.98 to 11.35°C, 60.00 to 63.50 per cent and 28.50 to 120.65 mm, respectively. While lowest population of larvae were recorded in the month of February (0.90) when average colony strength and brood area was 2.73 bee frames and 1144.88 cm^2 and the temperature, relative humidity and rainfall were 11.25°C, 60.00 per cent and 70.80 mm, respectively which was statistically at par with November (1.40).

The highest number of pupae were recorded in the month of August (7.50) when average colony strength and brood area was 4.37 bee frames and 1373.85 cm^2 and the temperature, relative humidity and rainfall were 24.69°C, 82.50 per cent and 187.20 mm, respectively which was statistically at par with July (7.10) and June (6.50) and May (5.80) followed by April (5.40) and September (5.30). The pupal incidence in the month of October was 3.50 which was statistically at par with March (3.40) followed by November (1.70). Significantly minimum pupal population was recorded in the month of February (0.60) when

average colony strength and brood area was 2.73 bee frames and 1144.88 cm^2 and the temperature, relative humidity and rainfall were 11.25°C, 60.00 per cent and 70.80 mm.

Maximum number of adults in the pooled data was observed in the month of July (3.70) when average colony strength and brood area was 5.28 bee frames and 1754.40 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm, respectively which was statistically at par with June (3.00) and August (2.00). Minimum adult population was recorded in the month of September (0.30) which was statistically at par with March (0.70), April (0.10) and May (1.40). No adult incidence was observed in the month of October, November, December, January and February.

During the two years of study period, the mean population of all stages of wax moth was found highest (7.47) in the month of July followed by August (6.67), June (6.17), May (4.80), September (4.00), April (3.90), March (2.60), October (2.30), November (1.03). Minimum number of wax moth (*G. mellonella*) larvae, pupae and adults were found in the month of February (0.50). The correlation between number of larvae, adults and weather parameters were found positive and significant for temperature [(r=0.92) and (r=0.73, respectively) and rainfall [(r=0.62), (r=0.67), respectively]. The pupal correlation with weather parameters was positive and significant for temperature (r=0.95).

In our studies the mean population of all stages of wax moth was maximum in July and the months of higher infestation were May, June, July and August. This may be due to higher temperature and rainfall during these months which are favourable for wax moth infestation (Sohali *et al.,* 2017; Lalita *et al.,* 2018; Swamy *et al.,* 2005; Mandal and Vishwakarma 2016 and Raghunandan and Basavarajappa, 2014). The wax moth incidence was found highest in summer months (July, August, June and May) when the temperature was high and lowest incidence was recorded in the months of February and November due to low ambient temperature which found support from the results of Nagaraja and Rajagopal (2009) who reported that wax moth population fluctuated according to weather conditions. Brar *et al.* (1985) reported that greater wax moth infestation started in June and acquired peak in September and thereafter decreased up to November in *A. mellifera* colonies in Punjab, similarly in Haryana maximum infestation of wax moth in *A. mellifera* colonies was recorded during July to September and most favourable temperature was 35°C combined with 70 per cent relative humidity (Gupta, 1987).

Table 50. Pooled data on incidence of *G. mellonella* in *A. mellifera* colonies under stationary conditions at Nauni during January, 2019 to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January	0.00 (1.00)*	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.23	661.13 (25.73)	8.98	63.50	120.65
February	0.90 (1.38)	0.60 (1.26)	0.00 (1.00)	0.50 (1.22)	2.73	1144.88 (33.85)	11.25	60.00	70.80
March	3.70 (2.17)	3.40 (2.10)	0.70 (1.30)	2.60 (1.90)	3.96	1873.73 (43.30)	13.83	58.00	113.20
April	5.20 (2.49)	5.40 (2.53)	1.10 (1.45)	3.90 (2.21)	4.82	2844.45 (53.34)	19.59	50.00	42.25
May	7.20 (2.86)	5.80 (2.61)	1.40 (1.55)	4.80 (2.41)	6.01	3308.85 (57.53)	22.33	48.50	48.05
June	9.00 (3.16)	6.50 (2.74)	3.00 (2.00)	6.17 (2.68)	4.18	1905.98 (43.67)	24.92	58.50	78.60
July	11.60 (3.55)	7.10 (2.85)	3.70 (2.17)	7.47 (2.91)	5.28	1754.40 (41.90)	24.44	80.00	248.10
August	10.50 (3.39)	7.50 (2.92)	2.00 (1.73)	6.67 (2.77)	4.37	1373.85 (37.08)	24.69	82.50	187.20
September	6.40 (2.72)	5.30 (2.51)	0.30 (1.14)	4.00 (2.24)	3.96	1291.19 (35.95)	23.79	77.00	78.70
October	3.40 (2.10)	3.50 (2.12)	0.00 (1.00)	2.30 (1.82)	3.31	1257.75 (35.48)	19.44	65.00	2.80
November	1.40 (1.55)	1.70 (1.64)	0.00 (1.00)	1.03 (1.43)	2.62	961.05 (31.02)	15.05	65.00	34.95
December	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	2.33	503.10 (22.45)	11.35	60.00	28.50
Mean	4.94 (2.44)	3.90 (2.21)	1.01 (1.42)						
$CD_{0.05}$	(0.27)	(0.29)	(0.53)		0.54	(2.70)			

*Figures in parentheses are square root (x+1) transformed values.

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature × wax moth incidence	=	0.92*	0.95*	0.73*
Relative Humidity× wax moth incidence	=	0.43	0.31	0.27
Rainfall × wax moth incidence	=	0.62*	0.46	0.67*
Colony strength × wax moth incidence	=	0.82	0.86	0.70
Brood area × wax moth incidence	=	0.52	0.63	0.45

(*Significant at 5%)

Verma and Raj (2000) observed maximum population of *G. mellonella* during first fortnight of August in Kangra valley of Himachal Pradesh. Minimum incidence of wax moth was observed in the month of February and no infestation was observed in the month of December. Our results are in line with Bhatnagar *et al.* (2020) who reported that in colder months of February and March there was no infestation of wax moth. The infestation was first noticed in the month of April which increased in May-June and highest was recorded in the month of July.

4.2.6.6 Pooled data under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions (2019-2020)

The (*G. mellonella*) incidence in *A. mellifera* colonies during two years of time period under stationary (Nauni, Solan) and migratory conditions in (Hisar, Haryana) is presented in Table 51 and Fig. 17. During this period the temperature, relative humidity and rainfall varied from 11.43 to 24.92, 8.00 to 97.50 per cent and 2.25 to 248.10 mm, respectively. Pooled data from January, 2019 to December, 2020 demonstrated that the highest larval population (10.30) was recorded in the month of July when average colony strength and brood area were 5.96 bee frames and 1702.80 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 80.00 per cent and 248.10 mm, respectively which was statistically at par with August (8.90). The wax moth larval incidence in September was 7.60 which was statistically at par with June (7.20) followed by May (5.70), April (3.60) which was statistically at par with March (2.80). The wax moth larval incidence in the month of October was 2.10 which was statistically at par with February (1.60) and November (1.40). Whereas, lowest population of larvae were recorded in the month of December (0.70) when the colony strength and brood area was 5.35 bee frames and 1612.50 cm^2 and temperature, relative humidity and rainfall were 12.35℃, 93.00 per cent and 2.25 mm, respectively which was statistically at par with January (0.90). The wax moth larval incidence during migratory conditions varied from 0.70-1.60 larvae per hive and maximum larval incidence was found in the month of February (1.60).

The highest number of pupae were recorded in the month of July (6.10) when average colony strength and brood area was 5.96 bee frames and 1702.80 cm^2 and the temperature, relative humidity and rainfall were 24.44°C, 8.00 per cent and 248.10 mm, respectively which was statistically at par with June (5.60), August (5.30), May (4.10), September (3.50) and April (3.10) followed by October (2.40) which was statistically at par with March (2.00). The greater wax moth pupal incidence in the month of February was 1.40 which was statistically at par with November (1.20). Minimum number of pupae were recorded in the month of December (0.30) which was statistically at par with January (0.50).

The highest number of Adults were recorded in the month of July (3.20) when the temperature, relative humidity and rainfall were 24.44°C, 8.00 per cent and 248.10 mm, respectively which was statistically at par with June (2.60) and August (2.30) followed by

May (2.30) which was statistically at par with April (1.90). The wax moth adult incidence in the month of March was 1.30 which was statistically at par with September (0.90) and February (0.70). Minimum number of population of adults were recorded in the month of November (0.30) when average colony strength and brood area was 4.76 bee frames and 1041.68 cm^2 and the temperature, relative humidity and rainfall were 18.95°C, 89.00 per cent and 16.10 mm, respectively which was statistically at par with October (0.50), February (0.30) and September (0.90).

During two years of study period, the mean population of all stages of wax moth was found highest (6.53) in the month of July followed by August (5.50), June (5.13), May (4.03), September (4.00), April (2.87), March (2.03), October (1.63), February (1.23), November (0.97) and January (0.47). Lowest mean population of wax moth population was found in the month of December (0.33). The correlation between number of larvae and weather parameters were found positive and significant for temperature (r=0.87) and rainfall (r=0.85) and negative (non-significant) for brood area (r=-0.28) and relative humidity (significant) (r=-0.62). Number of pupae and weather parameters showed positive and significant correlation for temperature (r=0.91) and rainfall (r=0.78) and negative correlation with brood area (r=-0.23) and relative humidity (r=-0.71). Adults incidence showed positive and significant correlation with temperature (r=0.76) and rainfall (r=0.77) and negative correlation for relative humidity (significant) (r=-0.80) and brood area (r=-0.05) (non-significant).

Mean population of all the stages of wax moth (larvae, pupae and adults) started increasing March onward and become maximum in July when the temperature and rainfall were high while relative humidity was low. Our results are in close proximity with those of Bhatnagar *et al*.(2020), Lalita *et al.* (2018), Mandal and Vishawkarma (2016), Gupta (1987) and Brar *et al.* (1985). In present studies the months of maximum infestation were May, June, July and August owing to higher ambient temperatures during these months. Sohail *et al.* (2017) studied the seasonal abundance of *G. mellonela* larvae in hives of honey bees located in district Sargodha, Punjab, Pakistan and reported that the maximum moth abundance occurred during the regional dearth period May to November with peak (14.8 ± 3.9 larvae/hive) in August. During present investigation the greater wax moth infestation was observed throughout the year.

Table 51. Pooled data on incidence of *G. mellonella* in *A. mellifera* colonies under migratory (Hisar, Haryana) and stationary (Nauni, Solan) conditions during January, 2019 to December, 2020

Months	Developmental stages of wax moth				Colony parameters		Weather parameters		
	Average no. of larvae	Average no. of pupae	Average no. of adults	Mean population of wax moth	Average colony strength (bee frame)	Average brood area (cm^2)	Temperature (°C)	Relative humidity (%)	Rainfall (mm)
January**	0.90 (1.38)*	0.50 (1.22)	0.00 (1.00)	0.47 (1.21)	6.49	2741.25 (52.37)	11.43	97.50	9.65
February**	1.60 (1.61)	1.40 (1.55)	0.70 (1.30)	1.23 (1.49)	7.33	4040.93 (63.58)	15.68	95.00	9.80
March	2.80 (1.95)	2.00 (1.73)	1.30 (1.52)	2.03 (1.74)	4.39	2560.65 (50.61)	13.83	58.00	113.20
April	3.60 (2.14)	3.10 (2.02)	1.90 (1.70)	2.87 (1.97)	3.17	2215.58 (47.08)	19.59	50.00	42.25
May	5.70 (2.59)	4.10 (2.26)	2.30 (1.82)	4.03 (2.24)	6.05	2322.00 (48.20)	22.33	48.50	48.05
June	7.20 (2.86)	5.60 (2.57)	2.60 (1.90)	5.13 (2.48)	5.75	2073.68 (45.55)	24.92	58.50	78.60
July	10.30 (3.36)	6.10 (2.66)	3.20 (2.05)	6.53 (2.74)	5.96	1702.80 (41.28)	24.44	8.00	248.10
August	8.90 (3.15)	5.30 (2.51)	2.30 (1.82)	5.50 (2.55)	5.66	1535.10 (39.19)	24.69	82.50	187.20
September	7.60 (2.93)	3.50 (2.12)	0.90 (1.38)	4.00 (2.24)	5.13	1402.88 (37.47)	23.79	77.00	78.70
October	2.10 (1.76)	2.40 (1.84)	0.50 (1.22)	1.67 (163)	3.66	1364.18 (36.95)	19.44	65.00	2.80
November**	1.40 (1.55)	1.20 (1.48)	0.30 (1.14)	0.97 (1.40)	4.76	1041.68 (32.29)	18.95	89.00	16.10
December**	0.70 (1.30)	0.30 (1.14)	0.00 (1.00)	0.33 (1.15)	5.35	1612.50 (40.17)	12.35	93.50	2.25
Mean	4.40 (2.32)	2.95 (1.98)	1.33 (1.52)						
$CD_{0.05}$	(0.22)	(0.19)	(0.24)		0.90	(4.00)			

*Figures in parentheses are square root (x+1) transformed values
**Months of migration period of colonies to Haryana

Pearson correlation Matrix (r) =

		No. of larvae	No. of pupae	No. of adults
Temperature× wax moth incidence	=	0.87*	0.91*	0.76*
Relative Humidity× wax moth incidence	=	-0.62*	-0.71*	-0.80*
Rainfall × wax moth incidence	=	0.85*	0.78*	0.77*
Colony strength× wax moth incidence	=	0.13	0.04	0.05
Brood area × wax moth incidence	=	-0.28	-0.23	-0.05

(*Significant at 5%)

Same observations were recorded by earlier investigators who reported that moth infestations in almost all months of the year due to overlapping generations of the wax moth (Garg and Kashyap, 1998; Hood *et al.,* 2003). The active period of wax moth was from March to October. Same observations were recorded by Garg and Kashyap (1998) and Brar *et al.* (1985). Wax moth abundance and the per cent of hives with moth-damaged combs were directly proportional to one other. The nature of severity was differentiated and categorized in

to three groups as 35, 47 and 2 per cent combs infestation *viz.*, light, moderate and severely affected, respectively (Kebede *et al*., 2015). Minimum mean population of wax moth was observed in the month of November and no infestation in December, this may be due to low temperature and rainfall conditions. Raghunandan and Basavarajappa (2014) also found more infestation in the semi-arid region during summer (30.8%) followed by a rainy season (23.4%). However, the infestation was less (6.6%) in winter season.

4.3 Management of greater wax moth (*G. mellonella*) in *A. mellifera* and *A. cerana* combs under laboratory conditions

4.3.1 Area damaged and per cent area infestation per comb in *A. mellifera* wax combs due to greater wax moth (*G. mellonella)* under laboratory conditions

a) During 2020

Data on comb area damaged by *G. mellonella* in *A. mellifera* combs presented in Table 52 indicated that the minimum comb area damaged after 7 day of treatment was recorded in *Bt* (4.31 cm^2) which was statistically at par with neem seed kernel extract (7.29 cm^2) followed by sulphur fumigation (9.53 cm^2) which was statistically at par with neem oil (10.25 cm^2) and neem leaf powder treatment (15.33 cm^2). The damaged comb area in acetic acid treatment was 21.62 cm^2 which was statistically at par with karanj oil treatment (23.26 cm^2) and formic acid (29.83 cm^2) followed by *T. viridae* (48.48 cm^2) which was at par with *B. bassiana* (53.55 cm^2) and *M. anisopliae* (57.85 cm^2). The damaged area in alsi seed extract was 67.85 cm^2 which was statistically at par with pumpkin seed extract (74.19 cm^2). Significantly maximum infested area was found in control (120.90 cm^2). After 7 day of treatment the minimum per cent area infestation was recorded in *Bt* (1.59 %) which was statistically at par with neem seed kernel extract (2.68 %) followed by sulphur fumigation (3.50 %) which was statistically at par with neem oil (3.77 %) and neem leaf powder treatment (5.64 %). The per cent area infestation in acetic acid treatment was 7.95 % which was statistically at par with karanj oil treatment (8.55 %) and formic acid (10.97 %) followed by *T. viridae* (17.83%) which was statistically at par with *B. bassiana* (19.69 %) and *M. anisopliae* (21.27%). In alsi seed extract treatment the per cent infested area was 24.94% which was at par with pumpkin seed extract (27.28%). Significantly maximum infested area was found in control (44.45%). No infestation was found in deep freezing (-8°C to -10°C) treatment and all the third instar larvae got killed after 5 hours in deep freezer treatment.

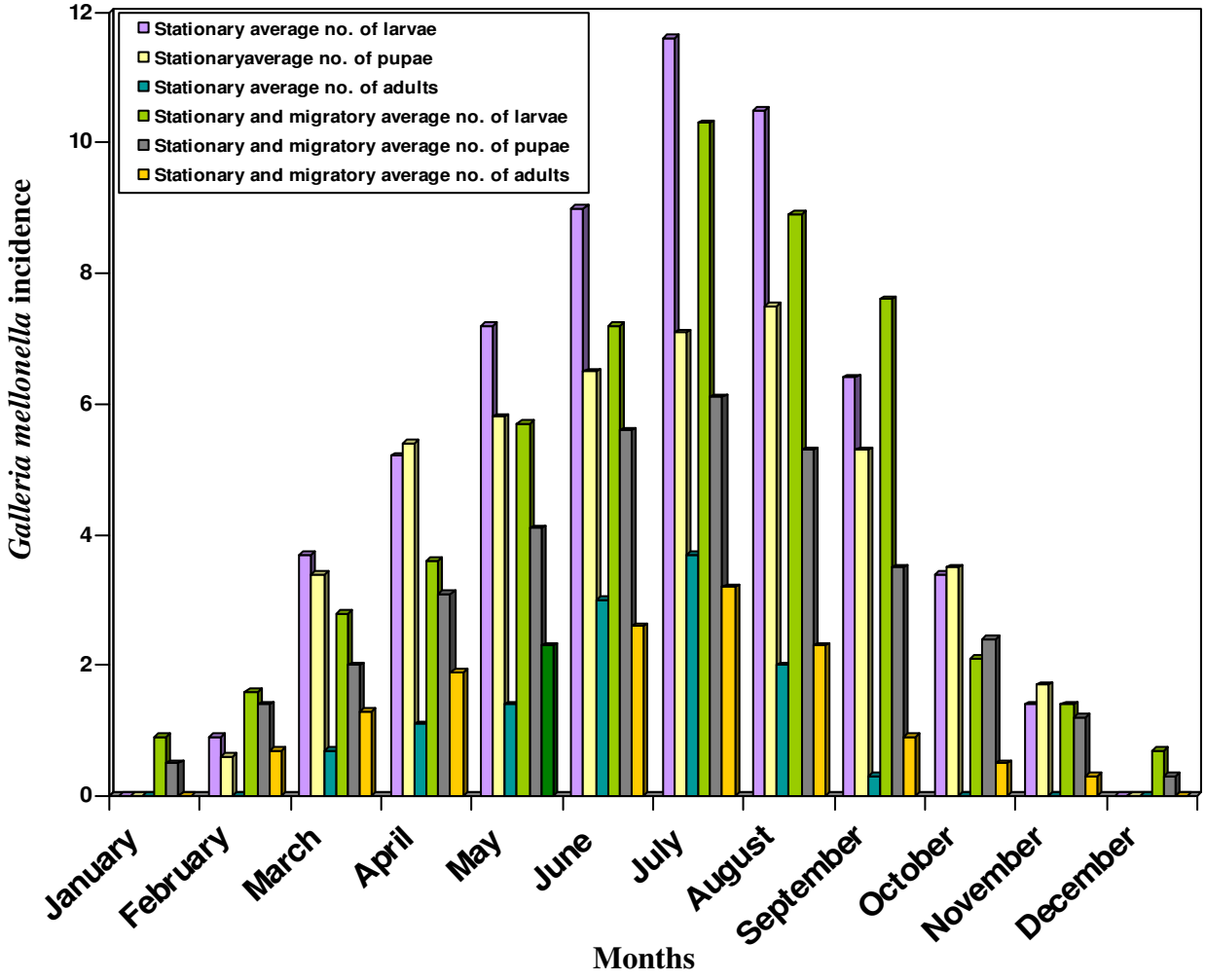


Fig 17. Incidence of greater wax moth (*Galleria mellonella)* in *A. mellifera* under stationary and migratory conditions (2019-2020)

The observations recorded on fourteen day after treatment showed that the minimum comb area damaged was recorded in *Bt* (8.01 cm^2) which was statistically at par with neem seed kernel extract (11.67 cm^2) and sulphur fumigation (12.46 cm^2) followed by damaged area in neem oil treatment (16.85cm^2) which was statistically at par with neem leaf powder treatment (22.90 cm^2). The damaged comb area in acetic acid treatment was 25.32 cm^2 which was statistically at par with karanj oil treatment (27.74 cm^2) followed by formic acid treatment (33.53 cm^2), *T. viridae* (80.13 cm^2) which was statistically at par with *B. bassiana* (88.63 cm^2) and *M. anisopliae* (90.69 cm^2).The infested area in alsi seed extract was 117.54 cm^2 followed by pumpkin seed extract (132.82 cm^2) treatment. Significantly maximum infested area was found in control (170.63 cm^2). Fourteen day after treatment of the combs, minimum per cent damaged comb area was recorded in *Bt* (2.95%) which was statistically at par with neem seed kernel extract (4.29%) followed by sulphur fumigation (5.94 %) which was statistically at par with neem oil treatment (6.20%). The per cent damaged area in neem leaf powder treatment was 8.42% which was statistically at par with karanj oil treatment (10.20%) and acetic acid treatment (10.67%) followed by formic acid (13.69%), *T. viridae* (29.46%) which was statistically at par with *B. bassiana* (32.59 %) and *M. anisopliae* (33.34%) treatment. The per cent infested area in alsi seed extract was 43.22% followed by pumpkin seed extract (48.83%). Significantly maximum infested area was found in control (62.73%).

After 21 day of treatment, *Bt* showed significantly minimum damaged comb area (8.65 cm^2) followed by neem seed kernel extract (14.09 cm^2) which was statistically at par with sulphur fumigation (17.81 cm^2). The damaged area in neem oil treatment was 20.22cm^2 followed by neem leaf powder (26.96 cm^2) which was statistically at par with acetic acid treatment (32.15 cm^2) followed by karanj oil treatment (33.91cm^2) which was further statistically at par with formic acid treatment (37.81 cm^2). The per cent infested area in *T. viridae* was 102.71 cm^2 followed by *B. bassiana* (110.79 cm^2) which was statistically at par with *M. anisopliae* (114.57 cm^2). In alsi seed extract, the per cent damaged area was 162.55 cm^2 followed by pumpkin seed extract (185.48 cm^2). Significantly maximum damaged area was found in control (222.07 cm^2).

Table 52. Area damaged and per cent area infestation per comb in *A. mellifera* wax combs due to greater wax moth (*G. mellonella)* during 2020

Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	10.25 (3.35)#	3.77 (2.18)	16.85 (4.22)	6.20 (2.68)	20.22 (4.61)	7.44 (2.90)
Dried neem leaf powder	8.33g	0.00	15.33 (4.04)	5.64 (2.58)	22.90 (4.89)	8.42 (3.07)	26.96 (5.29)	9.91 (3.30)
Neem seed kernel extract	5%	0.00	7.29 (2.88)	2.68 (1.92)	11.67 (3.56)	4.29 (2.30)	14.09 (3.88)	5.18 (2.49)
Karanj oil	3%	0.00	23.26 (4.93)	8.55 (3.09)	27.74 (5.36)	10.20 (3.35)	33.91 (5.91)	12.47 (3.67)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	67.84 (8.30)	24.94 (5.09)	117.54 (10.89)	43.22 (6.65)	162.55 (12.79)	59.76 (7.79)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	74.19 (8.67)	27.28 (5.32)	132.82 (11.57)	48.83 (7.06)	185.48 (13.66)	68.19 (8.32)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	4.31 (2.30)	1.59 (1.61)	8.01 (3.00)	2.95 (1.99)	8.65 (3.11)	3.18 (2.04)
Trichoderma viridae	2ml/ l water	0.00	48.48 (7.03)	17.83 (4.34)	80.13 (9.01)	29.46 (5.52)	102.71 (10.18)	37.76 (6.23)
Bauveria bassiana	2ml/ l water	0.00	53.55 (7.39)	19.69 (4.55)	88.63 (9.47)	32.59 (5.80)	114.57 (10.75)	42.12 (6.57)
Metarhizium anisopliae	2ml/ l water	0.00	57.85 (7.67)	21.27 (4.72)	90.69 (9.58)	33.34 (5.86)	110.79 (10.57)	40.73 (6.46)
Acetic acid spray	2ml/ l water	0.00	21.62 (4.76)	7.95 (2.99)	25.32 (5.13)	10.67 (3.42)	32.15 (5.76)	13.09 (3.75)
Formic acid spray	0.8ml/l water	0.00	29.83 (5.55)	10.97 (3.46)	33.53 (5.88)	13.69 (3.83)	37.81 (6.23)	15.17 (4.02)
Use of sulphur fumigation	5g	0.00	9.53 (3.24)	3.50 (2.12)	12.46 (3.67)	5.94 (2.63)	17.81 (4.34)	7.82 (2.97)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control		0.00	120.90 (11.04)	44.45 (6.74)	170.63 (13.10)	62.73 (7.98)	228.07 (15.14)	83.85 (9.21)
C.D. (0.05)			(0.84)	(0.48)	(0.67)	(0.39)	(0.53)	(0.31)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

The data on per cent damaged comb area after 21 day of treatments revealed that minimum per cent comb area damaged was recorded in *Bt* (3.18%) followed by neem seed kernel extract (5.18%), neem oil (7.44%) which was statistically at par with sulphur fumigation (7.82%). The per cent damaged area in neem leaf powder treatment was 9.91% followed by karanj oil treatment (12.47%) which was statistically at par with acetic acid treatment (13.09%). The per cent infested area in formic acid treatment was 15.17% followed by *T. viridae* (37.76%), *B. bassiana* (40.73%) which was statistically at par with *M. anisopliae* (42.12%). The per cent damage in alsi seed extract treatment was 59.76% followed by pumpkin seed extract (68.19%). Significantly maximum infested area was found in control (83.85%).

b) During 2021

Data on comb area damaged by *G. mellonella* in *A. mellifera* combs presented in Table 53 indicated that the minimum damaged comb area after 7 day of treatment was recorded in *Bt* (7.60 cm^2) which was statistically at par with neem seed kernel extract (10.58 cm^2) followed by neem oil treatment (12.42 cm^2) which was statistically at par with sulphur fumigation (13.50 cm^2) and neem leaf powder (14.82 cm^2). The damaged comb area in acetic acid treatment was 24.24 cm^2 which was statistically at par with karanj oil treatment (27.23 cm^2) and formic acid treatment (31.15 cm^2). The damaged area in *T. viridae* was 44.27cm^2 followed by *B. bassiana* (55.52 cm^2) which was statistically at par with *M. anisopliae* (55.93 cm^2) followed by alsi seed extract (71.81 cm^2) which was statistically at par with pumpkin seed extract (82.61 cm^2). Significantly maximum infested area was found in control (130.27 cm^2). After 7 day of treatment the minimum per cent damaged comb area was recorded in *Bt* (2.80%) which was statistically at par with neem seed kernel extract (3.89 %) followed by neem oil treatment (4.57%) which was statistically at par with sulphur fumigation (4.96%) and neem leaf powder treatment (5.45%). The per cent damaged comb area in acetic acid treatment was 8.91% which was statistically at par with karanj oil treatment (10.01 %) and formic acid treatment (11.45%). The per cent damaged area in *T. viridae* was 16.28% followed by *B. bassiana* (20.41%) which was statistically at par with *M. anisopliae* (20.56%) followed by alsi seed extract treatment (26.40%) which was statistically at par with pumpkin seed extract (30.37%). Significantly maximum per cent infested area was found in control (47.89%). No infestation was found in deep freezing treatment.

Fourteen day after treatment, the results showed that the minimum infested comb area was recorded in *Bt* (9.62 cm^2) which was statistically at par with sulphur fumigation (10.80 cm^2) followed by neem seed kernel extract (16.76 cm^2) which was statistically at par with neem oil treatment (18.42 cm^2) and neem leaf powder treatment (22.08 cm^2). The damaged comb area in acetic acid treatment was 28.97 cm^2 which was statistically at par with karanj oil treatment (35.57 cm^2) and formic acid treatment (37.18 cm^2) followed by *T. viridae* (78.75cm^2), *B. bassiana* (85.08 cm^2) which was statistically at par with *M. anisopliae* (94.34 cm^2). The damaged comb area in alsi seed extract was 112.23 cm^2 followed by pumpkin seed extract (136.47 cm^2). Significantly maximum infested area was found in control (174.27 cm^2). Data on area damaged on 14 day after treatment revealed that minimum per cent comb area damaged was recorded in *Bt* (3.54%) which was statistically at par with sulphur fumigation (5.33%) followed by neem seed kernel extract (6.16%) which was statistically at par with neem oil (6.77%) and neem leaf powder treatment (8.12%). The per cent damaged area in acetic acid treatment was 12.01 cm^2 which was statistically at par with karanj oil treatment (13.08%) and formic acid treatment (15.03%) followed by *T. viridae* (28.95%) which was statistically at par with *B. bassiana* (31.28 %) and *M. anisopliae* (34.68%). In alsi seed extract the per cent damaged area was 41.26% followed by pumpkin seed extract (50.17%). Significantly maximum per cent infested area was observed in control (64.07%).

After 21 day of treatment, significantly minimum damaged comb area was observed in *Bt* treated combs (12.81 cm^2) followed by neem seed kernel extract (18.25 cm^2) which was statistically at par with sulphur fumigation (21.97 cm^2). The damaged area in neem oil treatment was 24.38 cm^2 followed by neem leaf powder treatment (31.12 cm^2), karanj oil treatment (38.07) which was statistically at par with acetic acid treatment (38.11 cm^2) and formic acid treatment (45.55 cm^2). The damaged area in *T. viridae* was 95.26 cm^2 followed by *B. bassiana* (109.85 cm^2) which was statistically at par with *M. anisopliae* (114.95 cm^2) followed by alsi seed extract (172.15 cm^2) which was statistically at par with pumpkin seed extract (182.34 cm^2). Significantly maximum infested area was found in control (238.67 cm^2).The data on per cent damaged comb area after 21 day of treatment revealed that minimum per cent damaged comb area was recorded in *Bt* (4.71 %) which was statistically at par with neem seed kernel extract (6.71 %) followed by neem oil treatment (8.97 %) which was statistically at par with sulphur fumigation (9.35 %) and neem leaf powder treatment (11.44 %).

Table 53. Area damaged and per cent area infestation per comb in *A. mellifera* wax combs due to greater wax moth (*G. mellonella)* during 2021

Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	12.42 (3.66)[#]	4.57 (2.36)	18.42 (4.41)	6.77 (2.79)	24.38 (5.04)	8.97 (3.16)
Dried neem leaf powder	8.33g	0.00	14.82 (3.98)	5.45 (2.54)	22.08 (4.80)	8.12 (3.02)	31.12 (5.67)	11.44 (3.53)
Neem seed kernel extract	5%	0.00	10.58 (3.40)	3.89 (2.21)	16.76 (4.21)	6.16 (2.68)	18.25 (4.39)	6.71 (2.78)
Karanj oil	3%	0.00	27.23 (5.31)	10.01 (3.32)	35.57 (6.05)	13.08 (3.75)	38.07 (6.25)	14.00 (3.87)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	71.81 (8.53)	26.40 (5.23)	112.23 (10.64)	41.26 (6.50)	172.15 (13.16)	63.29 (8.02)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	82.61 (9.14)	30.37 (5.60)	136.47 (11.72)	50.17 (7.15)	182.34 (13.54)	67.04 (8.25)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	7.60 (2.93)	2.80 (1.95)	9.62 (3.26)	3.54 (2.13)	12.81 (3.72)	4.71 (2.39)
Trichoderma viridae	2ml/ l water	0.00	44.27 (6.73)	16.28 (4.16)	78.75 (8.93)	28.95 (5.47)	95.26 (9.81)	35.02 (6.00)
Bauveria bassiana	2ml/ l water	0.00	55.52 (7.52)	20.41 (4.63)	85.08 (9.28)	31.28 (5.68)	109.85 (10.53)	40.39 (6.43)
Metarhizium anisopliae	2ml/ l water	0.00	55.93 (7.55)	20.56 (4.64)	94.34 (9.76)	34.68 (5.97)	114.95 (10.77)	42.26 (6.58)
Acetic acid spray	2ml/ l water	0.00	24.24 (5.02)	8.91 (3.15)	28.97 (5.47)	12.01 (3.61)	38.11 (6.25)	15.28 (4.03)
Formic acid spray	0.8ml/l water	0.00	31.15 (5.67)	11.45 (3.53)	37.18 (6.18)	15.03 (4.00)	45.55 (6.82)	18.02 (4.36)
Use of sulphur fumigation	5g	0.00	13.50 (3.81)	4.96 (2.44)	10.80 (3.43)	5.33 (2.52)	21.97 (4.79)	9.35 (3.22)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control		0.00	130.27 (11.46)	47.89 (6.99)	174.27 (13.24)	64.07 (8.07)	238.67 (15.48)	87.75 (9.42)
C.D. (0.05)			(0.65)	(0.38)	(0.72)	(0.52)	(0.57)	(0.39)

[#]Figures in parentheses are square root (x+1) transformed values

*Days after treatment

The per cent damaged area in karanj oil treatment was 14.00% which was statistically at par with acetic acid treatment (15.28%) followed by formic acid treatment (18.02%), *T. viridae* (35.02%),' *B. bassiana* (40.39 %) which was statistically at par with *M. anisopliae* (42.26 %). The per cent infested area in alsi seed extract treatment was 63.29% which was statistically at par with pumpkin seed extract (67.04%). Significantly maximum damaged area after 21 days of treatment was observed in control (87.75%).

c) Pooled data (2020-2021)

Data on comb area damaged by *G. mellonella* in *A. mellifera* combs is presented in Table 54 and Fig.18. It indicated that significantly minimum damaged comb area after seven day of treatment was recorded in *Bt* (5.03 cm^2) followed by neem seed kernel extract (8.87 cm^2) which was statistically at par with sulphur fumigation (11.14 cm^2) and neem oil treatment (11.77 cm^2). The damaged comb area in neem leaf powder treatment was 16.62 cm^2followed by karanj oil treatment (23.17 cm^2) which was statistically at par with acetic acid treatment (24.90 cm^2). The damaged area in formic acid treatment was 30.23 cm^2 followed by *T. viridae* (47.27 cm^2) which was statistically at par with *B. bassiana* (51.88 cm^2). In *M. anisopliae,* the damaged area was 60.97 cm^2 which was at par with alsi seed extract (65.09 cm^2) followed by pumpkin seed extract (75.99 cm^2). Significantly maximum infested area was found in control (122.02 cm^2). After 7 day of treatment, the minimum per cent damaged comb area was recorded in *Bt* (2.19%) which was statistically at par with neem seed kernel extract (3.29%) followed by neem oil treatment (4.17%) which was statistically at par with sulphur fumigation (4.23%) and neem leaf powder treatment (5.54%). The per cent damaged comb area in acetic acid treatment was 8.43 % which was statistically at par with karanj oil treatment (9.28%) followed by formic acid treatment (11.21%), *T. viridae* (17.05%) , *B. bassiana* (20.05%) which was statistically at par with *M. anisopliae* (20.92%). Per cent damaged area in alsi seed extract was 25.67% which was statistically at par with pumpkin seed extract (28.83%). Significantly maximum infested area was found in control (46.17%). No infestation was found in deep freezing (-8°C to -10°C) treatment and all the third instar larvae got killed after 5 hours in deep freezer treatment (Plate 26).

Fourteen day after treatment, the results showed that the minimum damaged comb area was recorded in *Bt* (8.81 cm^2) which was statistically at par with sulphur fumigation (11.63 cm^2) followed by neem seed kernel extract (14.21 cm^2), neem oil treatment

(17.64 cm^2) which was statistically at par with neem leaf powder treatment (22.49 cm^2). The damaged comb area in acetic acid treatment was 27.15 cm^2 which was statistically at par with karanj oil treatment (31.66 cm^2) followed by formic acid treatment (35.35 cm^2), *T. viridae* (79.44 cm^2), *B. bassiana* (86.86 cm^2) which was statistically at par with *M. anisopliae* (92.25 cm^2). The per cent infested area in alsi seed extract was 114.89 cm^2 followed by pumpkin seed extract (134.65cm^2). Significantly maximum damaged area was found in control (172.45 cm^2). After 7 day of treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (3.24 %) followed by neem seed kernel extract (5.23 %) which was statistically at par with sulphur fumigation (5.64 %) and neem oil treatment (6.48 %). The per cent damaged area in neem leaf powder treatment was 8.27 % followed by acetic acid treatment (11.34 %) which was statistically at par with karanj oil treatment (11.64 %) followed by formic acid treatment (14.36%), *T. viridae* (29.21%) , *B. bassiana* (31.93%) which was statistically at par with *M. anisopliae* (34.01%). Per cent damaged area in alsi seed extract was 42.24% followed by pumpkin seed extract (49.50%). Significantly maximum infested area was found in control (63.40%).

After 21 day of treatment, the minimum damaged comb area was recorded in *Bt* treated combs (12.27 cm^2) which was statistically at par with neem seed kernel extract (15.90 cm^2) followed by neem oil treatment (20.39 cm^2) which was statistically at par with sulphur fumigation (20.44 cm^2). The damaged comb area damaged in neem leaf powder treatment was 28.74 cm^2 which was statistically at par with karanj oil treatment (32.13 cm^2) followed by acetic acid treatment (35.52 cm^2), formic acid treatment (46.73 cm^2), *T. viridae* (98.59 cm^2), *M. anisopliae* (110.13 cm^2) which was statistically at par with *B. bassiana* (114.47 cm^2). The damaged area in alsi seed extract was 164.03 cm^2 followed by pumpkin seed extract (180.87cm^2). Significantly maximum infested area was found in control (232.37 cm^2). After 21 day of treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (3.95 %) followed by neem seed kernel extract (5.95 %), neem oil treatment (8.20 %) which was statistically at par with sulphur fumigation (8.58 %). The per cent damaged area in neem leaf powder treatment was 10.68 % followed by karanj oil treatment (13.23 %) which was at par with acetic acid treatment (14.19 %) followed by formic acid treatment (16.59%), *T. viridae* (36.39%) , *B. bassiana* (41.25%) which was statistically at par with *M. anisopliae* (41.50%). Per cent damaged area in alsi seed extract was 61.65% followed by pumpkin seed extract (67.61%). Significantly maximum infested area was found in control (85.80%).

Table 54. Pooled data on area damaged and per cent area infestation per comb in *A. mellifera* wax combs due to greater wax moth (*G. mellonella)* during 2020-2021

Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	11.77 (3.57)#	4.17 (2.27)	17.64 (4.32)	6.48 (2.74)	20.39 (4.63)	8.20 (3.03)
Dried neem leaf powder	8.33g	0.00	16.62 (4.20)	5.54 (2.56)	22.49 (4.85)	8.27 (3.04)	28.74 (5.45)	10.68 (3.42)
Neem seed kernel extract	5%	0.00	8.87 (3.14)	3.29 (2.07)	14.21 (3.90)	5.23 (2.49)	15.90 (4.11)	5.95 (2.64)
Karanj oil	3%	0.00	23.17 (4.92)	9.28 (3.21)	31.66 (5.71)	11.64 (3.56)	32.13 (5.76)	13.23 (3.77)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	65.09 (8.13)	25.67 (5.16)	114.89 (10.77)	42.24 (6.58)	164.03 (12.85)	61.53 (7.91)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	75.99 (8.77)	28.83 (5.46)	134.65 (11.65)	49.50 (7.11)	180.87 (13.49)	67.61 (8.28)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	5.03 (2.45)	2.19 (1.79)	8.81 (3.13)	3.24 (2.06)	12.27 (3.64)	3.95 (2.22)
Trichoderma viridae	2ml/ l water	0.00	47.27 (6.95)	17.05 (4.25)	79.44 (8.97)	29.21 (5.50)	98.59 (9.98)	36.39 (6.11)
Bauveria bassiana	2ml/ l water	0.00	51.88 (7.27)	20.05 (4.59)	86.86 (9.37)	31.93 (5.74)	114.47 (10.75)	41.25 (6.50)
Metarhizium anisopliae	2ml/ l water	0.00	60.97 (7.87)	20.92 (4.68)	92.51 (9.67)	34.01 (5.92)	110.13 (10.54)	41.50 (6.52)
Acetic acid spray	2ml/ l water	0.00	24.90 (5.09)	8.43 (3.07)	27.15 (5.31)	11.34 (3.51)	35.52 (6.04)	14.19 (3.90)
Formic acid spray	0.8ml/l water	0.00	30.23 (5.59)	11.21 (3.49)	35.35 (6.03)	14.36 (3.92)	46.73 (6.91)	16.59 (4.19)
Use of sulphur fumigation	5g	0.00	11.14 (3.48)	4.23 (2.29)	11.63 (3.55)	5.64 (2.58)	20.44 (4.63)	8.58 (3.10)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control		0.00	122.02 (11.09)	46.17 (6.87)	172.45 (13.17)	63.40 (8.02)	232.37 (15.28)	85.80 (9.32)
C.D. (0.05)			(0.53)	(0.30)	(0.54)	(0.31)	(0.56)	(0.33)

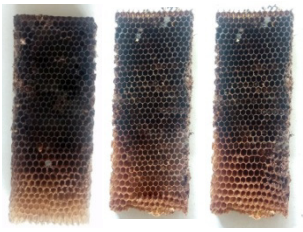
#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

Different management tactics such as use of plant products *viz.,* neem seed kernal extract (5%), alsi seed extract (20g/100 ml acetone), Pumpkin seed extract (20g/ 100 ml acetone); essential oils like neem oil (3ml/100 ml water), karanj oil (3ml/100 ml water); fungi like *T. viridae* (2 ml/l water)**,** *B. bassiana* (2 ml/l water), *M. anisopliae* (2 ml/l water) and organic acids *viz.,* acetic acid (2ml/ l water) and formic acid (0.8ml/l water) were sprayed or dusted on *A. mellifera* combs. Similarly, *B. thuringiensis* var. *kurstaki* @ 5g/ l water was sprayed in the wax combs; mechanical practices such as keeping the comb in deep freezer at -8°C to -10°C for 5 hours along with sulphur fumigation of combs were evaluated. Only third instar larvae of *G. mellonella* were released on the treated combs as this instar was reported to be most motile and injurious to the honey bees combs (Rahman *et al.,* 2017). In the present studies, 100% wax moth larval mortality was recorded within 5 hours of deep freezing treatment. So, per cent damaged comb area and per cent comb infestation was nil. Shimanuki (1981) reported similar results while using deep freezer for the control of wax moth and specified minimum cold temperature and storage time 20ºF (-7ºC) for 4.5 hours, 10ºF (-12ºC) for three hours, or 5ºF (-15ºC) to kill all life stages of wax moths in honey-extracted combs. The British Beekeepers Association (2012) also supported deep freezing of combs as an effective method of wax moth control. According to them, if a large chest freezer is available the combs can be frozen and this prevents egg hatching, kills caterpillars and prevents combs from being re-infested. Our findings got support from that of Kumar and Khan (2019) who found that the highest wax moth larval mortality (100%) was observed in case of deep freezer (-8°C to -10°C) when all the larvae got killed. Earlier workers also recommended deep freezing as an effective method of wax moth control (Cusman, 2002; Goodman, 2003, Rusty, 2011; Katna *et al.,* 2012). Kwadha *et al.* (2017) reported 100 per cent mortality of wax moth larvae with no damage to the combs exposed to cold rooms or refrigerator such as home freezers set at -7°C to -15 °C for 2-4.5 hours as the growth and development of greater wax moth is dependent on environmental factors such as temperature. The per cent area infestation per comb in greater wax moth infested combs with cold treatment at -15°C had minimum per cent infestation (0.04%) (Pawar, 2018).

Variations in the damaged comb area and per cent infested comb were observed with all other treatments. In present studies, lowest damaged comb area and per cent infestation per comb was recorded in *Bt* (12.27 cm^2 and 3.95%). Present findings are in conformity with that of Verma (1995) who reported highest mortality (98.74%) of wax moth in honey bee

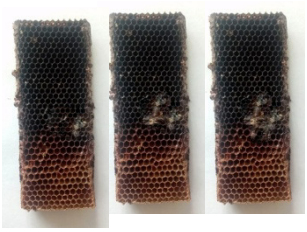
colonies sprayed with a suspension of Dipel (10%) and observed no death of bees brood in treated colonies. Viraktamath *et al.* (2005) reported 56 to 80% larval mortality of *G. mellonella* by using different *Bt* formulations *viz.*, Dipel, Delfin, Biobit, Biolep and Bioasp. Combs of honey bees sprayed with *Bt* var *kurstaki* @ 5g per 100 ml of water resulted in 85% mortality of third instar larvae of wax moth (Magno *et al.,* 2005). The present investigations are also in agreement with the Bhopale *et al*. (2013) reported that cell damage ranged from 12.00% to 20.00% using *Bt kurstaki* (Halt), *Bt* local strain-1 and neem oil with minimum cell damage in *Bt kurstaki* (Halt). They found that dried neem leaf powder and *Bt* local strain-2 performed poorly with 32.22 to 37 per cent cell damage, respectively. Rahman *et al.* (2017) reported that *Bt* var *kurstaki* at 1 per cent concentration was found to be most effective in minimizing the per cent comb infestation and cumulative mortality of the larvae were found to be 93.32 per cent after 96 hours of treatment. Vijaykumar *et al.* (2019) compared the efficacy of two *Bt* products, commercial V-*Bt* and local strain (HD-1) against different instars of greater wax moth at 3 concentrations, *viz.,* 3, 6, 9 g per litre and showed that the early instar larvae of greater wax moth were highly susceptible to commercial V-*Bt* when compared to the local *Bt* strain HD-1, V-*Bt* caused higher mortality of third instar at its highest concentration 9 g per litre (96.67%). Azadirachtin showed anti- feedant effects and among insect order Lepidoptera was found to be most susceptible as compared to other orders *viz.,* Coleoptera, Hemiptera and Homoptera. The application of each low dosages of azadirachtin caused loss of vigour or fitness of insects. Beside azadirachtin, a large number of other compounds present in neem seed extract reported to exhibit biological actions (Dorn *et al*., 1987; Ascher, 1993 and Mordue and Blackwell, 1993).The observed mortality of wax moth larvae with these neem formulations were dose dependent. Bisht *et al.* (2017) demonstrated that the neem formulations at various concentrations exhibited varying degree of activity against the larvae of *G. mellonella*. The highest mortality was recorded for 5% neem oil (65.33% at 72 h) followed by 5% NSKE (51.67%).

Abou- Shara (2020) investigated the effect of three doses of *B. bassiana* (1,3 and 6 g per larva) on the development of greater wax moth under laboratory conditions and showed that lowest dose resulted in 80% normal development. While 3g per larva caused 20% mortality but 70 per cent of pupae failed to emerge as adults and the highest dose showed most deleterious effects and completely impaired development caused 100% larval death.



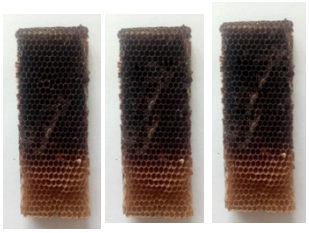
7 DAT 14 DAT 21 DAT

T1- Neem oil



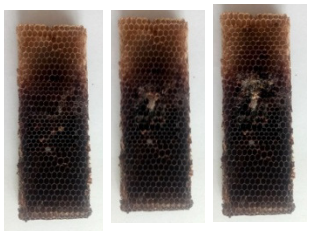
7 DAT 14 DAT 21 DAT

T2- Neem leaf powder



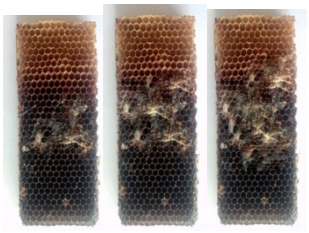
7 DAT 14 DAT 21 DAT

T3- Neem seed kernal extract



7 DAT 14 DAT 21 DAT

T4- Karanj oil

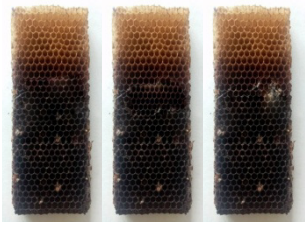


7 DAT 14 DAT 21 DAT

T5- Alsi seed extract

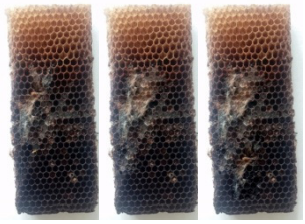
7 DAT 14 DAT 21 DAT

T6- Pumpkin seed extract



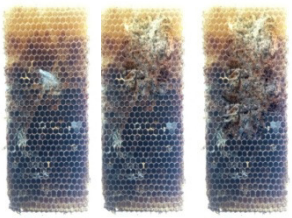
7 DAT 14 DAT 21 DAT

T7- *Bacillus thuringiensis*



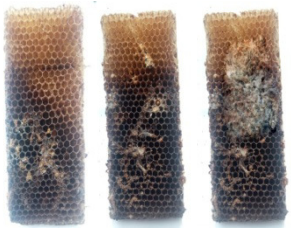
7 DAT 14 DAT 21 DAT

T8- *Trichoderma viridae*



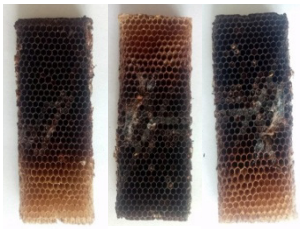
7 DAT 14 DAT 21 DAT

T9-Beauveria bassiana



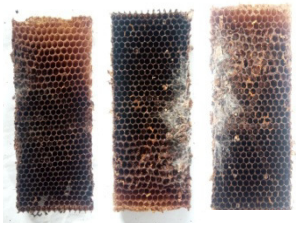
7 DAT 14 DAT 21 DAT

T10- *Metarrhizium anisopliae*



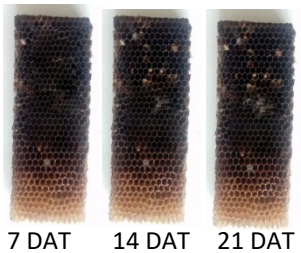
7 DAT 14 DAT 21 DAT

T11-Acetic acid



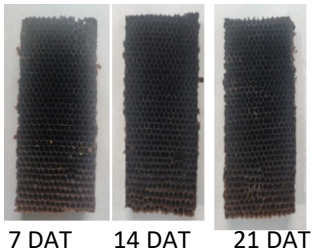
7 DAT 14 DAT 21 DAT

T12-Formic acid



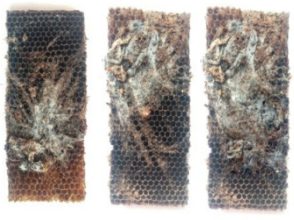
7 DAT 14 DAT 21 DAT

T13-Sulphur fumigation



7 DAT 14 DAT 21 DAT

T14-Deep freezing



7 DAT 14 DAT 21 DAT

T15- Control

Plate 26. Damage and infestation of greater wax moth in *A. mellifera* combs at 7,14 and 21 days after treatments (DAT)

The efficacy of commercial bio-pesticides of three fungal pathogens (*M. anisopliae*, *B. bassiana* and *T. viride)* were tested individually or in combination with an indigenous entomopathogenic nematode, *Heterorhabditis indica* on greater wax moth under laboratory conditions and pathogenic interaction was assessed at every 12 hours interval after storage *B. bassiana* imposed greater mortality (40%) when tested in isolation compared to other bio-pesticides after 24 hours of storage (Sankara *et al.,* 2009). Calderone (2000) advocated that sulphur fumigation could be used for controlling wax moth. Kumari and Jha (2013) observed that maximum comb area with *G. mellonella* infestation occurred in bee frames stored in chambers kept in a store without fumigation, whereas minimum was in case of frames stored in the polythene sheet with fumigation. Frames stored in polythene sprayed with 3 per cent neem oil after drying in shade proved its superiority as the next best treatment in minimizing the per cent wax moth infestation. Maximum infestation (85.80%) in present studies was reported in control. Munshimbwe *et al*. (2011) suggested that 80% solution of acetic acid could be used to control wax moths in stored frames on top-bars. Charriere and Imdorf (1999) reported that acetic acid and formic acid vapours instantly kills eggs, larvae and moths of *G. mellonella.* The larvae, especially in the cocoon are although resistant to chemical and must be exposed to the vapours for longer period. Alsi seed extract and pumpkin seed extract are less effective treatment and showed maximum infested area after control (61.53% and 67.61%). In our studies, the per cent damaged area in karanj oil treatment was 13.23% which was equally effective as acetic acid treatment with 14.14% infestation and formic acid with 16.59% damaged area.

4.3.2 Area damaged and per cent area infestation per comb in *A. cerana* wax combs due to greater wax moth (*G. mellonella)* under laboratory conditions

a) During 2020

Data on damaged comb area by *G. mellonella* in *A. cerana* combs presented in Table 55 indicated that the minimum damaged comb area after 7 days of treatment was recorded in *Bt* treatment (8.21 cm^2) which was statistically at par with neem seed kernel extract (11.89 cm^2) and sulphur fumigation (13.16 cm^2) followed by neem oil treatment (14.82 cm^2) which was statistically at par with neem leaf powder treatment (19.54 cm^2). The damaged comb area in acetic acid treatment was 25.48 cm^2 followed by formic acid (32.56 cm^2) treatment which was statistically at par with karanj oil treatment (35.99 cm^2). The damaged area in *T. viridae* was 45.30 cm^2 which was statistically at par with *B. bassiana* (50.56 cm^2)

and *M. anisopliae* (58.21 cm^2) followed by alsi seed extract (69.39 cm^2) which was statistically at par with pumpkin seed extract (82.16cm^2). Significantly maximum infested area was found in control (126.28 cm^2). After 7 days of treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (3.02%) which was statistically at par with neem seed kernel extract (4.37%) and sulphur fumigation (4.84%) followed by neem oil treatment (5.45%) which was statistically at par with neem leaf powder treatment (7.19%). The per cent damaged area in acetic acid treatment treatment was 9.37% which was statistically at par with formic acid treatment (11.97%) followed by karanj oil treatment (13.23%) which was at par with *T. viridae* (16.66%). The per cent area infested in *B. bassiana* was 18.59% which was statistically at par with *M. anisopliae* (21.40%) followed by alsi seed extract (25.51%) which was at par with pumpkin seed extract (30.21%). Significantly maximum infested area was found in control (46.43%). No infestation was found in deep freezing (-8°C to -10°C) treatment.

The observations recorded on fourteen days after treatment, showed that the minimum damaged comb area was recorded in *Bt* (11.17 cm^2) which was statistically at par with neem seed kernel extract (15.10 cm^2) followed by neem oil treatment (17.86cm^2) which was statistically at par with sulphur fumigation (19.10 cm^2). The damaged area in neem leaf powder was 25.33 cm^2 which was statistically at par with acetic acid treatment (26.76 cm^2) followed by formic acid treatment (34.59 cm^2) treatment which was statistically at par with karanj oil treatment (34.89 cm^2). The damaged area in *T. viridae* was 90.47 cm^2 followed by *B. bassiana* (101.66 cm^2) which was statistically at par with *M. anisopliae* (108.33 cm^2). Damaged area in alsi seed extract was 135.67 cm^2 followed by pumpkin seed extract (169.40cm^2). Significantly maximum damaged area was found in control (183.90 cm^2). Fourteen days after treatment, minimum per cent damaged comb area was recorded in *Bt* (4.11%) which was statistically at par with neem seed kernel extract (5.55%) followed by neem oil treatment (6.57%) which was at par with sulphur fumigation (7.02%). The per cent damaged area in neem leaf powder treatment was 9.31% which was statistically at par with acetic acid treatment (9.84%) followed by karanj oil treatment (12.72%) which was statistically at par with formic acid treatment (12.81%). The area damaged in *T. viridae* was 33.26% followed by the per cent area infested in *B. bassiana* (37.38%) which was statistically at par with *M. anisopliae* (40.01%). Maximum infested area was found in control (67.61%) which was at par with pumpkin seed extract (62.28%).

Table 55. Area damaged and per cent area infestation per comb in *A. cerana* wax combs due to greater wax moth (*G. mellonella)* during 2020

Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	14.82 (3.98)#	5.45 (2.54)	17.86 (4.34)	6.57 (2.75)	30.55 (5.62)	9.73 (3.28)
Dried neem leaf powder	8.33g	0.00	19.54 (4.53)	7.19 (2.86)	25.33 (5.13)	9.31 (3.21)	33.21 (5.85)	12.38 (3.66)
Neem seed kernel extract	5%	0.00	11.89 (3.59)	4.37 (2.32)	15.10 (4.01)	5.55 (2.56)	21.38 (4.73)	7.78 (2.96)
Karanj oil	3%	0.00	35.99 (6.08)	13.23 (3.77)	34.59 (5.97)	12.72 (3.70)	41.07 (6.49)	14.59 (3.95)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	69.39 (8.39)	25.51 (5.15)	135.67 (11.69)	49.88 (7.13)	150.23 (12.30)	57.33 (7.64)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	82.16 (9.12)	30.21 (5.59)	169.40 (13.05)	62.28 (7.95)	184.25 (13.61)	71.18 (8.50)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	8.21 (3.03)	3.02 (2.00)	11.17 (3.49)	4.11 (2.26)	12.95 (3.73)	4.94 (2.44)
Trichoderma viridae	2ml/ l water	0.00	45.30 (6.80)	16.66 (4.20)	90.47 (9.56)	33.26 (5.85)	113.04 (10.68)	44.32 (6.73)
Bauveria bassiana	2ml/ l water	0.00	50.56 (7.18)	18.59 (4.43)	101.66 (10.13)	37.38 (6.19)	118.13 (10.91)	48.26 (7.02)
Metarhizium anisopliae	2ml/ l water	0.00	58.21 (7.69)	21.40 (4.73)	108.83 (10.48)	40.01 (6.40)	139.35 (11.85)	51.00 (7.21)
Acetic acid spray	2ml/ l water	0.00	25.48 (5.15)	9.37 (3.22)	26.76 (5.27)	9.84 (3.29)	38.22 (6.26)	16.09 (4.13)
Formic acid spray	0.8ml/l water	0.00	32.56 (5.79)	11.97 (3.60)	34.84 (5.99)	12.81 (3.72)	87.72 (9.42)	21.77 (4.77)
Use of sulphur fumigation	5g	0.00	13.16 (3.76)	4.84 (2.42)	19.10 (4.48)	7.02 (2.83)	23.12 (4.91)	7.64 (2.94)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control		0.00	126.28 (11.28)	46.43 (6.89)	183.90 (13.60)	67.61 (8.28)	240.64 (15.54)	90.21 (9.55)
C.D. (0.05)			(0.89)	(0.52)	(0.58)	(0.35)	(0.69)	(0.41)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

After 21 days of treatment, significantly minimum damaged area was recorded in *Bt* (12.95 cm^2) followed by neem seed kernel extract (21.38 cm^2) which was statistically at par with sulphur fumigation (23.12 cm^2). In neem oil treatment, the damaged area was 30.55 cm^2 followed by neem leaf powder (33.21cm^2) which was statistically at par with acetic acid treatment (38.22 cm^2) and karanj oil treatment (41.07 cm^2) .The damaged area in formic acid treatment was 87.92 cm^2 followed by *T. viridae* (113.04 cm^2), *B. bassiana* (118.13 cm^2) which was statistically at par with *M. anisopliae* (139.35 cm^2) and alsi seed extract (150.23 cm^2) followed by pumpkin seed extract (184.25 cm^2). Significantly maximum infested area was found in control (240.64 cm^2). Twenty one days after treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (4.94%) followed by sulphur fumigation (7.64%) which was statistically at par with neem seed kernel extract (7.78%) and neem oil treatment (9.73%). The per cent damaged area in neem leaf powder treatment was 12.38% which was statistically at par with karanj oil treatment (14.59%) followed by acetic acid treatment (16.09%), formic acid treatment (21.77%), *T. viridae* (44.32%), *B. bassiana* (48.26%) which was statistically at par with *M. anisopliae* (51.00%). The per cent infested area in alsi seed extract was 57.33% followed by pumpkin seed extract (71.18%). Significantly maximum infested area was found in control (90.21%).

b) During 2021

Data in Table 56 demonstrated that significantly minimum damaged comb area after seven days of treatment was recorded in *Bt* (7.01 cm^2) followed by neem seed kernel extract (14.38 cm^2) which was statistically at par with neem oil treatment (18.75 cm^2) and sulphur fumigation (19.77 cm^2). The damaged area in acetic acid treatment was 22.22 cm^2 which was at par with neem leaf powder treatment (26.15 cm^2) followed by formic acid (39.17 cm^2) which was statistically at par with *T. viridae* (41.43 cm^2) and karanj oil treatment (42.60 cm^2). The damaged area in *B. bassiana* treatment was 51.31 cm^2 which was at par with *M. anisopliae* (61.58 cm^2) followed by alsi seed extract (64.82 cm^2) which was statistically at par with pumpkin seed extract (77.34cm^2). Significantly maximum infested area was found in control (132.89 cm^2). Seven days after treatment significantly minimum per cent damaged comb area was recorded in *Bt* (2.58%) followed by neem seed kernel extract (5.29%) which was at par with neem oil treatment (6.90%) and sulphur fumigation (7.27%). The per cent damaged area in acetic acid treatment was 8.17 % which was statistically at par with neem leaf powder treatment (9.62%) followed by formic acid treatment (14.40%) which was at par

with *T. viridae* (15.23%) and karanj oil treatment (15.66 %). The per cent area infested in *B. bassiana* was 18.87% which was statistically at par with alsi seed extract (22.64%) followed by *M. anisopliae* (23.83%) which was at par with pumpkin seed extract (28.44%). Significantly maximum infested area was found in control (48.86%). No infestation was found in deep freezing (-8°C to -10°C) treatment because all the third instar larvae died after 5 hours in deep freezer.

The data recorded on fourteen days after treatment showed that significantly minimum damaged comb area was recorded in *Bt* (10.34 cm^2) followed by neem seed kernel extract (18.14 cm^2) which was statistically at par with sulphur fumigation (21.70 cm^2) and neem oil treatment (22.62 cm^2). The area damaged in acetic acid treatment was 29.16 cm^2 which was statistically at par with neem leaf powder (30.09 cm^2) followed by karanj oil treatment (39.35 cm^2) which was at par with formic acid treatment (39.70 cm^2). The damaged area in *T. viridae* was 89.82 cm^2 followed by *B. bassiana* (97.04 cm^2), *M. anisopliae* (113.59 cm^2) which was statistically at par with alsi seed extract (126.50 cm^2). Maximum infested area was found in control (188.66 cm^2) which was at par with pumpkin seed extract (174.16%). Fourteen days after treatment significantly minimum per cent damaged comb area was recorded in *Bt* treatment (3.80%) followed by neem seed kernel extract (6.67%) which was statistically at par with sulphur fumigation (7.98%) and neem oil treatment (8.32%). The per cent area damaged in neem leaf powder treatment was 10.12 % which was statistically at par with acetic acid treatment (10.72%) followed by karanj oil treatment (14.47%) which was statistically at par with formic acid treatment (14.56%). The area damaged in *T. viridae* was 33.02% which was at par with *B. bassiana* (35.68%) followed by *M. anisopliae* (41.76%) which was statistically at par with alsi seed extract (46.51%). Maximum infested area was found in control (69.38%) which was at par with pumpkin seed extract (64.03%).

Data further revealed that after 21 days of treatment, significantly minimum damaged area was recorded in *Bt* (14.67 cm^2) followed by neem seed kernel extract (22.72 cm^2) which was statistically at par with sulphur fumigation (25.52 cm^2), neem oil treatment (28.01 cm^2) and neem leaf powder treatment (30.53 cm^2). The damaged area in karanj oil treatment was 44.42 cm^2 which was statistically at par with acetic acid treatment (47.78 cm^2) and formic acid treatment (54.39 cm^2) followed by *T. viridae* (110.13 cm^2) which was at par with *B. bassiana* (127.68 cm^2).

Table 56. Area damaged and per cent area infestation per comb in *A. cerana* wax combs due to greater wax moth (*G. mellonella)* during 2021

Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	18.75 (4.44)#	6.90 (2.81)	22.62 (4.86)	8.32 (3.05)	28.10 (5.39)	10.33 (3.37)
Dried neem leaf powder	8.33g	0.00	26.15 (5.21)	9.62 (3.26)	30.09 (5.58)	10.12 (3.33)	30.53 (5.61)	11.22 (3.50)
Neem seed kernel extract	5%	0.00	14.38 (3.92)	5.29 (2.51)	18.14 (4.37)	6.67 (2.77)	22.72 (4.87)	8.35 (3.06)
Karanj oil	3%	0.00	42.60 (6.60)	15.66 (4.08)	39.35 (6.35)	14.47 (3.93)	44.42 (6.74)	16.33 (4.16)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	61.58 (7.91)	22.64 (4.86)	126.50 (11.29)	46.51 (6.89)	150.80 (12.32)	55.44 (7.51)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	77.34 (8.85)	28.44 (5.43)	174.16 (13.23)	64.03 (8.06)	187.11 (13.72)	68.79 (8.35)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	7.01 (2.83)	2.58 (1.89)	10.34 (3.37)	3.80 (2.19)	14.67 (3.96)	5.39 (2.53)
Trichoderma viridae	2ml/ l water	0.00	41.43 (6.51)	15.23 (4.03)	89.82 (9.53)	33.02 (5.83)	110.13 (10.54)	40.49 (6.44)
Bauveria bassiana	2ml/ l water	0.00	51.31 (7.23)	18.87 (4.46)	97.04 (9.90)	35.68 (6.06)	127.68 (11.34)	46.94 (6.92)
Metarhizium anisopliae	2ml/ l water	0.00	64.82 (8.11)	23.83 (4.98)	113.59 (10.70)	41.76 (6.54)	143.45 (12.02)	52.74 (7.33)
Acetic acid spray	2ml/ l water	0.00	22.22 (4.82)	8.17 (3.03)	29.16 (5.49)	10.72 (3.42)	47.78 (6.98)	17.57 (4.31)
Formic acid spray	0.8ml/l water	0.00	39.17 (6.34)	14.40 (3.92)	39.60 (6.37)	14.56 (3.94)	54.39 (7.44)	20.00 (4.58)
Use of sulphur fumigation	5g	0.00	19.77 (4.56)	7.27 (2.88)	21.70 (4.76)	7.98 (3.00)	25.52 (5.15)	9.38 (3.22)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0 (1.00)	0.00 (1.00)	0 (1.00)
Control		0.00	132.89 (11.57)	48.86 (7.06)	188.66 (13.77)	69.36 (8.39)	250.10 (15.85)	91.95 (9.64)
C.D. (0.05)			(0.80)	(0.47)	(0.62)	(0.43)	(0.74)	(0.44)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

The damaged area in *M. anisopliae* was 143.45 cm^2 which was at par with alsi seed extract (150.80 cm^2) followed by pumpkin seed extract (187.11cm^2). Significantly maximum infested area was found in control (250.10 cm^2). Twenty one days after treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (5.39%) followed by neem seed kernel extract (8.35%) which was statistically at par with sulphur fumigation (9.38 %), neem oil treatment (10.33%) and neem leaf powder treatment (11.22%). The damaged area in karanj oil treatment was 16.33% which was at par with acetic acid treatment (17.57%) and formic acid treatment (20.00%) followed by *T. viridae* (40.49%), *B. bassiana* (46.94%), *M. anisopliae* (52.74%) which was statistically at par with alsi seed extract (55.44%) followed by pumpkin seed extract (68.79%). Maximum infested area was found in control (91.95%).

c) Pooled data (2020-2021)

Data presented in Table 57 and Fig. 19 revealed that significantly minimum damaged comb area after seven days of treatment was recorded in *Bt* treatment (7.61 cm^2) followed by neem seed kernel extract (13.13 cm^2) which was statistically at par with sulphur fumigation (16.47 cm^2) and neem oil treatment (16.79 cm^2). The damaged area in neem leaf powder treatment was 22.85 cm^2 which was at par with acetic acid treatment (23.85 cm^2) followed by formic acid treatment (35.86 cm^2) which was statistically at par with karanj oil treatment (39.29 cm^2) and *T. viridae* (43.37 cm^2). The damaged area in *B. bassiana* was 50.94 cm^2 followed by *M. anisopliae* (61.51 cm^2) which was at par with alsi seed extract (65.48 cm^2). Significantly maximum infested area was found in control (129.58 cm^2). Seven days after treatment, significantly minimum per cent comb area damaged was recorded in *Bt* (2.80%) followed by neem seed kernel extract (4.83%) which was at par with sulphur fumigation (6.06%) and neem oil treatment (6.17%). The per cent damaged area in neem leaf powder treatment was 8.40% which was statistically at par with acetic acid treatment (8.77%) followed by formic acid treatment (13.19%) which was at par with karanj oil treatment (14.45 %) and *T. viridae* (15.94%). The per cent area infested in *B. bassiana* was 18.73% which was statistically at par with *M. anisopliae* (22.62%) followed by alsi seed extract (24.08%). Significantly maximum infested area was found in control (48.86%). No infestation was found in deep freezing (-8°C to -10°C) treatment (Plate 27).

Observations recorded on fourteen days after treatment showed that significantly minimum damaged comb area was recorded in *Bt* (10.75 cm^2) followed by neem seed kernel extract (16.62 cm^2) which was statistically at par with neem oil treatment (20.24 cm^2) and

sulphur fumigation (20.40 cm^2). The damaged area in neem leaf powder treatment was 27.71 cm^2 which was at par with acetic acid treatment 27.96 cm^2 followed by karanj oil treatment (36.97 cm^2) which was at par with formic acid treatment (37.22 cm^2). The damaged area in *T. viridae* was 90.15 cm^2 followed by *B. bassiana* (99.35 cm^2) which was statistically at par with *M. anisopliae* (111.21 cm^2). The damaged area in alsi seed extract was 131.09 cm^2 followed by pumpkin seed extract (171.78 cm^2). Significantly maximum infested area was found in control (186.26 cm^2). Fourteen days after treatment, significantly minimum per cent damaged comb area was recorded in *Bt* (3.95 %) followed by neem seed kernel extract (6.11%), neem oil treatment (7.44 %) which was statistically at par with sulphur fumigation (7.50 %). The per cent damaged area in neem leaf powder treatment was 9.72% which was statistically at par with acetic acid treatment (10.28%) followed by karanj oil treatment (13.59 %) which was statistically at par with formic acid treatment (13.69%). The damaged area in *T. viridae* was 33.14% followed by *B. bassiana* (36.53%), *M. anisopliae* (40.89%) alsi seed extract (48.19%) and pumpkin seed extract (64.03%).Significantly maximum infested area was found in control (68.49%).

Data further showed that after 21 days of treatment, significantly minimum damaged area was recorded in *Bt* (13.81 cm^2) followed by neem seed kernel extract (22.05 cm^2) which was statistically at par with sulphur fumigation (24.32 cm^2). The damaged comb area in neem oil treatment was 29.32 cm^2 which was statistically at par with neem leaf powder treatment (31.87 cm^2) followed by karanj oil treatment (42.74 cm^2) which was statistically at par with acetic acid treatment (43.00 cm^2). The area damaged area in formic acid treatment was 71.05 cm^2 followed by *T. viridae* (111.58 cm^2) which was at par with *B. bassiana* (122.91 cm^2). The damaged area in *M. anisopliae* was 141.40 cm^2 which was at par with alsi seed extract (150.51 cm^2) followed by pumpkin seed extract (185.68cm^2). Significantly maximum infested area was found in control (245.37 cm^2). Twenty one days after treatment significantly minimum per cent damaged comb area was recorded in *Bt* (5.17%) followed by neem seed kernel extract (8.06%) which was statistically at par with sulphur fumigation (8.15%). The damaged comb area in neem oil treatment was 10.03% which was at par with neem leaf powder treatment (11.80%) followed by karanj oil treatment (15.46%) which was at par with acetic acid treatment (17.83 %). The per cent infested area in formic acid treatment was 20.88% followed by *T. viridae* (42.40%) which was statistically at par with *B. bassiana* (47.60%). In *M. anisopliae* the damaged area was 51.87% followed by alsi seed

Table 57. Pooled data on area damaged and per cent area infestation per comb in *A. cerana* wax combs due to greater wax moth (*G. mellonella)* during 2020–2021

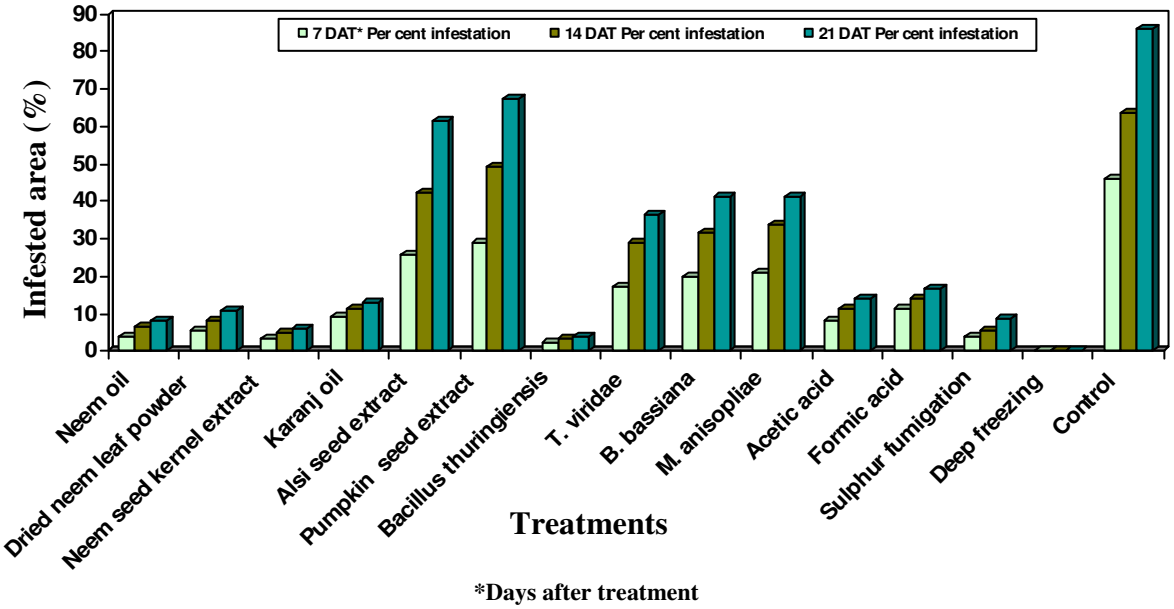
Treatment	Dose	Initial infestation before treatment	Damage and infestation of greater wax moth					
			7 DAT*		14DAT		21DAT	
			Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation	Area of comb infested (cm^2)	Per cent infestation
Neem oil	3%	0.00	16.79 (4.22)[#]	6.17 (2.68)	20.24 (4.61)	7.44 (2.91)	29.32 (5.51)	10.03 (3.32)
Dried neem leaf powder	8.33g	0.00	22.85 (4.88)	8.40 (3.07)	27.71 (5.36)	9.72 (3.27)	31.87 (5.73)	11.80 (3.58)
Neem seed kernel extract	5%	0.00	13.13 (3.76)	4.83 (2.41)	16.62 (4.20)	6.11 (2.67)	22.05 (4.80)	8.06 (3.01)
Karanj oil	3%	0.00	39.29 (6.35)	14.45 (3.93)	36.97 (6.16)	13.59 (3.82)	42.74 (6.61)	15.46 (4.06)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	0.00	65.48 (8.15)	24.08 (5.01)	131.09 (11.49)	48.19 (7.01)	150.51 (12.31)	56.39 (7.58)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	0.00	79.75 (8.99)	29.32 (5.51)	171.78 (13.14)	63.16 (8.01)	185.68 (13.66)	69.99 (8.43)
Bacillus thuringiensis var. *kurstaki*	5g/l water	0.00	7.61 (2.93)	2.80 (1.95)	10.75 (3.43)	3.95 (2.23)	13.81 (3.85)	5.17 (2.48)
Trichoderma viridae	2ml/ l water	0.00	43.37 (6.66)	15.94 (4.12)	90.15 (9.55)	33.14 (5.84)	111.58 (10.61)	42.40 (6.59)
Bauveria bassiana	2ml/ l water	0.00	50.94 (7.21)	18.73 (4.44)	99.35 (10.02)	36.53 (6.13)	122.91 (11.13)	47.60 (6.97)
Metarhizium anisopliae	2ml/ l water	0.00	61.51 (7.91)	22.62 (4.86)	111.21 (10.59)	40.89 (6.47)	141.40 (11.93)	51.87 (7.27)
Acetic acid spray	2ml/ 1 water	0.00	23.85 (4.98)	8.77 (3.13)	27.96 (5.38)	10.28 (3.36)	43.00 (6.63)	16.83 (4.22)
Formic acid spray	0.8ml/l water	0.00	35.86 (6.07)	13.19 (3.77)	37.22 (6.18)	13.69 (3.83)	71.05 (8.49)	20.88 (4.68)
Use of sulphur fumigation	5g	0.00	16.47 (4.18)	6.06 (2.66)	20.40 (4.63)	7.50 (2.92)	24.32 (5.03)	8.51 (3.08)
Deep freezing	(-8°C to -10°C)	0.00	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control		0.00	129.58 (11.43)	47.64 (6.97)	186.28 (13.68)	68.49 (8.34)	245.37 (15.70)	91.08 (9.60)
C.D. (0.05)			(0.76)	(0.45)	(0.49)	(0.28)	(0.52)	(0.30)

[#]Figures in parentheses are square root (x+1) transformed values

*Days after treatment

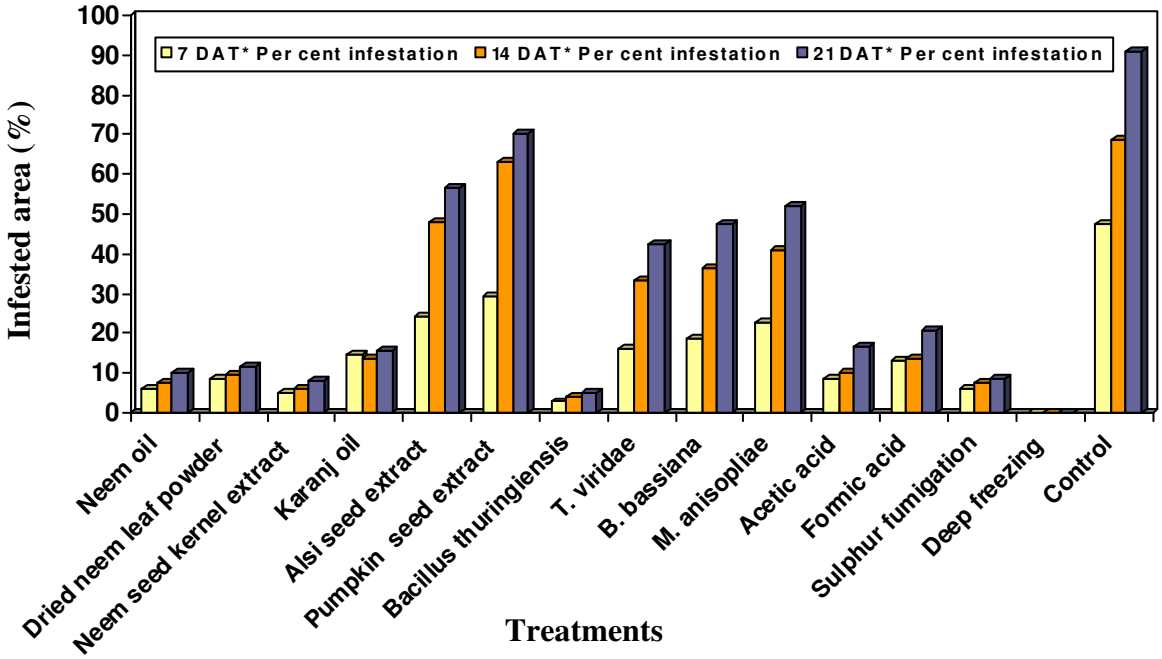
extract (56.39%) and pumpkin seed extract (69.99%). Significantly maximum damaged area was found in control (91.08%). No infestation was found in deep freezing (-8℃ to -10℃) treatment.

Among all treatments used for the control of greater wax moth *G. mellonella* infesting *A. cerana* wax combs moth under laboratory conditions, deep freezing was the best treatment followed by *Bt*, NSKE and Neem oil treatment. Least effective treatments were plant extracts (alsi seed extract and pumpkin seed extract) and biopesticides, *T. viridae*, *B. bassiana*, *M. anisopliae*. All wax moth stages got destroyed when cold treatment of the combs at -7℃ for 4-5 hour, -15℃ for 2 hours or –12°C for 3 hours was done (Charrière and Elmendorf, 1997). Burgers (1978) observed most rapid wax moth control by deep freezing at -l7°C. Longer exposures were also effective at temperatures above but near 0°C, such as those in cold-rooms and domestic refrigerators. The disruption of the greater wax moth growth period can be achieved by exposing the greater wax moth resistance spectrum of beekeeping equipment and bee combs to temperatures beyond (heating technique) or even below (freezing technique) (Ritter, 1996). Many workers (Cusman, 2002; Goodman, 2003; Rusty, 2011) in different parts of the world recommended freezing as an effective method of wax moth control as it is free of any chemical residues. Deep freezer treatment with 1.33% infestation for 5 h and sulphur fumigation with 6.74% infestation in combs were found to be the best treatment for management of wax moth under storage condition (Katna *et al.,* 2012). Vijaykumar *et al*. (2019) showed that the incidence of *G. mellonella* in *Bt* (commercial product) treated combs was significantly low (2.29-5.24 larvae per hive) depending upon the concentration of *Bt* as compared to control in which 34 larvae per hive were observed. Krieg (1959) demonstrated that spores of *B. thuringiensis* Berliner when dusted on wax combs at 5 mg/dm^2 caused mortality of greater wax moth larvae ranging from 80-100 per cent. The formulation of *Bt aizawai* spores (B-401) containing 10^6 viable spores/mg when sprayed on combs or wax foundations gave protection from *G. mellonella* infestation for up to 12 months (Goodwin, 1985). Vishwas and Gowda (2006) also reported that 500ppm of *Bt kurstaki* UAS strains (Dipel and Halt) resulted in 80 and 63.33 per cent mortality of 3rd instar larvae of *G. mellonella*. Bhopale *et al.* (2013) subjected different larval instars of *G. mellonela* to botanicals and microbial pesticides viz., dried neem leaf, 3% neem oil, *Bt* kurstaki (Halt), *Bt* local strain-1 and *Bt* (Halt) local strain-2, 3% pongamia oil and 5% NSKE.



*Days after treatment

Fig 18. Per cent infested area of *A. mellifera* treated combs due to *G. mellonella* feeding (2020-2021)



*Days after treatment

Fig 19. Per cent infested area of *A. cerana* treated combs due to *G. mellonella* feeding (2020-2021)

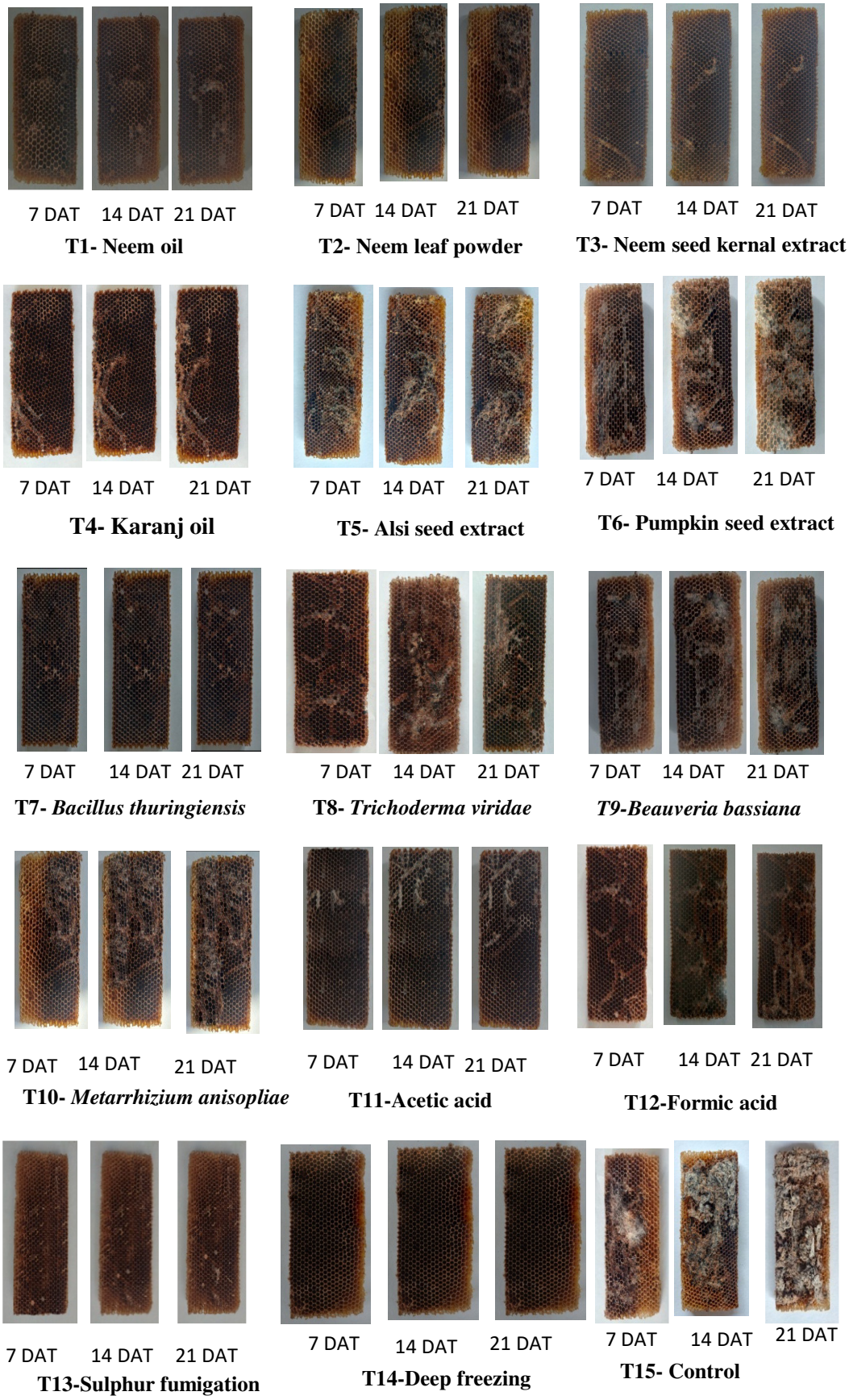


Plate 27. Damage and infestation of greater wax moth in *A. cerana* combs at 7, 14 and 21 days after treatments (DAT)

Among these *Bt kurstaki* and pongamia oil gave maximum larval mortality (93.33%) after 10 days of treatment. *G. mellonella* larval mortality (56.0 to 80.0%) has been reported by using different *Bt* formulation viz; Dipel, Biobit, Biolep and Bioasp (Viraktamath *et al.,* 2005). The insect growth regulation effects of azadirachtin manifest as developmental aberrations in immature insects and are both dose and time dependent; can cause death before and during the moult, or delay of the moult (Rembold, 1995). Sezer and Ozalp (2011) studied the adverse effects of azadirachtin extract of *Melia azedarach* and *Azadirachta indica* on total glycogen amount of *G. mellonella* and observed maximum up to 50% decrease in total glycogen synthesized. Azadirachtin showed anti-feedant effects on insects and lepidopterans were found to be the most susceptible to it (Ascher, 1993; Mordue and Blackwell, 1993).

In the present studies, after 21 days of treatment per cent area infestation per comb in *A. cerana* ranged between 5.17% (*Bt*) to 91.08% (control). Izae-ul- Haq *et al.* (2008) fed 4% neem seed kernel extract and 0.5% neem seed extracts in artificial diet to *G. mellonella* larvae and reported 50% to 83.33% mortality while, 4% neem azal-T/S treated colonies resulted in 100% mortality during first week of treatment (Elbehery *et al.,* 2016). Basedow *et al.* (2012) reported 100% larval mortality of wax moth after the use of Neem Azal® -T/S (80 ppm) in four weeks. These studies showed that greater wax moth mortality depends on time and dose of treatments. Verma *et al.* (1997) reported that the use of sulphur and cleaning of bee hives assisted in reducing wax moth population. The results are also in agreement with those of Sattigi *et al.* (1990) who reported the effectiveness of smearing of hive with lime sulphur paste against greater wax moth, *G. mellonella*.

4.3.3 Effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions

a) During 2020

Data in Table 58 showed the effect of tested material on the larval feeding and were evaluated on the basis of reduction in weight of the comb. The observations were recorded at 7, 14 and 21 days after treatment until the larvae complete their development and entered in to pupation stage. After 7 days of treatment, the minimum per cent comb weight loss was recorded in *Bt* (1.55%) which was statistically at par with sulphur fumigation (2.18%) and neem seed kernel extract (2.45%) followed by neem oil treatment (3.21%) which was statistically at par with neem leaf powder treatment (4.29%).

Table 58. Effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions during 2020

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	53.57 (7.39)#	51.85 (7.27)	3.21 (2.05)	50.75 (7.19)	5.37 (2.52)	49.27 (7.09)	7.86 (2.98)
Dried neem leaf powder	8.33g	50.46 (7.17)	48.29 (7.02)	4.29 (2.30)	47.33 (6.95)	6.25 (2.69)	45.41 (6.81)	9.98 (3.31)
Neem seed kernel extract	5%	48.19 (7.01)	47.00 (6.93)	2.45 (1.86)	46.58 (6.90)	3.32 (2.08)	45.67 (6.83)	5.17 (2.48)
Karanj oil	3%	46.47 (6.89)	43.08 (6.64)	7.20 (2.86)	42.27 (6.58)	9.41 (3.23)	40.76 (6.46)	12.58 (3.69)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	52.54 (7.32)	41.13 (6.49)	21.69 (4.76)	35.89 (6.07)	31.70 (5.72)	26.26 (5.22)	49.99 (7.14)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	45.25 (6.80)	33.51 (5.87)	25.93 (5.19)	26.80 (5.27)	40.78 (6.46)	16.61 (4.20)	63.27 (8.02)
Bacillus thuringiensis var. *kurstaki*	5g/l water	49.46 (7.10)	48.70 (7.05)	1.55 (1.60)	48.01 (7.00)	2.89 (1.97)	47.53 (6.97)	3.90 (2.21)
Trichoderma viridae	2ml/ l water	51.23 (7.23)	43.76 (6.69)	14.58 (3.95)	36.72 (6.14)	28.27 (5.41)	33.21 (5.85)	35.30 (6.02)
Bauveria bassiana	2ml/ l water	47.34 (6.95)	39.41 (6.36)	16.91 (4.23)	33.02 (5.83)	30.26 (5.59)	26.99 (5.29)	43.10 (6.64)
Metarhizium anisopliae	2ml/ l water	50.21 (7.16)	41.21 (6.50)	17.91 (4.35)	33.79 (5.90)	32.77 (5.81)	29.98 (5.57)	40.30 (6.43)
Acetic acid spray	2ml/ l water	54.17 (7.43)	50.61 (7.18)	6.60 (2.76)	48.99 (7.07)	10.93 (3.45)	47.05 (6.93)	13.17 (3.76)
Formic acid spray	0.8ml/l water	44.47 (6.74)	40.19 (6.42)	9.62 (3.26)	39.34 (6.35)	12.88 (3.73)	37.69 (6.22)	15.25 (4.03)
Use of sulphur fumigation	5g	52.89 (7.34)	51.73 (7.26)	2.18 (1.78)	51.28 (7.23)	4.38 (2.32)	49.51 (7.11)	6.38 (2.72)
Deep freezing	(-8°C to -10°C)	53.87 (7.41)	53.87 (7.41)	0 (1.00)	53.87 (7.41)	0 (1.00)	53.87 (7.41)	0.00 (1.00)
Control		55.6 (7.52)	36.28 (6.11)	34.74 (5.98)	22.91 (4.89)	58.86 (7.74)	11.67 (3.56)	79.00 (8.94)
C.D. (0.05)		NS	(0.63)	(0.44)	(0.70)	(0.39)	(0.58)	(0.52)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

The per cent weight loss of comb in acetic acid treatment was 6.60% followed by karanj oil treatment (7.20%) which was statistically at par with formic acid treatment (9.62%). The per cent decrease in weight loss of the comb in *T. viridae* treatment was 14.58% which was at par with *B. bassiana* treatment (16.91%) and *M. anisopliae* (17.91%) followed by alsi (21.69%) which was at par with pumpkin seed extract (29.32%). Significantly maximum per cent reduction in weight of the comb in control (34.74%). No change in the weight of the comb was observed in deep freezing (-8°C to -10°C) as all the larvae got died.

Fourteen days after treatment, the minimum per cent weight loss of the was recorded in *Bt* (2.89%) which was statistically at par with neem seed kernel extract (3.32%) and sulphur fumigation (4.38%) followed by neem oil treatment (5.37%) which was statistically at par with neem leaf powder treatment (6.25%). The per cent decrease in weight of the comb in karanj oil treatment was 9.41% which was statistically at par with acetic acid treatment (10.93%) followed by formic acid treatment (12.88%), *T. viridae* (28.27%), *B. bassiana* (30.26%) which was statistically at par with alsi seed extract treatment (31.70%) and *M. anisopliae* (32.77%). Significantly maximum per cent comb weight loss was found in control (58.86%).

After 21 days of treatment, the minimum per cent reduction in weight of the combs was observed in *Bt* (3.90%) which was statistically at par with neem seed kernel extract (5.17 %) and sulphur fumigation (6.38%) followed by neem oil treatment (7.86%) which was statistically at par with neem leaf powder treatment (9.98%). The per decrease in weight of the combs in karanj oil treatment was 12.58% which was at par with acetic acid treatment (13.17%) and formic acid treatment (15.25%) followed by *T. viridae* treatment (35.30%) which was at par with *M. anisopliae* (40.30%). In *B. bassiana* treatment the reduction in weight of the comb was 43.10% which was statistically at par with alsi seed extract (49.99%) followed by pumpkin seed extract (63.25%). Statistically maximum per cent decrease in the weight of the combs was found in control (79.00%).

b) During 2021

Data in Table 59 revealed that after 7 days of treatment, the minimum per cent weight loss of treated combs was recorded in *Bt* (2.29%) which was statistically at par with neem seed kernel extract (2.45%) followed by sulphur fumigation (3.46%) which was at par with neem oil treatment (4.49%).

Table 59. Effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions during 2021

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7 DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	52.71 (7.33)#	50.35 (7.17)	4.49 (2.34)	49.11 (7.08)	6.87 (2.81)	47.70 (6.98)	9.44 (3.23)
Dried neem leaf powder	8.33g	48.82 (7.06)	46.45 (6.89)	4.80 (2.41)	44.97 (6.78)	7.94 (2.99)	43.78 (6.69)	10.37 (3.37)
Neem seed kernel extract	5%	51.69 (7.26)	50.14 (7.15)	3.01 (2.00)	49.33 (7.09)	4.57 (2.36)	48.55 (7.04)	6.07 (2.66)
Karanj oil	3%	55.65 (7.53)	50.93 (7.21)	8.48 (3.08)	49.49 (7.11)	11.10 (3.48)	48.16 (7.01)	13.44 (3.80)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	56.55 (7.59)	45.03 (6.78)	20.38 (4.62)	39.72 (6.38)	29.91 (5.56)	34.74 (5.98)	38.52 (6.29)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	54.37 (7.44)	39.58 (6.37)	27.21 (5.31)	33.19 (5.85)	38.95 (6.32)	22.28 (4.82)	59.06 (7.75)
Bacillus thuringiensis var. *kurstaki*	5g/l water	55.35 (7.51)	54.08 (7.42)	2.29 (1.81)	53.21 (7.36)	3.87 (2.21)	53.08 (7.35)	4.08 (2.25)
Trichoderma viridae	2ml/ l water	55.05 (7.49)	46.92 (6.92)	14.75 (3.97)	40.26 (6.42)	26.96 (5.29)	34.85 (5.99)	36.88 (6.15)
Bauveria bassiana	2ml/ l water	51.94 (7.28)	43.95 (6.70)	15.42 (4.05)	35.35 (6.03)	31.95 (5.74)	31.76 (5.72)	38.82 (6.31)
Metarhizium anisopliae	2ml/ l water	49.67 (7.12)	40.11 (6.41)	19.19 (4.49)	32.27 (5.77)	34.46 (5.95)	28.72 (5.45)	41.88 (6.55)
Acetic acid spray	2ml/ l water	47.95 (7.00)	45.29 (6.80)	5.69 (2.59)	43.86 (6.70)	9.60 (3.26)	42.58 (6.60)	11.55 (3.54)
Formic acid spray	0.8ml/l water	54.02 (7.42)	49.80 (7.13)	7.84 (2.97)	47.69 (6.98)	13.05 (3.75)	45.47 (6.82)	15.89 (4.11)
Use of sulphur fumigation	5g	46.73 (6.91s)	45.12 (6.79)	3.46 (2.11)	44.53 (6.75)	6.07 (2.66)	43.35 (6.66)	7.25 (2.87)
Deep freezing	(-8°C to -10°C)	50.94 (7.21)	50.94 (7.21)	0 (1.00)	50.94 (7.21)	0 (1.00)	50.94 (7.21)	0.00 (1.00)
Control		57.08 (7.62)	39.32 (6.35)	31.15 (5.67)	21.06 (4.70)	63.12 (8.01)	10.30 (3.36)	82.00 (9.11)
C.D. (0.05)		NS	(0.62)	(0.26)	(0.60)	(0.36)	(0.63)	(0.37)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

Per cent weight loss in neem leaf powder treatment was 4.80% which was statistically at par with acetic acid treatment (5.69%) followed by formic acid treatment (7.84%) which was statistically at par with karanj oil (8.48%). In *T. viridae* treatment 14.75% loss in comb weight was observed which was statistically at par with *B. bassiana* treatment (15.42 %) followed by *M. anisopliae* (19.19%) which was at par with alsi seed extract (20.38%). The comb's per cent weight loss in pumpkin seed extract was 27.21%. Significantly maximum weight loss was observed in untreated combs (31.15%). No change in the weight of the comb was observed in deep freezing (-8°C to -10°C) as all the larvae died after 5 hours of treatment.

Fourteen days after treatment the minimum per cent decrease in weight of the treated combs was recorded in *Bt* (3.87%) which was statistically at par with neem seed kernel extract (4.57%) followed by sulphur fumigation (6.07%) which was statistically at par with neem oil treatment (6.87%) and neem leaf powder treatment (7.94%) which was further at par with acetic acid treatment (9.60%). The per cent decrease in weight of the comb in karanj oil treatment was 11.10% which was statistically at par with formic acid treatment (13.05%) followed by *T. viridae* (26.96%) which was at par with alsi seed extract (29.91%). The per cent decrease in weight of the comb in *B. bassiana* treatment was 31.95% which was at par with *M. anisopliae* (34.46%) followed by pumpkin seed extract (38.95%). Significantly maximum per cent reduction in weight of the comb was found in control (63.12%).

After 21 days of treatment, significantly minimum per cent comb weight loss was observed in *Bt* treatment (4.08%) followed by neem seed kernel extract (6.07%) which was statistically at par with sulphur fumigation (7.25%). Reduction in weight of the comb in neem oil treatment was 9.44 % which was statistically at par with neem leaf powder treatment (10.37%) and acetic acid treatment (11.55%). The per cent decrease in weight of the comb in karanj oil treatment was 13.44% which was at par with formic acid treatment (15.89%) followed by *T. viridae* treatment (36.88%), alsi seed extract (38.52%) which was at par with *B. bassiana* (38.82%) and *M. anisopliae* (41.88%). Statistically maximum weight loss was found in control (82.00%).

c) Pooled data (2020-2021)

Pooled data in Table 60 and Fig. 20 on the effects of different treatments on weight loss of the comb by feeding of greater wax moth (*G. mellonella*) larvae in *A. mellifera* combs

during 2020 and 2021 revealed that per cent weight loss of comb after seven days of treatment was minimum in *Bt* (1.92%) which was statistically at par with neem seed kernel extract (2.73%) and sulphur fumigation (2.82%) followed by neem oil treatment (3.85%) which was statistically at par with neem leaf powder treatment (4.55%). The per cent weight loss of the comb in acetic acid treatment was 6.14% which was statistically at par with karanj oil treatment (7.84%) followed by formic acid treatment (8.73%), *T. viridae* (14.66%) which was statistically at par with *B. bassiana* treatment (16.16%). In *M. anisopliae* the per cent weight loss of the comb was 18.55% which was at par with alsi seed extract (21.03%) followed by pumpkin seed extract treatment (26.57%). Significantly maximum reduction in comb weight was observed in control (32.94%). No change in the weight of the comb was observed in deep freezing (-8℃ to -10℃).

Fourteen days after treatment the minimum per cent decrease in comb weight was recorded in *Bt* (3.38%) which was statistically at par with neem seed kernel extract (3.94%) and sulphur fumigation (5.23%) followed by neem oil treatment (6.12%) which was at par with neem leaf powder treatment (7.10%). The per cent weight loss in comb in karanj oil was 10.25% which was statistically at par with acetic acid (10.27%) and formic acid treatment (12.97%) followed by *T. viridae* (27.61%) which was at par with alsi seed extract (30.81%) and *B. bassiana* (31.11%). The per cent reduction in weight of the comb in *M. anisopliae* was 33.61% followed by pumpkin seed extract (39.86%). Significantly maximum decrease in weight of the combs was observed in control (60.99%).

After 21 days of treatment, minimum per cent comb weight loss was observed in *Bt* (3.99%) which was statistically at par with neem seed kernel extract (5.62%) followed by sulphur fumigation (6.81%) which was at par with neem oil treatment (8.65%). The per cent weight loss of the comb in neem leaf powder treatment was 10.17% which was statistically at par with acetic acid (12.36%) and karanj oil treatment (13.01%) followed by formic acid treatment (15.57%), *T. viridae* treatment (36.09%) which was statistically at par with *B. bassiana* (40.96%) and *M. anisopliae* (41.09%). In alsi seed extract the per cent decrease in weight of the comb was (44.25%) followed by pumpkin seed extract (61.16%). Significantly maximum weight loss of the comb was observed in control (80.50%).

In the present investigations the per cent weight loss of treated *A. mellifera* combs due to greater wax moth larval feeding in laboratory conditions was lowest in *Bt* treatment.

Table 60. Pooled data on effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions during 2020-2021

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7 DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	53.14 (7.36)[#]	51.10 (7.22)	3.85 (2.20)	49.93 (7.14)	6.12 (2.67)	48.49 (7.03)	8.65 (3.11)
Dried neem leaf powder	8.33g	49.64 (7.12)	47.37 (6.95)	4.55 (2.35)	46.15 (6.87)	7.10 (2.85)	44.59 (6.75)	10.17 (3.34)
Neem seed kernel extract	5%	49.94 (7.14)	48.57 (7.04)	2.73 (1.93)	47.96 (7.00)	3.94 (2.22)	47.11 (6.94)	5.62 (2.57)
Karanj oil	3%	51.06 (7.22)	47.00 (6.93)	7.84 (2.97)	45.88 (6.85)	10.25 (3.35)	44.46 (6.74)	13.01 (3.74)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	54.54 (7.45)	43.08 (6.64)	21.03 (4.69)	37.80 (6.23)	30.81 (5.64)	30.50 (5.61)	44.25 (6.73)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	49.81 (7.13)	36.55 (6.13)	26.57 (5.25)	29.99 (5.57)	39.86 (6.39)	19.44 (4.52)	61.16 (7.88)
Bacillus thuringiensis var. *kurstaki*	5g/l water	52.41 (7.31)	51.39 (7.24)	1.92 (1.71)	50.61 (7.18)	3.38 (2.09)	50.31 (7.16)	3.99 (2.23)
Trichoderma viridae	2ml/ l water	53.14 (7.36)	45.34 (6.81)	14.66 (3.96)	38.49 (6.28)	27.61 (5.35)	34.03 (5.92)	36.09 (6.09)
Bauveria bassiana	2ml/ l water	49.64 (7.12)	41.68 (6.53)	16.16 (4.14)	34.19 (5.93)	31.11 (5.67)	29.37 (5.51)	40.96 (6.48)
Metarhizium anisopliae	2ml/ l water	49.94 (7.14)	40.66 (6.45)	18.55 (4.42)	33.03 (5.83)	33.61 (5.88)	29.35 (5.51)	41.09 (6.49)
Acetic acid spray	2ml/ l water	51.06 (7.22)	47.95 (7.00)	6.14 (2.67)	46.42 (6.89)	10.27 (3.36)	44.81 (6.77)	12.36 (3.66)
Formic acid spray	0.8ml/l water	49.25 (7.09)	44.99 (6.78)	8.73 (3.12)	43.52 (6.67)	12.97 (3.74)	41.58 (6.53)	15.57 (4.07)
Use of sulphur fumigation	5g	49.81 (7.13)	48.42 (7.03)	2.82 (1.96)	47.91 (6.99)	5.23 (2.50)	46.43 (6.89)	6.81 (2.80)
Deep freezing	(-8°C to -10°C)	52.41 (7.31)	52.41 (7.31)	0.00 (1.00)	52.41 (7.31)	0.00 (1.00)	52.41 (7.31)	0.00 (1.00)
Control		56.34 (7.57)	37.80 (6.23)	32.94 (5.83)	21.99 (4.79)	60.99 (7.87)	10.99 (3.46)	80.50 (9.03)
C.D. (0.05)		NS	(0.69)	(0.37)	(0.70)	(0.42)	(0.76)	(0.53)

[#]Figures in parentheses are square root (x+1) transformed values
*Days after treatment

The effectiveness of NSKE and sulphur fumigation treatments for per cent weight loss was equally good to *Bt*. It was also observed that per cent weight loss due to larval feeding was lowest in NSKE compared to all neem formulations used in the present studies. Similar results were reported by Kumar and Khan (2019) who recorded minimum comb's weight loss 26.41% in NSKE and maximum in control (38.09%) after 14 days of treatment.

The per cent weight loss of treated combs with various neem products; neem oil (8.65%), neem leaf powder (10.17%) and neem seed kernel extract (5.62%) was at par. The difference in per cent weight reduction between treated and untreated combs and within the treated combs may be the result of feeding deterrent property of the tested material.

Azadirachtin has antifeedant property and moreover it affects the moulting of insect by causing death before and during the moult or delay the moult. If it is given at 2% concentration in diet to greater wax moth second instars then these instars could proceed to fifth instar but failed to moult to sixth instar (Rambold, 1995; Elbehery *et al.,* 2016). In deep freezing treatment no weight loss of the comb was observed as all larvae got killed in 4-5 hours of exposure in deep freezer. However maximum weight loss (37.80%, 60.99% and 80.50%) was observed in untreated combs (control) on 7, 14 and 21 days of observations. Alsi seed extract and pumpkin seed extract are also not as effective as other treatments with 44.25% and 61.16% weight loss in the combs. B*. bassiana, M. anisopliae* and *T. viridae* also showed reduction in the weight of the comb ranging from 36.09% to 40. 96%, although *T. viridae* was most effective among these biopesticides. The least effectiveness of the biopesticides may be due to lower concentration of applied dose.

4.3.4 Effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions

a) During 2020

Data in Table 61 demonstrated the effect of tested material on weight of the comb by larval feeding showed that after seven days of treatment, the minimum per cent comb weight loss was recorded in *Bt* (2.03%) which was statistically at par with neem seed kernel extract (2.96%) followed by sulphur fumigation (2.16%) which was statistically at par with neem oil (4.18%) and neem leaf powder treatment (4.76%). The per cent decrease in comb weight in acetic acid treatment was 8.07% which was at par with karanj oil (9.42%) followed by formic acid (11.09%), *T. viridae* treatment (15.80%) which was at par with *B. bassiana*

Table 61. Effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions during 2020

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7 DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	47.90 (6.99)#	45.89 (6.85)	4.18 (2.28)	45.06 (6.79)	5.95 (2.64)	43.54 (6.67)	8.94 (3.15)
Dried neem leaf powder	8.33g	44.79 (6.77)	42.67 (6.61)	4.76 (2.40)	41.33 (6.51)	7.78 (2.96)	39.71 (6.38)	11.31 (3.51)
Neem seed kernel extract	5%	42.52 (6.60)	41.32 (6.51)	2.96 (1.99)	40.87 (6.47)	3.94 (2.22)	39.87 (6.39)	6.25 (2.69)
Karanj oil	3%	40.80 (6.47)	36.92 (6.16)	9.42 (3.23)	36.47 (6.12)	11.03 (3.47)	35.26 (6.02)	13.91 (3.86)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	46.87 (6.92)	37.13 (6.17)	20.75 (4.66)	32.37 (5.78)	30.93 (5.65)	24.50 (5.05)	47.60 (6.97)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	39.58 (6.37)	28.73 (5.45)	27.40 (5.33)	22.92 (4.89)	42.10 (6.57)	14.00 (3.87)	64.60 (8.10)
Bacillus thuringiensis var. *kurstaki*	5g/l water	43.79 (6.69)	42.89 (6.62)	2.03 (1.74)	42.35 (6.58)	3.32 (2.08)	41.83 (6.54)	4.48 (2.34)
Trichoderma viridae	2ml/ l water	45.56 (6.82)	38.36 (6.27)	15.80 (4.10)	31.91 (5.74)	29.89 (5.56)	29.31 (5.51)	35.82 (6.07)
Bauveria bassiana	2ml/ l water	41.67 (6.53)	34.62 (5.97)	16.80 (4.22)	28.39 (5.42)	31.88 (5.73)	24.59 (5.06)	40.97 (6.48)
Metarhizium anisopliae	2ml/ l water	44.54 (6.75)	35.90 (6.07)	19.38 (4.51)	29.25 (5.50)	34.39 (5.95)	26.01 (5.20)	41.63 (6.53)
Acetic acid spray	2ml/ l water	48.50 (7.04)	44.60 (6.75)	8.07 (3.01)	43.43 (6.67)	11.80 (3.58)	40.87 (6.47)	15.70 (4.09)
Formic acid spray	0.8ml/l water	38.80 (6.31)	34.49 (5.96)	11.09 (3.48)	33.70 (5.89)	14.50 (3.94)	31.89 (5.74)	17.86 (4.34)
Use of sulphur fumigation	5g	47.22 (6.94)	45.48 (6.82)	3.65 (2.16)	45.38 (6.81)	5.25 (2.50)	44.38 (6.74)	6.00 (2.65)
Deep freezing	(-8°C to -10°C)	48.20 (7.01)	48.20 (7.01)	0 (1.00)	48.20 (7.01)	0 (1.00)	48.20 (7.01)	0.00 (1.00)
Control		49.93 (7.14)	31.84 (5.73)	36.21 (6.10)	19.39 (4.52)	61.23 (7.89)	9.82 (3.29)	81.68 (9.09)
C.D. (0.05)		NS	(0.64)	(0.39)	(0.69)	(0.36)	(0.74)	(0.44)

#Figures in parentheses are square root (x+1) transformed values

*Days after treatment

treatment (16.80%). In *M. anisopliae* treatment the per cent weight loss was 19.38% which was statistically at par with alsi seed extract (20.75%) followed by pumpkin seed extract (27.40%). Significantly maximum weight loss was found in control (36.21%). No change in the weight of the comb was observed in deep freezing (-8°C to -10°C) as all the larvae died after 5 hours of treatment.

Fourteen days after treatment, the minimum per cent comb weight loss was recorded in *Bt* (3.32%) which was statistically at par with neem seed kernel extract (3.94%) followed by sulphur fumigation (5.25%) which was at par with neem oil treatment (5.95%). The reduction in weight of the comb in neem leaf powder treatment was 7.78% followed by karanj oil treatment (11.03%) which was statistically at par with acetic acid treatment (11.80%). In formic acid treatment the weight loss was 14.50% followed by *T. viridae* (29.89%) which was statistically at par with alsi seed extract treatment (30.93%) and *B. bassiana* (31.88%) which was further at par with *M. anisopliae* (34.89%) followed by pumpkin seed extract (42.10%). Significantly maximum reduction in weight of the comb was found in control (58.86%).

After 21 days of treatment, the minimum per cent comb weight loss was observed in *Bt* (4.48%) which was statistically at par with sulphur fumigation (6.00%), neem seed kernel extract (6.25%) followed by neem oil treatment (7.86%) which was statistically at par with neem leaf powder treatment (11.31%). The per cent decrease in weight of comb in karanj oil treatment was 13.91% which was at par with acetic acid treatment (15.70%) followed by formic acid treatment (17.86%), *T. viridae* (35.82%) which was at par with *B. bassiana* treatment (40.97%). In *M. anisopliae* the per cent weight loss was 41.63% which was statistically at par with alsi seed extract (47.60%) followed by Pumpkin seed extract (64.60%). Significantly maximum infested area was found in control (81.68%).

b) During 2021

Data in Table 62 revealed that after seven days of treatment, the minimum per cent weight loss of comb was recorded in *Bt* (2.94%) which was statistically at par with neem seed kernel extract (3.34%), sulphur fumigation (3.99%) and neem oil treatment (4.01%) followed by neem leaf powder treatment (5.41%) which was statistically at par with acetic acid treatment (6.97%).

Table 62. Effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions during 2021

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7 DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	46.40 (6.88)#	44.54 (6.75)	4.01 (2.24)	44.14 (6.72)	6.41 (2.72)	41.90 (6.55)	9.72 (3.27)
Dried neem leaf powder	8.33g	48.13 (7.01)	45.53 (6.82)	5.41 (2.53)	44.61 (6.75)	8.85 (3.14)	42.96 (6.63)	10.75 (3.43)
Neem seed kernel extract	5%	46.10 (6.86)	44.58 (6.75)	3.34 (2.08)	44.46 (6.74)	5.15 (2.48)	43.14 (6.64)	6.53 (2.74)
Karanj oil	3%	42.99 (6.63)	38.78 (6.31)	9.76 (3.28)	37.57 (6.21)	14.20 (3.90)	36.69 (6.14)	14.69 (3.96)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	40.72 (6.46)	33.44 (5.87)	18.55 (4.42)	29.60 (5.53)	28.97 (5.47)	23.47 (4.95)	41.80 (6.54)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	39.00 (6.32)	29.17 (5.49)	25.28 (5.13)	23.38 (4.94)	42.05 (6.56)	13.14 (3.76)	65.38 (8.15)
Bacillus thuringiensis var. *kurstaki*	5g/l water	43.76 (6.69)	42.47 (6.59)	2.94 (1.99)	42.82 (6.62)	3.73 (2.17)	41.55 (6.52)	5.06 (2.46)
Trichoderma viridae	2ml/ l water	39.87 (6.39)	34.06 (5.92)	14.69 (3.96)	28.55 (5.44)	30.06 (5.57)	25.39 (5.14)	36.60 (6.13)
Bauveria bassiana	2ml/ l water	42.74 (6.61)	35.60 (6.05)	16.70 (4.21)	28.42 (5.42)	35.05 (6.00)	26.09 (5.20)	39.04 (6.33)
Metarhizium anisopliae	2ml/ l water	46.70 (6.91)	38.15 (6.26)	18.34 (4.40)	29.87 (5.56)	37.56 (6.21)	26.91 (5.28)	42.41 (6.59)
Acetic acid spray	2ml/ l water	37.00 (6.16)	34.42 (5.95)	6.97 (2.82)	33.38 (5.86)	12.70 (3.70)	31.69 (5.72)	14.37 (3.92)
Formic acid spray	0.8ml/l water	45.42 (6.81)	41.29 (6.50)	9.12 (3.18)	39.40 (6.36)	16.15 (4.14)	37.93 (6.24)	16.45 (4.18)
Use of sulphur fumigation	5g	45.07 (6.79)	43.27 (6.65)	3.99 (2.23)	43.42 (6.66)	6.55 (2.75)	41.79 (6.54)	7.30 (2.88)
Deep freezing	(-8°C to -10°C)	37.78 (6.23)	37.78 (6.23)	0.00 (1.00)	37.78 (6.23)	0.00 (1.00)	37.78 (6.23)	0.00 (1.00)
Control		41.99 (6.56)	27.95 (5.38)	33.37 (5.86)	15.48 (4.06)	64.91 (8.12)	6.22 (2.69)	85.18 (9.28)
C.D. (0.05)		NS	(0.66)	(0.33)	(0.58)	(0.35)	(0.48)	(0.37)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

The per cent reduction in weight of the comb in formic acid treatment was 9.12% which was at par with karanj oil treatment (9.76%) followed by *T. viridae* treatment (14.69%) which was at par with *B. bassiana* (16.70%). In alsi seed extract the per cent weight loss was 18.34% which was statistically at par with *M. anisopliae* (18.55%). Significantly maximum weight loss was found in control (33.37%). No change in the weight of the comb was observed in deep freezing (-8^0C to -10^0C) as all the larvae died after 5 hours of treatment.

Fourteen days after treatment, the minimum per cent comb weight loss was recorded in *Bt* (3.73%) which was statistically at par with neem seed kernel extract (5.15%) followed by neem oil treatment (6.41%) which was at par with sulphur fumigation (6.55%). The per cent reduction in weight of the comb in neem leaf powder treatment was 8.85% followed by acetic acid treatment (12.70%) which was statistically at par with karanj oil treatment (14.20%). In formic acid treatment the weight loss was 16.15% followed by alsi seed extract treatment (28.97%) which was at par with *T. viridae* (30.06%). The per cent weight loss in *B. bassiana* was 35.05% which was statistically at par with *M. anisopliae* (37.56%) which was further at par with pumpkin seed extract (42.05%). Significantly maximum decrease in weight of the comb was found in control (64.91%).

After 21 days of treatment, the minimum per cent comb weight loss was observed in *Bt* (5.06%) which was statistically at par with neem seed kernel extract (6.53%) followed by sulphur fumigation (7.30%), neem oil treatment (9.72%) which was statistically at par with neem leaf powder treatment (10.75%). The per cent comb weight loss in acetic acid treatment was 14.37% which was at par with karanj oil treatment (14.69%) and formic acid treatment (16.45%) followed by *T. viridae* (36.60%) which was at par with *B. bassiana* treatment (39.04%). In alsi seed extract the per cent weight loss was 41.80% which was at par with *M. anisopliae* (42.41%) followed by Pumpkin seed extract (65.38%). Significantly maximum reduction in comb weight was recorded in control (85.18%).

c) Pooled data (2020-2021)

Data in Table 63 and Fig. 21 revealed that after seven days of treatment, the minimum per cent comb weight loss was recorded in *Bt* (2.49%) which was statistically at par with neem seed kernel extract (3.15%) followed by sulphur fumigation (3.82%) which was statistically at par with neem oil treatment (4.09%) and neem leaf powder treatment (5.08%). The per cent weight loss in acetic acid treatment was 6.97% followed by per cent reduction in

comb weight in karanj oil treatment (9.59%) which was at par with formic acid treatment (10.11%). In *T. viridae* treatment the per cent weight loss was 15.24% which was at par with *B. bassiana* (16.75 %). In *M. anisopliae* the weight loss was 18.86% which was statistically at par with alsi seed extract (19.65%) followed by pumpkin seed extract (26.34%). Significantly maximum per cent reduction in comb weight was found in control (34.79%). In deep freezing (-8℃ to -10℃) all the larval instars got killed so no change in the weight of the comb was observed.

Fourteen days after treatment, the minimum per cent comb weight loss was recorded in *Bt* (3.52%) which was statistically at par with neem seed kernel extract (4.54%) followed by sulphur fumigation (5.90%) which was statistically at par with neem oil treatment (6.18%). The per cent weight loss in neem leaf powder treatment was 8.31% followed by acetic acid treatment (12.25%) which was statistically at par with karanj oil treatment (12.61%). In formic acid treatment, the weight loss was 15.33% followed by alsi seed extract treatment (29.95%) which was at par with *T. viridae* (29.97%). The per cent weight loss in *B. bassiana* was 33.47 % at par with *M. anisopliae* (35.97%). The per cent comb weight loss in pumpkin seed extract was 42.08%. Significantly maximum comb weight loss was found in control (63.07%).

Twenty one days after treatment, the minimum per cent comb weight loss was observed in *Bt* (4.77%) which was statistically at par with neem seed kernel extract (6.39%) followed by sulphur fumigation (6.65%), neem oil treatment (9.33%) which was statistically at par with neem leaf powder treatment (11.03%). The per cent weight loss of comb in karanj oil treatment was 14.30 % which was at par with acetic acid treatment (15.04 %) followed by formic acid treatment (17.15%), *T. viridae* (36.21%) which was at par with *B. bassiana* treatment (40.00%). In *M. anisopliae* the reduction in the weight of comb was 42.02% which was at par with alsi seed extract (44.70%) followed by Pumpkin seed extract (64.99%). Significantly maximum decrease in comb weight was found in control (83.43%).

The efficacy of various non-chemical and chemical treatments against larval feeding of greater wax moth on treated *A. cerana* combs showed a similar trends of treatments as observed in treated *A. mellifera* combs. No feeding in *A. cerana* the combs was found in deep freezing treatment.

Table 63. Pooled data on effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions during 2020-2021

Treatment	Dose	Weight of wax comb (g) before treatment	Effect of larval feeding on weight of treated combs					
			7 DAT*		14DAT		21DAT	
			Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)	Weight of wax comb (g)	Weight loss (%)
Neem oil	3%	47.15 (6.94) #	45.21 (6.80)	4.09 (2.26)	44.60 (6.75)	6.18 (2.68)	42.72 (6.61)	9.33 (3.21)
Dried neem leaf powder	8.33g	46.46 (6.89)	44.10 (6.72)	5.08 (2.47)	42.97 (6.63)	8.31 (3.05)	41.34 (6.51)	11.03 (3.47)
Neem seed kernel extract	5%	44.31 (6.73)	42.95 (6.63)	3.15 (2.04)	42.66 (6.61)	4.54 (2.35)	41.51 (6.52)	6.39 (2.72)
Karanj oil	3%	41.90 (6.55)	37.85 (6.23)	9.59 (3.25)	37.02 (6.17)	12.61 (3.69)	35.98 (6.08)	14.30 (3.91)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	43.80 (6.69)	35.29 (6.02)	19.65 (4.54)	30.99 (5.66)	29.95 (5.56)	23.99 (5.00)	44.70 (6.76)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	39.29 (6.35)	28.95 (5.47)	26.34 (5.23)	23.15 (4.91)	42.08 (6.56)	13.57 (3.82)	64.99 (8.12)
Bacillus thuringiensis var. *kurstaki*	5g/l water	43.78 (6.69)	42.68 (6.61)	2.49 (1.87)	42.58 (6.60)	3.52 (2.13)	41.69 (6.53)	4.77 (2.40)
Trichoderma viridae	2ml/ l water	42.72 (6.61)	36.21 (6.10)	15.24 (4.03)	30.23 (5.59)	29.97 (5.57)	27.35 (5.32)	36.21 (6.10)
Bauveria bassiana	2ml/ l water	42.21 (6.57)	35.11 (6.01)	16.75 (4.21)	28.41 (5.42)	33.47 (5.87)	25.34 (5.13)	40.00 (6.40)
Metarhizium anisopliae	2ml/ l water	45.62 (6.83)	37.03 (6.17)	18.86 (4.46)	29.56 (5.53)	35.97 (6.08)	26.46 (5.24)	42.02 (6.56)
Acetic acid spray	2ml/ l water	42.75 (6.61)	39.51 (6.36)	7.52 (2.92)	38.40 (6.28)	12.25 (3.64)	36.28 (6.11)	15.04 (4.00)
Formic acid spray	0.8ml/l water	42.11 (6.57)	37.89 (6.24)	10.11 (3.33)	36.55 (6.13)	15.33 (4.04)	34.91 (5.99)	17.15 (4.26)
Use of sulphur fumigation	5g	46.15 (6.87)	44.38 (6.74)	3.82 (2.20)	44.40 (6.74)	5.90 (2.63)	43.08 (6.64)	6.65 (2.77)
Deep freezing	(-8°C to -10°C)	42.99 (6.63)	42.99 (6.63)	0.00 (1.00)	42.99 (6.63)	0.00 (1.00)	42.99 (6.63)	0.00 (1.00)
Control		45.96 (6.85)	29.90 (5.56)	34.79 (5.98)	17.44 (4.29)	63.07 (8.00)	8.02 (3.00)	83.43 (9.19)
C.D. (0.05)		NS	(0.71)	(0.29)	(0.59)	(0.30)	(0.74)	(0.32)

#Figures in parentheses are square root (x+1) transformed values
*Days after treatment

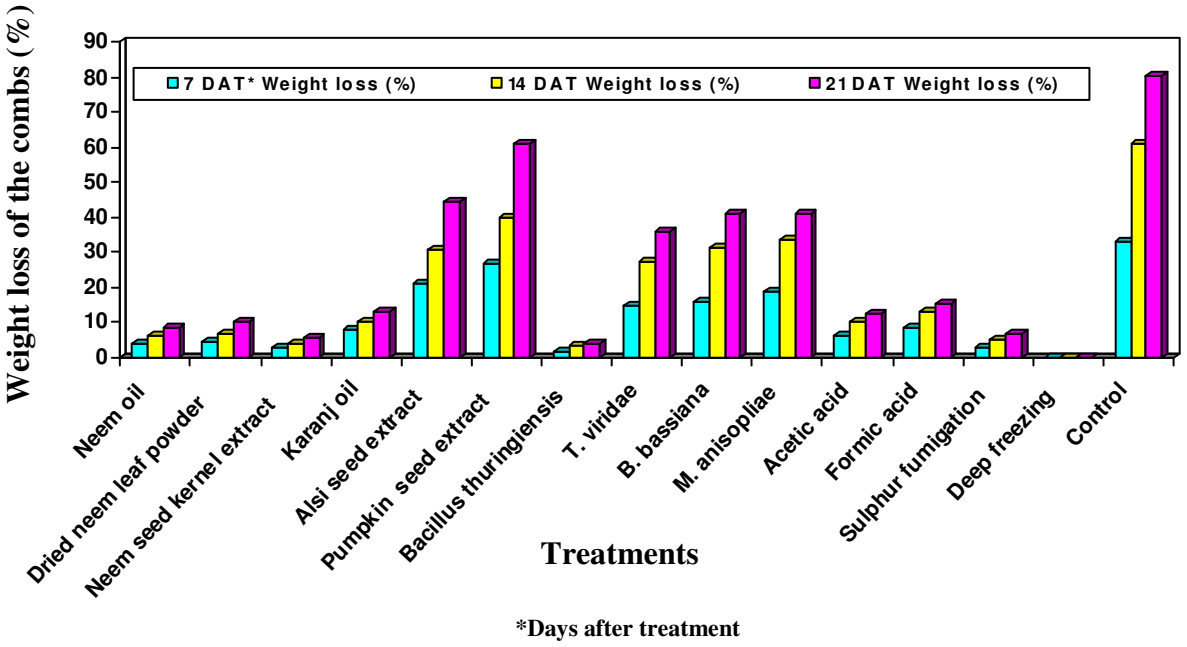


Fig 20. Effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions (2020-2021)

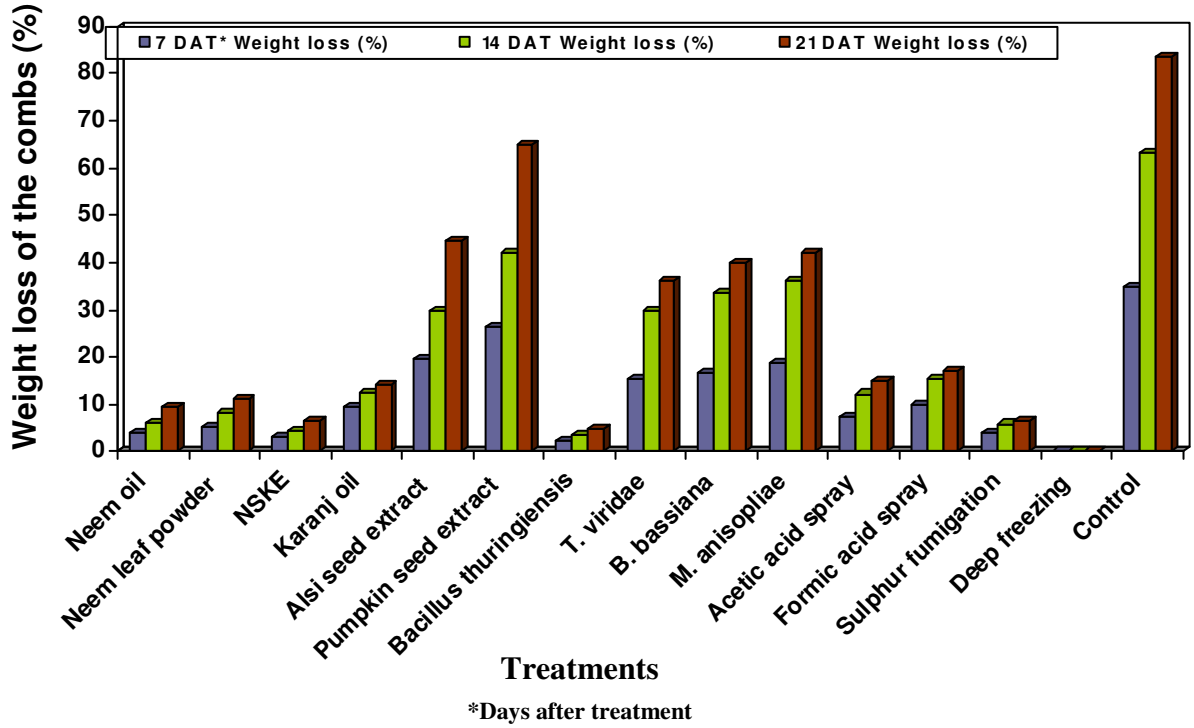


Fig 21. Effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions (2020-2021)

After 21 days of treatment, among other treatments, *Bt* showed lowest weight loss (4.77%) and was found to be equally good to NSKE (6.39%). Next to these, was sulphur fumigation (6.65%) equally effective as neem oil (9.33%) and neem leaf powder treatment (11.03%). The per cent weight loss in three biopesticides used in present studies ranged from 36.21% in *T. viridae* to 42.02% in *M. anisopliae* and karanj oil treatment with 14.30% weight loss of the comb showed same effects as acetic acid treatment as formic acid treatment. The per cent weigh loss of treated and untreated combs due to larval feeding of greater wax moth was observed higher in *A. cerana* combs in comparison to *A. mellifera* combs in present studies. It showed the preference of greater wax moth larvae for *A. cerana* wax combs.

4.3.5 Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*Galleria mellonella*) by using *Apis mellifera* combs

a) During 2020

Data in Table 64 revealed that significantly maximum per cent larval mortality was recorded in deep freezing (100%) followed by *Bt* (87.50%), neem seed kernel extract (75.00%) which was statistically at par with sulphur fumigation (72.50%) and neem oil treatment (67.50%). The per cent larval mortality in acetic acid treatment was 62.50% statistically at par with neem leaf powder treatment (60.00%) followed by karanj oil treatment (55.00%) which was at par with formic acid treatment (52.50%). In *T. viridae* treatment the per cent larval mortality was 35.00% which was at par with *B. bassiana* (32.50%) and *M. anisopliae* (30.00%). Per cent larval mortality in alsi seed extract was 25.00% followed by pumpkin seed extract (12.50%). Significantly minimum per cent larval mortality was found in control (5.00%).

Maximum per cent pupal mortality was recorded in *Bt* (83.33%) which was statistically at par with neem seed kernel extract (73.15%) and sulphur fumigation (72.92%) followed by neem oil treatment (63.75%) which was statistically at par with acetic acid treatment (58.33%). The per cent pupal mortality in neem leaf powder treatment was 51.19% which was statistically at par with formic acid treatment (45.83%) and karanj oil treatment (45.48%) followed by *T. viridae* (35.38%) which was at par with alsi seed extract (33.96%). The pupal mortality in *B. bassiana* treatment was 27.62% which was statistically at par with *M. anisopliae* (23.75%) followed by pumpkin seed extract (17.22%). Significantly minimum per cent pupal mortality was observed in control (2.50%).

Significantly minimum per cent adult emergence was recorded in *Bt* (16.67%) followed by neem seed kernel extract (25.76%) which was statistically at par with sulphur fumigation (27.08 %) followed by neem oil treatment (36.25%) which was at par with acetic acid treatment (41.67%). The per cent adult emergence in neem leaf powder treatment was 48.81% which was statistically at par with formic acid treatment (54.17%) and karanj oil treatment (54.52%) followed by *T. viridae* (64.52%) which was statistically at par with alsi seed extract (66.04%), *B. bassiana* treatment (72.38%) and *M. anisopliae* (76.25%). Significantly maximum per cent adult emergence was observed in control (97.50%) which was statistically at par with pumpkin seed extract (82.78%).

Table 64. Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. mellifera* combs during 2020

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	67.50 (8.28)*	63.75 (8.05)	36.25 (6.10)
Dried neem leaf powder	8.33g	60.00 (7.81)	51.19 (7.22)	48.81 (7.06)
Neem seed kernel extract	5%	75.00 (8.72)	73.15 (8.61)	25.76 (5.30)
Karanj oil	3%	55.00 (7.48)	45.48 (6.82)	54.52 (7.45)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	25.00 (5.10)	33.96 (5.91)	66.04 (8.19)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	12.50 (3.67)	17.22 (4.27)	82.78 (9.15)
Bacillus thuringiensis var. *kurstaki*	5g/l water	87.50 (9.41)	83.33 (9.18)	16.67 (4.20)
Trichoderma viridae	2ml/ l water	35.00 (6.00)	35.48 (6.04)	64.52 (8.09)
Bauveria bassiana	2ml/ l water	32.50 (5.79)	27.62 (5.35)	72.38 (8.57)
Metarhizium anisopliae	2ml/ l water	30.00 (5.57)	23.75 (4.97)	76.25 (8.79)
Acetic acid spray	2ml/ l water	62.50 (7.97)	58.33 (7.70)	41.67 (6.53)
Formic acid spray	0.8ml/l water	52.50 (7.31)	45.83 (6.84)	54.17 (7.43)
Use of sulphur fumigation	5g	72.50 (8.57)	72.92 (8.60)	27.08 (5.30)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control		5.00 (2.45)	2.50 (1.87)	97.50 (9.92)
C.D. (0.05)		(0.48)	(0.63)	(0.92)

*Figures in parentheses are square root (x+1) transformed values

b) During 2021

Data in Table 65 demonstrated that significantly maximum per cent larval mortality was recorded in deep freezing (100%) followed by *Bt* (85.00%) which was statistically at par with neem seed kernel extract (77.50%) and sulphur fumigation (75.00 %) followed by acetic acid treatment (65.00%) which was statistically same with neem oil treatment (65.00%) and was statistically at par with neem leaf powder treatment (57.50%).

Table 65. Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. mellifera* combs during 2021

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	65.00 (8.12)*	67.50 (8.28)	32.50 (5.79)
Dried neem leaf powder	8.33g	57.50 (7.65)	65.08 (8.13)	34.95 (6.00)
Neem seed kernel extract	5%	77.50 (8.86)	83.33 (9.18)	16.67 (4.20)
Karanj oil	3%	55.00 (7.48)	56.67 (7.59)	43.33 (6.66)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	27.50 (5.34)	31.73 (5.72)	68.27 (8.32)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	15.00 (4.00)	12.50 (3.67)	87.50 (9.41)
Bacillus thuringiensis var. *kurstaki*	5g/l water	85.00 (9.27)	88.89 (9.48)	11.11 (3.48)
Trichoderma viridae	2ml/ l water	32.50 (5.79)	32.14 (5.76)	67.86 (8.30)
Bauveria bassiana	2ml/ l water	30.00 (5.57)	24.29 (5.03)	75.71 (8.76)
Metarhizium anisopliae	2ml/ l water	27.50 (5.34)	23.06 (4.90)	76.94 (8.83)
Acetic acid spray	2ml/ l water	65.00 (8.12)	59.17 (7.76)	40.43 (6.47)
Formic acid spray	0.8ml/l water	47.50 (6.96)	53.45 (7.38)	46.55 (6.90)
Use of sulphur fumigation	5g	75.00 (8.72)	79.17 (8.95)	20.83 (4.67)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control		2.50 (1.87)	2.50 (1.87)	97.50 (9.92)
C.D. (0.05)		(0.59)	(0.68)	(0.72)

*Figures in parentheses are square root (x+1) transformed values

The per cent larval mortality in karanj oil treatment was 55.00% which was statistically at par with formic acid treatment (47.50%) followed by *T. viridae* (32.50%) which was statistically at par with *B. bassiana* (30.00 %), alsi seed extract (27.50%) and *M. anisopliae* (27.50%). The per cent larval mortality in pumpkin seed extract was 15.00%. Significantly minimum per cent larval mortality was found in control (2.50%).

Maximum per cent pupal mortality was recorded in *Bt* (88.89%) which was statistically at par with neem seed kernel extract (83.33%) and sulphur fumigation (79.17%) followed by neem oil treatment (67.50%) which was statistically at par with neem leaf powder treatment (65.08%) and acetic acid treatment (59.17%). The per cent pupal mortality in karanj oil treatment was 56.67% which was statistically at par with formic acid treatment (53.45%) followed by *T. viridae* (32.14%) which was at par with alsi seed extract (31.73%). The pupal mortality in *B. bassiana* treatment was 24.29% which was statistically at par with *M. anisopliae* (23.06%) followed by pumpkin seed extract (12.50%). Significantly minimum per cent pupal mortality was observed in control (2.50%).

Minimum per cent adult emergence was recorded in *Bt* (11.11%) which was statistically at par with neem seed kernel extract (16.67%) followed by sulphur fumigation (20.83%), neem oil treatment (32.50%) which was statistically at par with neem leaf powder treatment (34.95%) and acetic acid treatment (40.43%). The per cent adult emergence in karanj oil treatment was 43.33% which was statistically at par with formic acid treatment (46.55%) followed by *T. viridae* (67.86%) which was statistically at par with alsi seed extract (68.27%), *B. bassiana* (75.71 %) and *M. anisopliae* (76.94%). Significantly maximum per cent adult emergence was observed in control (97.50%) which was statistically at par with pumpkin seed extract (87.50%).

c) Pooled data (2020-2021)

Data in Table 66 and Fig. 22 demonstrated that significantly maximum per cent larval mortality was recorded in deep freezing (100%) followed by *Bt* (86.25%), neem seed kernel extract (76.25%) which was statistically at par with sulphur fumigation (73.75%) followed by neem oil treatment (66.25%) which was at par with acetic acid treatment (63.75%) and neem leaf powder treatment (58.75%). The per cent larval mortality in karanj oil treatment was 55.00% which was statistically at par with formic acid treatment (50.00%) followed by *T.*

viridae (33.75%) which was statistically at par with *B. bassiana* (31.25%) and *M. anisopliae* (28.75%). The per cent larval mortality in alsi seed extract was 26.25% followed by pumpkin seed extract (13.75%). Significantly minimum per cent larval mortality was found in control (3.75%).

Maximum per cent pupal mortality was recorded in *Bt* (86.11%) which was statistically at par with neem seed kernel extract (78.13%) and sulphur fumigation (76.04%) followed by neem oil treatment (65.63%) which was at par with acetic acid treatment (58.75%) and neem leaf powder treatment (58.13%). The per cent pupal mortality in karanj oil treatment was 51.07% which was statistically at par with formic acid treatment (49.64%) followed by *T. viridae* (33.81%) which was at par with alsi seed extract (32.84%). The pupal mortality in *B. bassiana* treatment was 25.95% which was statistically at par with *M. anisopliae* (23.40%) followed by pumpkin seed extract (14.86%). Significantly minimum per cent pupal mortality was observed in control (2.50%).

Significantly minimum per cent adult emergence was recorded in *Bt* (12.50%) followed by neem seed kernel extract (21.88%) which was statistically at par with sulphur fumigation (23.96%). The per cent adult emergence in neem oil treatment was 34.38 % which was statistically at par with neem leaf powder treatment (37.50%) and acetic acid treatment (41.25%) followed by karanj oil treatment (48.93%) which was statistically at par with formic acid treatment (50.36%). The per cent adult emergence in *T. viridae* was 66.99% which was statistically at par with alsi seed extract (67.16%), *B. bassiana* treatment (74.05 %) and *M. anisopliae* (76.60%) followed by pumpkin seed extract (85.14%). Significantly maximum per cent adult emergence was observed in control (97.50%) (Plate 28).

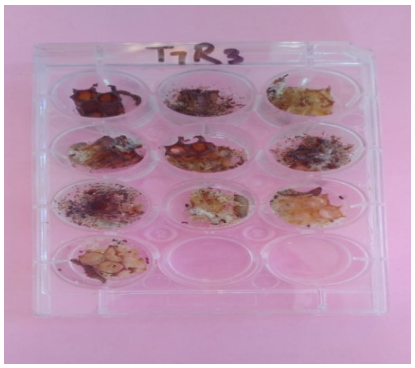
The highest larval mortality (100%) was observed in T4 (deep freezing at -8°C to -10°C) in 4-5 hours. Similar results were reported by (Shimanuki, 1981). They recommended 20ºF (-7ºC) for 4.5 hours, 10ºF (12ºC) for three hours, or 5ºF (-15ºC) for two hours to kill all life stages of wax moths in honey-extracted combs. Deep freezer treatment for 5 h was found best treatment for management of greater wax moth under storage conditions and all the instars got killed (Katna *et al.,* 2012). Zhu *et al.* (2016) proposed cold treatment < -15°C for a full 10h to kill all the stages of greater wax moth. Low temperature treatment avoids the comb “sagging” problem even fire damage which sometimes occurs when combs are treated with heat and thus cold treatment is a promising option for greater wax moth control, as it

avoids physical damage and was cheaper and pollution free. There are certain limitations of using this method on practical level, this method is suitable for a relatively small number of combs and combs need 24 hours at -18°C to destroy eggs and caterpillars (The British beekeepers association, 2012). Because of the limitations associated with the use of deep freeze method, other methods of control were evaluated and suggested by many workers (Sattigi *et al.*, 1990; Izar- ul-Haq *et al.*, 2008; Bhopale *et al.*, 2013 and Negi *et al.*, 2019).

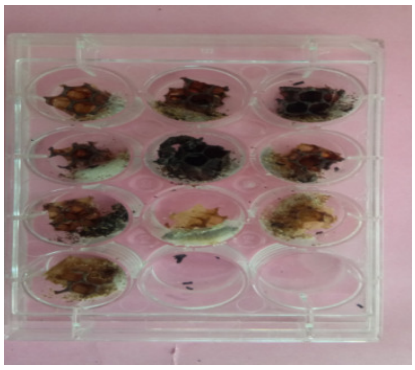
Table 66. Pooled data on effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. mellifera* combs during 2020-2021

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	66.25 (8.20)*	65.63 (8.16)	34.38 (5.95)
Dried neem leaf powder	8.33g	58.75 (7.73)	58.13 (7.69)	37.50 (6.20)
Neem seed kernel extract	5%	76.25 (8.79)	78.13 (8.90)	21.88 (4.78)
Karanj oil	3%	55.00 (7.48)	51.07 (7.22)	48.93 (7.07)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	26.25 (5.22)	32.84 (5.82)	67.16 (8.26)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	13.75 (3.84)	14.86 (3.98)	85.14 (9.28)
Bacillus thuringiensis var. *kurstaki*	5g/l water	86.25 (9.34)	86.11 (9.33)	12.50 (3.67)
Trichoderma viridae	2ml/ l water	33.75 (5.89)	33.81 (5.90)	66.19 (8.20)
Bauveria bassiana	2ml/ l water	31.25 (5.68)	25.95 (5.19)	74.05 (8.66)
Metarhizium anisopliae	2ml/ l water	28.75 (5.45)	23.40 (4.94)	76.60 (8.81)
Acetic acid spray	2ml/ l water	63.75 (8.05)	58.75 (7.73)	41.25 (6.50)
Formic acid spray	0.8ml/l water	50.00 (7.14)	49.64 (7.12)	50.36 (7.17)
Use of sulphur fumigation	5g	73.75 (8.65)	76.04 (8.78)	23.96 (5.00)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control		3.75 (2.18)	2.50 (1.87)	97.50 (9.92)
C.D. (0.05)		(0.52)	(0.70)	(0.63)

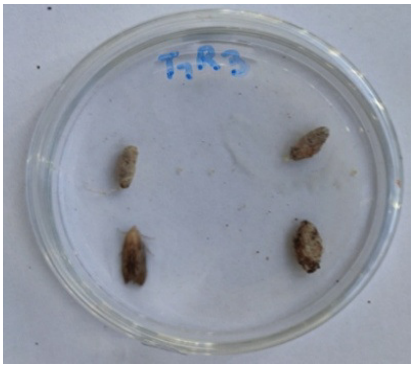
*Figures in parentheses are square root (x+1) transformed values



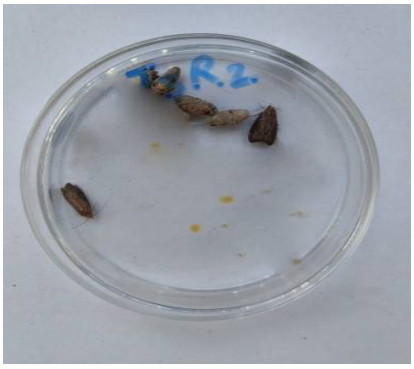
a) Larval mortality in the *Bt* treatment



b) Control



c) *Bacillus thuringiensis*



d) NSKE

e) Sulphur fumigation

f) Neem oil

g) Pupae and emerged adults in Control

Plate 28. Larval, pupal mortality and emerged adults in *A. mellifera* combs

In the present studies different treatments tested at various concentrations were shown to exhibit varying degree of activity against the larval, pupal mortality and adults emergence of *G. mellonella*. Next to deep freezing treatment, the highest larval, pupal mortality and lowest adult emergence was recorded in *Bt* (86.25%, 86.11% and 12.50%) followed by NSKE (76.25%, 78.13% and 21.88%) are in accordance with those of Bhopale *et al.* (2013) who subjected different larval instars of *G. mellonella* to botanicals and microbial pesticides viz., dried neem leaf, neem oil 3 per cent, *Bt* var. *kurstaki* (Halt), *Bt* local strain-1 and *Bt* (Halt) local strain-2, pongamia oil 3 per cent and NSKE 5 per cent and reported that *Bt kurstaki* and pongamia oil gave maximum larval mortality (93.33%).

Negi *et al.* (2019) used various concentrations of *Bt* (2.5, 5 and 8 g/lt of *Bt* var *kurstaki* 0.5% WP) and concluded that highest dose of *Bt* showed maximum 40.00% larval mortality. Izar-ul- Haq *et al.* (2008) examined different concentrations (0.5, 1, 2, 3 and 4%) of aqueous extract of neem seed against greater wax moth. The highest mortality (83.33%) of greater wax moth was observed with 4% neem seed kernel extract (NSKE) while 50% mortality was found with 0.5% neem seed extract. These results also coincide with those of Sattigi *et al.* (1990) who reported the effectiveness of smearing of hive with lime sulphur paste on wax moth infestation. The treatment provided 78% protection to colonies from wax moth attack. As a result of feeding of greater wax moth larvae on treated combs, the longevity and fecundity of adults may be affected adversely by the action of plant derivatives on the physiology and nervous system of insects and metabolism of larvae may also be affected so the process of conversion of pupa to adult may be either extended or complete pupal death may occur. The death of insect in pupal stage prevent adult emergence, greater the number of dead pupae lesser were the number of emerged adults. Therefore, adult emergence was minimum in the treatments in which the pupal mortality was high *viz., Bt*, NSKE and sulphur fumigation. Some secondary compounds are present in neem seed extract induce egg sterility, oviposition repellency and inhibition of chitin biosynthesis (Ascher, 1993).

4.3.6 Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) by using *A. cerana* combs

a) During 2020

Data in Table 67 showed that that significantly maximum per cent larval mortality was recorded in deep freezing (100%) followed by *Bt* (82.50%), neem seed kernel extract

(70.00%) which was statistically at par with sulphur fumigation (67.50%), acetic acid treatment (65.00%) and neem oil treatment (62.50%). The per cent larval mortality in neem leaf powder treatment was 55.00% which was statistically at par formic acid treatment (52.50%) and karanj oil treatment (50.00%) followed by *T. viridae* (32.50%) which was at par with *B. bassiana* (30.00%) and *M. anisopliae* (27.50%). Per cent larval mortality in alsi seed extract was 20.00%. Significantly minimum per cent larval mortality was found in pumpkin seed extract (12.50%). No larval mortality was recorded in control in which no treatment was given.

Table 67. Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. cerana* combs during 2020

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	62.50 (7.97)*	69.17 (8.38)	30.83 (5.64)
Dried neem leaf powder	8.33g	55.00 (7.48)	64.29 (8.08)	35.71 (6.06)
Neem seed kernel extract	5%	70.00 (8.43)	78.33 (8.91)	21.67 (4.76)
Karanj oil	3%	50.00 (7.14)	58.33 (7.70)	41.67 (6.53)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	20.00 (4.58)	22.92 (4.89)	77.08 (8.84)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	12.50 (3.67)	14.72 (3.97)	85.28 (9.29)
Bacillus thuringiensis var. *kurstaki*	5g/l water	82.50 (9.14)	91.67 (9.63)	8.33 (3.06)
Trichoderma viridae	2ml/ l water	32.50 (5.79)	34.58 (5.97)	65.42 (8.15)
Bauveria bassiana	2ml/ l water	30.00 (5.57)	26.79 (5.27)	73.21 (8.61)
Metarhizium anisopliae	2ml/ l water	27.50 (5.34)	25.83 (5.18)	74.17 (8.67)
Acetic acid spray	2ml/ l water	65.00 (8.12)	61.67 (7.92)	38.33 (6.27)
Formic acid spray	0.8ml/l water	52.50 (7.31)	50.00 (7.14)	50.00 (7.14)
Use of sulphur fumigation	5g	67.50 (8.28)	74.17 (8.67)	25.83 (5.18)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control		0.00 (1.00)	0.00 (1.00)	95.00 (9.80)
C.D. (0.05)		(0.61)	(0.58)	(0.83)

*Figures in parentheses are square root (x+1) transformed values

Significantly maximum per cent pupal mortality was recorded in *Bt* (91.67%) followed by neem seed kernel extract (78.33%) which was statistically at par with sulphur fumigation (74.17%) and neem oil treatment (69.17%). The per cent pupal mortality in neem leaf powder treatment was 64.29% which was statistically at par with acetic acid treatment (61.67%) and karanj oil treatment (58.33%) which was further at par with formic acid treatment (50.00%). The per cent pupal mortality in *T. viridae* was 34.58% followed by *B. bassiana* treatment (26.79 %) which was at par with *M. anisopliae* (25.83%) further at par with alsi seed extract (22.92%). The per cent pupal mortality in pumpkin seed extract was 14.72%. Significantly minimum per cent pupal mortality was observed in control (5.00%).

Significantly minimum per cent adult emergence was recorded in *Bt* (8.33%) followed by neem seed kernel extract (21.67%) which was statistically at par with sulphur fumigation (25.83%). The per cent adult emergence in neem oil treatment was 30.83 % which was statistically at par with neem leaf powder treatment (35.71%) and acetic acid treatment (38.33%). The per cent adult emergence in karanj oil treatment was 41.67% which was statistically at par with formic acid treatment (50.00%) followed by *T. viridae* (65.42%) which was at par with *B. bassiana* treatment (73.21%), *M. anisopliae* (74.17%) and alsi seed extract (77.08%). Maximum per cent adult emergence was observed in control (95.00%) which was statistically at par with pumpkin seed extract (85.28%).

b) During 2021

Data in Table 68 demonstrated that significantly maximum per cent larval mortality was recorded in deep freezing (100%) followed by *Bt* (90.00%), neem seed kernel extract (75.00%) which was statistically at par with sulphur fumigation (72.50%). The per cent larval mortality in neem oil treatment was 65.00% which was statistically at par with acetic acid treatment (62.50%) followed by neem leaf powder treatment (57.50%), karanj oil treatment (47.50%) which was statistically same with formic acid treatment (47.50%). The larval mortality in *T. viridae* was 27.50% which was statistically at par with *B. bassiana* (25.00%) followed by *M. anisopliae* (22.50%) which was statistically at par with alsi seed extract (20.00%). The per cent larval mortality in pumpkin seed extract was 10.00%. Significantly minimum per cent larval mortality was found in control (2.50%).

Maximum per cent pupal mortality was recorded in *Bt* (83.33%) which was statistically at par with neem seed kernel extract (79.17%) and sulphur fumigation (72.92%) followed by neem oil treatment (66.25%) which was at par with neem leaf powder treatment (62.70%) and acetic acid treatment. The per cent pupal mortality in karanj oil treatment was 54.17% followed by formic acid treatment (45.42%), *T. viridae* (31.73%) which was at par with *B. bassiana* treatment (30.12%) and alsi seed extract (26.19%). The pupal mortality in *M. anisopliae* was 24.40% followed by pumpkin seed extract (9.13%). Significantly minimum per cent pupal mortality was observed in control (2.50%).

Table 68. Effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. cerana* combs during 2021

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	65.00 (8.12)*	66.25 (8.20)	33.75 (5.89)
Dried neem leaf powder	8.33g	57.50 (7.65)	62.70 (7.98)	37.30 (6.19)
Neem seed kernel extract	5%	75.00 (8.72)	79.17 (8.95)	20.83 (4.67)
Karanj oil	3%	47.50 (6.96)	54.17 (7.43)	45.83 (6.84)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	20.00 (4.58)	26.19 (5.21)	73.81 (8.65)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	10.00 (3.32)	9.13 (3.18)	90.87 (9.59)
Bacillus thuringiensis var. *kurstaki*	5g/l water	90.00 (9.54)	83.33 (9.18)	16.67 (4.20)
Trichoderma viridae	2ml/ l water	27.50 (5.34)	31.73 (5.72)	68.27 (8.32)
Bauveria bassiana	2ml/ l water	25.00 (5.10)	30.12 (5.58)	69.88 (8.42)
Metarhizium anisopliae	2ml/ l water	22.50 (4.85)	24.40 (5.04)	75.60 (8.75)
Acetic acid spray	2ml/ l water	62.50 (7.97)	58.33 (7.70)	41.67 (6.53)
Formic acid spray	0.8ml/l water	47.50 (6.96)	45.42 (6.81)	54.58 (7.46)
Use of sulphur fumigation	5g	72.50 (8.72)	72.92 (8.60)	27.08 (5.30)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control	-	2.50 (1.87)	2.50 (1.87)	97.50 (9.92)
C.D. (0.05)		(0.42)	(0.59)	(0.64)

*Figures in parentheses are square root (x+1) transformed values

Significantly minimum per cent adult emergence was recorded in *Bt* (16.67%) which was statistically at par with neem seed kernel extract (20.23%) followed by sulphur fumigation (27.08%) which was at par with neem oil treatment (33.75%). The per cent adult emergence in neem leaf powder treatment was 37.30% which was statistically at par with acetic acid treatment (41.67%) followed by karanj oil treatment (45.83%) which was at par with formic acid treatment (54.58%). The per cent adult emergence in *T. viridae* was 68.27% which was statistically at par with *B. bassiana* treatment (69.88%), alsi seed extract (73.81%), and *M. anisopliae* (75.60%). Maximum per cent adult emergence was observed in control (97.50%) which was statistically at par with pumpkin seed extract (90.87%).

c) Pooled data (2020-2021)

Data in Table 69 and Fig. 23 showed that significantly maximum per cent larval mortality was recorded in deep freezing (100.00%) followed by *Bt* (86.25%), neem seed kernel extract (72.50%) which was statistically at par with sulphur fumigation (70.00%), acetic acid treatment (63.75%) and neem oil treatment (63.75%).The per cent larval mortality in neem leaf powder treatment was 56.25% which was statistically at par with formic acid treatment (50.00%) and karanj oil treatment (48.75%) followed by *T. viridae* (30.00%) which was statistically at par with *B. bassiana* (27.50%) and *M. anisopliae* (25.00%) which was at par with alsi seed extract (20.00%) followed by pumpkin seed extract (11.25%). Significantly minimum per cent larval mortality was found in control (1.25%).

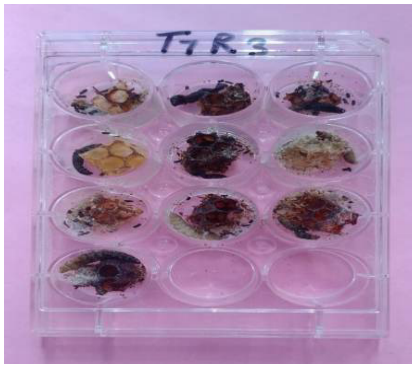
Maximum per cent pupal mortality was recorded in *Bt* (87.50%) which was statistically at par with neem seed kernel extract (78.75%) followed by sulphur fumigation (73.54%) which was statistically at par with neem oil treatment (67.71%) and neem leaf powder treatment (63.49%). The per cent pupal mortality in acetic acid treatment was 60.00% which was statistically at par with karanj oil treatment (56.25%) followed by formic acid treatment (47.71%), *T. viridae* (33.15%) which was at par with *B. bassiana* treatment (28.45%). The per cent pupal mortality in *M. anisopliae* was 25.12% which was statistically at par with alsi seed extract (24.55%). The per cent pupal mortality in pumpkin seed extract was 11.92%. Significantly minimum per cent pupal mortality was observed in control (3.75%) (Plate 29).

Significantly minimum per cent adult emergence was recorded in *Bt* (12.50%) followed by neem seed kernel extract (21.25%) which was statistically at par with sulphur fumigation (26.46 %). The per cent pupal mortality in neem oil treatment was 32.29% which was at par with neem leaf powder treatment was 36.5% followed by acetic acid treatment (40.00%) which was statistically at par with karanj oil treatment (43.75%). The per cent adult emergence in formic acid treatment was 52.29% followed by *T. viridae* (66.85%) which was statistically at par with *B. bassiana* treatment (71.55%), *M. anisopliae* (74.88%) and alsi seed extract (75.45%). Maximum per cent adult emergence was observed in control (96.25%) which was statistically at par with pumpkin seed extract (88.08%).

Table 69. Pooled data on effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) in *A. cerana* combs during 2020-2021

Treatment	Dose	Larval mortality (%)	Pupal mortality (%)	Adult emerged (%)
Neem oil	3%	63.75 (8.05)*	67.71 (8.29)	32.29 (5.77)
Dried neem leaf powder	8.33g	56.25 (7.57)	63.49 (8.03)	36.51 (6.12)
Neem seed kernel extract	5%	72.50 (8.57)	78.75 (8.93)	21.25 (4.72)
Karanj oil	3%	48.75 (7.05)	56.25 (7.57)	43.75 (6.69)
Alsi (*Linum usitatissimum*) seed extract	20g/ 100 ml acetone	20.00 (4.58)	24.55 (5.06)	75.45 (8.74)
Pumpkin (*Cucurbita moschata*) seed extract	20g / 100 ml acetone	11.25 (3.50)	11.92 (3.60)	88.08 (9.44)
Bacillus thuringiensis var. *kurstaki*	5g/l water	86.25 (9.34)	87.50 (9.41)	12.50 (3.67)
Trichoderma viridae	2ml/ l water	30.00 (5.57)	33.15 (5.84)	66.85 (8.24)
Bauveria bassiana	2ml/ l water	27.50 (5.34)	28.45 (5.43)	71.55 (8.52)
Metarhizium anisopliae	2ml/ l water	25.00 (5.10)	25.12 (5.11)	74.88 (8.71)
Acetic acid spray	2ml/ l water	63.75 (8.05)	60.00 (7.81)	40.00 (6.40)
Formic acid spray	0.8ml/l water	50.00 (7.14)	47.71 (6.98)	52.29 (7.30)
Use of sulphur fumigation	5g	70.00 (8.43)	73.54 (8.63)	26.46 (5.24)
Deep freezing	(-8°C to -10°C)	100.00 (10.05)	0.00 (1.00)	0.00 (1.00)
Control	-	1.25 (1.50)	3.75 (2.18)	96.25 (9.86)
C.D. (0.05)		(0.64)	(0.71)	(0.60)

*Figures in parentheses are square root (x+1) transformed values



a) Larval mortality in *Bt* treatment

b) Control

c) *Bacillus thuringiensis*

d) NSKE

e) Sulphur fumigation

f) Neem oil

g) Pupae and emerged adults in Control

Plate 29. Larval, pupal mortality and emerged adults in *A. cerana* combs

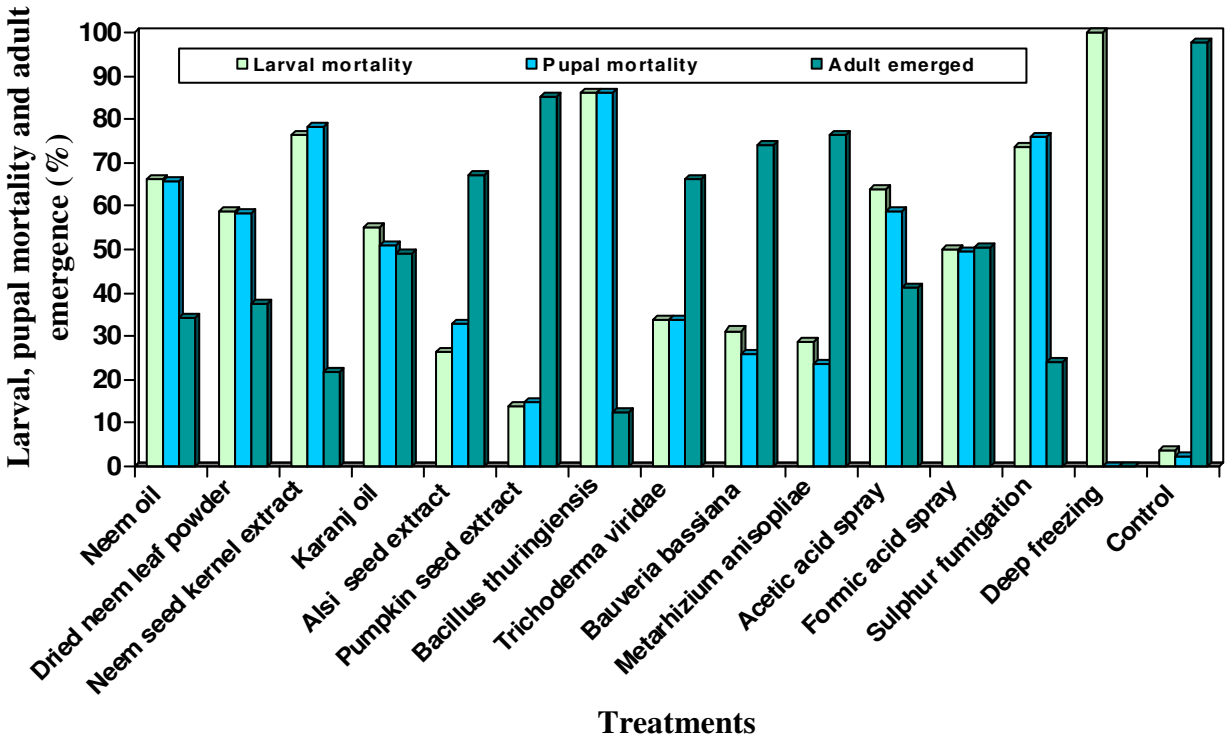


Fig 22. Per cent larval, pupal mortality and adult emergence in *A. mellifera* treated combs under laboratory conditions (2020-2021)

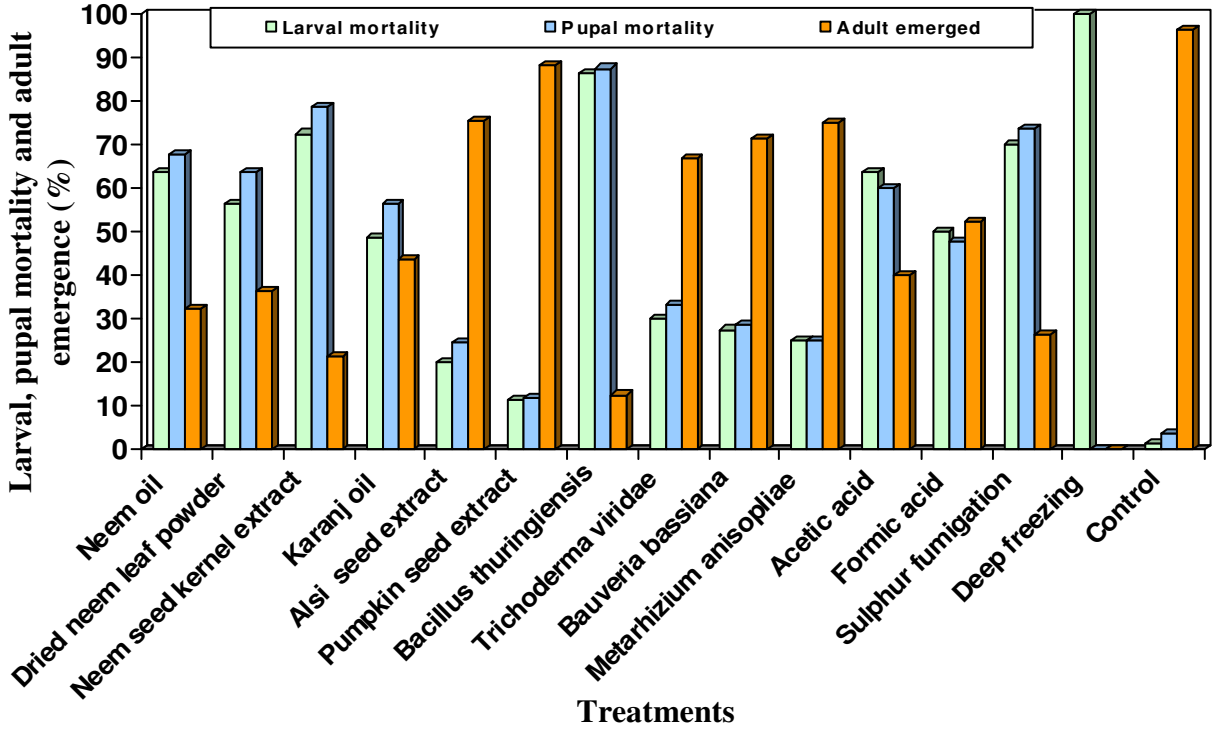


Fig 23. Per cent larval, pupal mortality and adult emergence in *A. cerana* treated combs under laboratory conditions (2020-2021)

In our studies the trend of larval, pupal mortality and adult emergence in *A. Cerana* combs is similar as observed in *A. mellifera* combs. Highest larval mortality was observed in deep freezing as all the third instar larvae found dead after 5 hours of treatment (Kumar and Khan, 2019 and Katna *et al.,* 2012). *Bt* showed maximum larval and pupal mortality and minimum adult emergence (86.25%, 87.50% and 12.50%) followed by NSKE (72.50%, 78.75% and 21.25%) which was equally effective as sulphur fumigation (70.00%, 73.54% and 26.46%).

Our results coincides with Rahman *et al.* (2017) who reported that *Bt.* var *Karstaki* at 1 percent concentration was found to be most effective in minimizing the percent comb infestation and cumulative mortality of larvae was found to be 93.32% followed by sulphur fumigation @ 28 g/hive (89.98%) and neem oil @ 3% (79.99%). Basedow *et al*. (2012) observed that when third instar larvae of *G. mellonella* were fed for five days on beeswax dipped for 20 sec into 0.5, 1 and 2 g/l water concentrations of the commercial preparations of *Bt* aizawai viz. XenTari® and 20, 40 and 80 ppm azadirachtin *viz.,* NeemAzal®-T/S. The larval mortality by *Bt* (2g/l), after four weeks was 77% and the emergence of moths was delayed by four days in *Bt* treatments, even when no mortality occurred. The highest concentration of NeemAzal T/S (80 ppm) caused 100% mortality within a month. Effectiveness of sulphur fumigation on *G. mellonella* was tested by Ahmed *et al.* (1993) and Calderone, 2000 observed that sulphur fumigation can be used for controlling wax moth. Dumani and Altuntas (2018) observed that sublethal Azadirachtin (0.5, 1, 1.5, and 2 µg/larva) given to *G. mellonella* larvae via insect force feeding method. At 72 h post force feeding with median lethal dose of Azadirachtin, a significant increase in DNA damage indicators was observed in larval hemocytes as compared with untreated groups. Consequently, this study showed that Azadirachtin caused significant damage in the genome of *G. mellonella* larvae even at sublethal doses. Neem oil, cedar oil, clove oil, peppermint oil, Karanj oil, and neem seed kernel extract highly effects mortality of greater wax moth (*G. mellonella*) in storage conditions. Aadirachtin disrupted the molting process in larva and pupa and moults of last instar larvae and also induced mortality (Malczeuska *et al.,* 1988).

4.4 Management of greater wax moth (*G. mellonella*) in *A. mellifera* and *A. cerana* colonies under field conditions

4.4.1 Management of Greater wax moth (*G. mellonella*) in *A. cerana* colonies

a) During 2020

Wax moth incidence under natural field conditions (in live colonies) in apiary were recorded under various treatments such as periodical cleaning of hives, treating cracks and

crevices with lime Sulphur paste, application of *Bt* product, neem oil, neem seed kernel extracts, integrated management practices and setting of traps (delta trap and wax moth trap). The observations were recorded at 30 days interval upto three months. Data in Table 70 revealed that during the year 2020, the initial infestation in the colonies varied non-significantly from 6.33 to 10.33 larvae per colony. One month after first treatment, minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with lime sulphur paste which was statistically at par with *Bt* (6g) treatment (1.33 larvae/hive) and neem seed kernel extract (1.67 larvae/hive). The number of wax moth larvae in *Bt* (3g) treatment were 2.33 larvae/hive which was statistically at par with neem oil treatment (2.67 larvae/hive) and bottom board cleaning (4.67 larvae/hive). Significantly maximum number of larvae/hive were found in untreated control (11 larvae/hive). No wax moth larval infestation was found in *Bt* (9g) treatment and colonies in which integrated management was done.

One month after second treatment, minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with neem seed kernel extract which was statistically at par with *Bt* (6g) treated colonies (1.00 larvae/hive), *Bt* (3g) treated colonies (2.00 larvae/hive) and neem oil treatment (2.00 larvae/hive). Significantly maximum infestation was found in untreated colonies (13 larvae/hive). No wax moth larval infestation was found in *Bt* (9gm), lime sulphur paste treatment and the colonies in which integrated management was done.

One month after third treatment minimum infestation was found in neem seed kernel extract treated colonies (0.33 larvae/hive) which was statistically at par with *Bt* (3g) with 0.67 larvae/hive followed by neem oil (1.67 larvae/hive) and bottom board cleaning (3.67 larvae per hive). Significantly maximum larval infestation was found in the colonies without any treatment (12.33 larvae/hive).

For mechanical control of wax moth (*G. mellonella*) the results presented in Table 70 revealed that during three months of treatment maximum adult wax moths male and females were trapped in wax moth trap followed by delta trap with *A. dorsata* comb, delta trap with *A. cerana* comb and delta trap with *A. mellifera* comb. One month after first treatment significantly maximum number of adults were trapped in wax moth trap (31.25 adult/trap) followed by delta trap with *A. dorsata* comb (20.25 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (11.00 females/trap) which was statistically at par to delta trap with *A. cerana* comb (13.75 females/trap). One month after second treatment maximum number of adults were trapped in wax moth trap (23.50

adults/trap) which was statistically at par to delta trap with *A. dorsata* comb (18.25 females/trap).

Table 70. Effect of different management practices on the incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during 2020

Control methods	Mean number of larvae / hive			
	Pre-count	1MAFT*	1MAST**	1MATT***
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	7.67 (2.94)#	2.33 (1.83)	2.00 (1.73)	0.67 (1.29)
Bt var. *kurstaki* @ 6g/l	8.33 (3.06)	1.33 (1.53)	1.00 (1.41)	0.00 (1.00)
Bt var. *kurstaki* @ 9g/l	6.33 (2.71)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non- chemical method				
Neem oil (3%)	7.67 (2.94)	2.67 (1.91)	2.00 (1.73)	1.67 (1.63)
Neem seed kernel extract (5%)	9.00 (3.16)	1.67 (1.63)	0.33 (1.15)	0.33 (1.15)
Sealing cracks and crevices with lime sulphur paste	6.67 (2.77)	0.33 (1.15)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	10.33 (3.37)	4.67 (2.38)	5.33 (2.52)	3.67 (2.16)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	8.67 (3.11)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	9.33 (3.21)	11.00 (3.46)	13.00 (3.74)	12.33 (3.65)
C.D.	NS	(0.57)	(0.73)	(0.44)

Mechanical method	**Moth catches in the trap/ month**		
	June	**July**	**August**
1. Delta trap with *A. dorsata* comb	20.25 (4.61)	18.25 (4.39)	15.75 (4.09)
2. Delta trap with *A. cerana* comb	13.75 (3.84)	11.25 (3.50)	9.25 (3.20)
3. Delta trap with *A. mellifera* comb	11.00 (3.46)	8.00 (3.00)	6.50 (2.74)
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	31.25 (5.68)	23.50 (4.95)	19.75 (4.56)
5. Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.(0.05)	(0.55)	(0.74)	(0.37)

#Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (8.00 females/trap) which was statistically at par with delta trap with *A. cerana* comb

(11.25 females/trap). One month after third treatment maximum number of adults were trapped in wax moth trap (19.75 adults/trap) followed by delta trap with *A. dorsata* comb (15.75 females/trap), delta trap with *A. cerana* comb (9.25 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (6.50 females/trap).

b) During 2021

Data on management of Greater wax moth (*G. mellonella*) in *A. cerana* colonies presented in Table 71 revealed that during the year 2021 the initial infestation in the colonies varies from 5.67 to 9.67 larvae. One month after first treatment, minimum number of larvae (0.67 larvae/hive) were found in the colonies treated with *Bt* (6g) which was statistically at par with *Bt* (3g) treatment (2.00 larvae/hive) and neem seed kernal extract (2.00 larvae/hive). The number of larvae found in neem oil treatment were 3.00 larvae/hive which was statistically at par with bottom board cleaning (4.33larvae/hive). Significantly maximum number oflarvae/hive were found in control in which no treatment was applied (14.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9gm) treatment, lime sulphur paste treatment and the colonies in which integrated management was done.

One month after second treatment, minimum number of larvae (1.00 larvae/hive) were found in the colonies treated with 3g *Bt* which was statistically same with neem seed kernal extract (1.00 larvae/hive) and was at par with neem oil treatment (1.67 larvae/hive) followed by bottom board cleaning (4.00 larvae/hive) Significantly maximum number oflarvae/hive were found in colonies without any treatment (16.33 larvae/hive).

One month after third treatment, minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with *Bt* (3g) which was statistically at par with neem seed kernal extract (0.67 larvae/hive) and neem oil treatment (1.00 larva/hive). Significantly maximum number oflarvae/hive were found in control (15.67 larvae/hive).

One month after first treatment, maximum number of adults were trapped in wax moth trap (26.75 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (23.25 females/trap) followed by delta trap with *A. cerana* comb (16.00 females/trap). Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (9.50 females/trap). One month after second treatment, maximum number of adults were trapped in wax moth trap (20.00 adults/trap) which was statistically at par with

delta trap with *A. dorsata* comb (17.50 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (8.00 females/trap) which was statistically at par with delta trap with *A. cerana* comb (14.50 females/trap).

Table 71. Effect of different management practices on the incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during 2021

Control methods	Mean number of larvae / hive			
	Pre-count	**1MAFT***	**1MAST****	**1MATT *****
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	7.00 (2.83)#	2.00 (1.73)	1.00 (1.41)	0.33 (1.15)
Bt var. *kurstaki* @ 6g/l	7.67 (2.94)	0.67 (1.29)	0.00 (1.00)	0.00 (1.00)
Bt var. *kurstaki* @ 9g/l	5.67 (2.58)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non- chemical method				
Neem oil(3%)	8.00 (3.00)	3.00 (2.00)	1.67 (1.63)	1.00 (1.41)
Neem seed kernel extract (5%)	8.33 (3.06)	2.00 (1.73)	1.00 (1.41)	0.67 (1.29)
Sealing cracks and crevices with lime sulphur paste	6.00 (2.65)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	9.67 (3.27)	4.33 (2.31)	4.00 (2.23)	3.00 (2.00)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	8.00 (3.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	6.67 (2.76)	14.67 (3.96)	16.33 (4.16)	15.67 (4.08)
C.D.	NS	(0.54)	(0.42)	(0.52)

Mechanical method	**Moth catches in the trap/ month**		
	June	**July**	**August**
1. Delta trap with *A. dorsata* comb	23.25 (4.92)	20.00 (4.58)	16.25 (4.15)
2. Delta trap with *A. cerana* comb	16.00 (4.12)	14.50 (3.39)	10.50 (3.39)
3. Delta trap with *A. mellifera* comb	9.50 (3.24)	8.00 (3.46)	5.50 (2.55)
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	26.75 (5.27)	17.50 (4.30)	20.00 (4.58)
5.Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.(0.05)	(0.67)	(0.54)	(0.53)

#Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

One month after third treatment, maximum number of adults were trapped in wax moth trap (20.00 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (16.25 females/trap) followed by delta trap with *A. cerana* comb (10.50 females/trap).

Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (5.50 females/trap).

c) Pooled data (2020-2021)

Data on management of Greater wax moth (*G. mellonella*) in *A. cerana* colonies presented in Table 72 and Fig. 24 demonstrated that pooled data during 2020 and 2021 showed that the initial infestation in the colonies varied from 6.00 to 10.00 larvae per colony and was statistically non-significant. One month after first treatment, minimum number of larvae (0.17 larvae/hive) were found in the colonies treated with lime sulphur paste which was statistically at par with colonies treated with *Bt* (6 g) treatment (1.00 larvae/hive) followed by neem seed kernel extract treatment (1.83 larvae/hive) and *Bt* (3g) treatment (2.17 larvae/hive). The number of larvae per hive in neem oil treatment were 2.83 larvae/hive which was statistically at par with bottom board cleaning of the hives (4.50 larvae/hive). No wax moth larval infestation was found in *Bt* (9g) treatment and the colonies in which integrated management was done. Significantly maximum number oflarvae/hive were found in control without any treatment (12.83 larvae/hive).

One month after second treatment, minimum number of larvae (0.50 larvae/hive) were found in the colonies treated *Bt* (6 g) which was statistically at par with neem seed kernel extract (0.67 larvae/hive), *Bt* (3g) treatment (1.50larvae/hive) and neem oil treatment (1.83larvae/hive) followed by bottom board cleaning of the hive (4.67 larvae/hive). Significantly maximum number of larvae/hive were found in control without any treatment (14.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g) treatment, lime sulphur paste treatment and the colonies in which integrated management was done.

One month after third treatment, minimum number of larvae (0.50 larvae/hive) were found in the colonies treated with neem seed kernel extract treatment which was statistically same with *Bt* (3g) (0.50 larvae/hive) and was at par with neem oil treatment (1.33 larvae/hive). Significantly maximum number oflarvae/hive were found in control without any treatment (13.83 larvae/hive). No wax moth larval infestation was found in *Bt* (6g and 9g) treatments, lime sulphur paste treatment and the colonies in which integrated management was done.

One month after first treatment, maximum number of adults were trapped in wax moth trap (29.00 adults/trap) which was statistically at par with delta trap with *A. dorsata*

comb (21.75 females/trap) (Table 72 and Fig. 25). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (10.25 females/trap) which was statistically at par with delta trap with *A. cerana* comb (14.88 females/trap).

Table 72. Pooled data on effect of different management practices on the incidence of greater wax moth (*G. Mellonella)* in *A. cerana* colonies during 2020-2021

Control methods	Mean number of larvae / hive			
	Pre-count	1MAFT*	1MAST**	1MATT ***
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	7.33 (2.89)[#]	2.17 (1.78)	1.50 (1.58)	0.50 (1.22)
Bt var. *kurstaki* @ 6g/l	8.00 (3.00)	1.00 (1.41)	0.50 (1.22)	0.00 (1.00)
Bt var. *kurstaki* @ 9g/l	6.00 (2.65)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non- chemical method				
Neem oil (3%)	7.83 (2.97)	2.83 (1.96)	1.83 (1.68)	1.33 (1.53)
Neem seed kernel extract (5%)	8.67 (3.11)	1.83 (1.68)	0.67 (1.29)	0.50 (1.22)
Sealing cracks and crevices with lime sulphur paste	6.33 (2.71)	0.17 (1.08)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	10.00 (3.32)	4.50 (2.35)	4.67 (2.38)	3.33 (2.08)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	8.33 (3.06)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	8.00 (3.00)	12.83 (3.72)	14.67 (3.96)	13.83 (3.85)
C.D.	NS	(0.40)	(0.51)	(0.39)

Mechanical method	**Moth catches in the trap/ moth**		
	June	**July**	**August**
1. Delta trap with *A. dorsata* comb	21.75 (4.77)	19.13 (4.49)	16.00 (4.12)
2. Delta trap with *A. cerana* comb	14.88 (3.98)	10.88 (3.45)	9.88 (3.30)
3. Delta trap with *A. mellifera* comb	10.25 (3.35)	9.50 (3.24)	6.00 (2.65)
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	29.00 (5.48)	20.50 (4.64)	19.88 (4.57)
5.Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.(0.05)	(0.64)	(0.80)	(0.28)

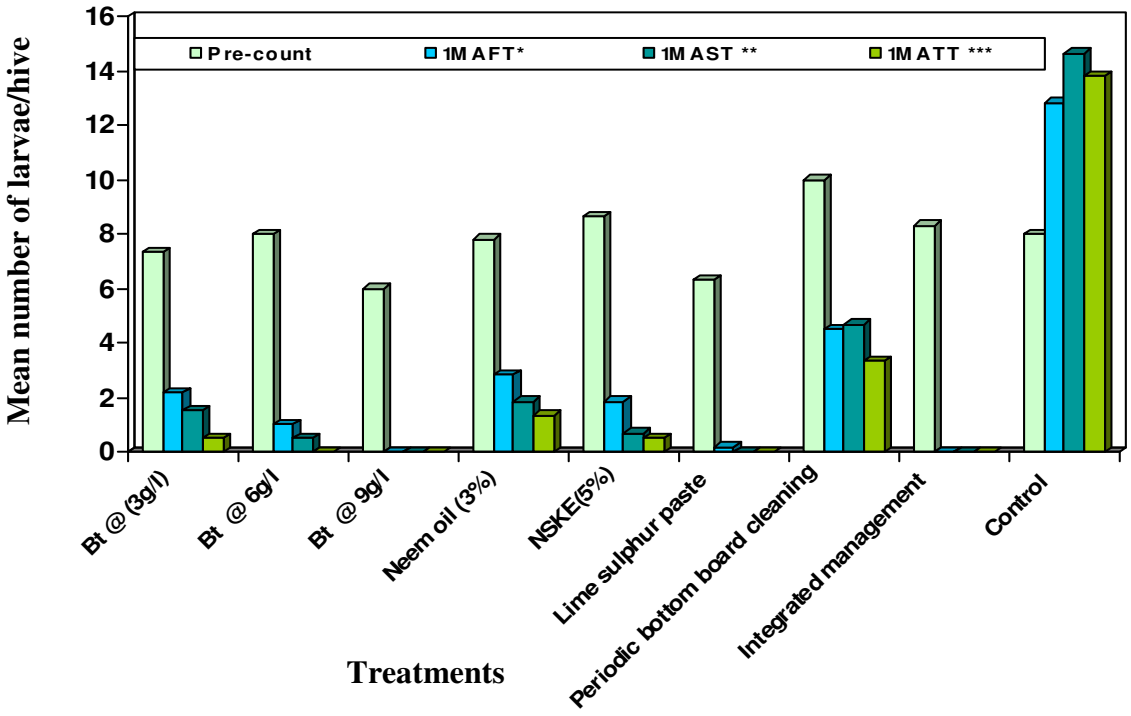
[#]Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

One month after second treatment, maximum number of adults were trapped in wax moth trap (20.50 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (19.13 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (9.50 females/trap) which was statistically at par with delta trap with *A. cerana* comb (10.88 females/trap). One month after third treatment, maximum number of adults were trapped in wax moth trap (19.88 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (16.00 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (6.00 females/trap) followed by delta trap with *A. cerana* comb (9.88 females/trap) (Plate 30).

In the present investigations 100% larval mortality of greater wax moth was observed with *Bt* var. *Kurstaki* 9g/l sprayed on brood combs of *A. cerana.* The present findings are in agreement with those of Verma (1995) who reported that the highest mortality (98.72%) of wax moth in honey bee colonies sprayed with a suspension of Dipel (10%) (*Bt* var. *kurstaki*). It protected the combs for a period of 5.5 months from wax moth infestation and no death of bee brood were observed in any of the treated colonies, therefore, population of young bees did not decrease. Basedow *et al.* (2012) reported 77.00% larval mortality of wax moth after the use of commercial formulation of *B. thuringiensis aizawai* XenTari® (20 ppm) and NeemAzal ® -T/S (20 ppm). Neem Azal® -T/S @ 80 ppm resulted in 100% mortality after 4 weeks of application and it was reported to control *V. destructor* also, without harming the bees. Local and commercial *Bt* formulations were tested @ 3, 6 and 9 g/l by spraying on *A. dorsata* combs for a period of three months and the results revealed that spraying V-*Bt* @ 9 g/l thrice at 30 days interval protected the combs from infestation by greater wax moth while single spray was ineffective and two sprays were less effective and three sprays were superior in protecting the combs (Madhu, 2013).

One month after third treatment, no wax moth larval infestation was found in *Bt* (6g and 9g) treatments, lime sulphur paste treatment and the colonies in which integrated management was done. Significantly maximum number of larvae/hive were found in control without any treatment. Minimum number of larvae were found in the colonies treated with neem seed kernel extract treatment which was equally effective as *Bt* (3g) treatment and neem oil treatment. Metwally *et al.* (1982) smeared the hives with lime sulphur paste to avoid wax moth infestation and found only one colony was infested by the wax moth out of ten



*one month after first treatment, ** one month after second treatment and *** one month after third treatment

Fig 24. Mean number of larvae per hive in *A. cerana* treated colonies (2020-2021)

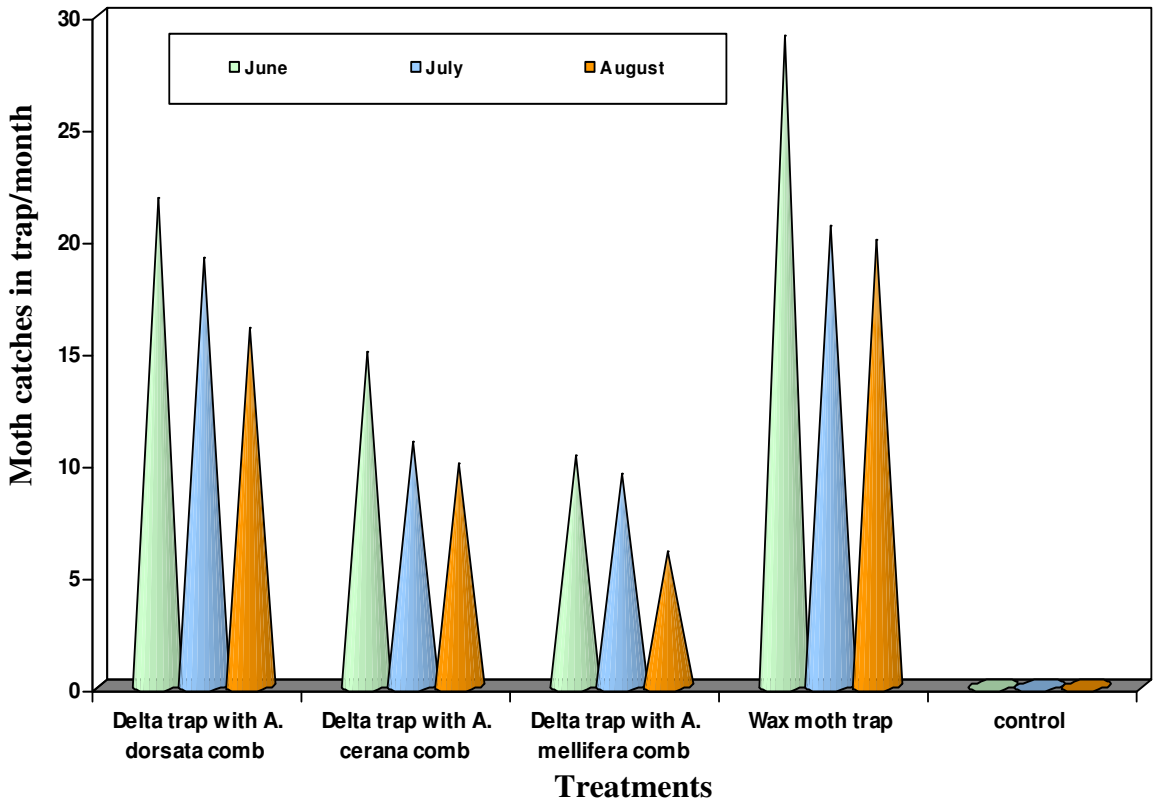


Fig 25. Mean number of moths catches per trap during June, July and August months in *A. cerana* colonies (2020-2021)

Plate 30 a) Greater wax moth adults trapped in delta trap with *A. dorsata* comb, b) Greater wax moth adults trapped in delta trap with *A. cerana* comb, c) Greater wax moth adults trapped in delta trap with *A. mellifera* comb and d) Greater wax moth adults trapped in wax moth trap (*A. cerana* apiary)

colonies resulting in 90% protection against zero protection in untreated colonies. They also reported that there was no effect on honeybees even if the bees came in direct contact with the lime sulphur paste as evident by no mortality of bees in any of the treated hives. Fletcher (1911), Morrison (1948) and Nessa *et al.* (1980) stated that minimizing the cracks and crevices, cleaning the hive at regular intervals helps to check the wax moth incidence. Madhu (2013) also reported that in the hives subjected to sealing the cracks and crevices with lime sulphur and IPM [cultural (periodic cleaning), mechanical (oviposition trap installation) and chemical (lime Sulphur application)] no infestation of greater wax moth was observed during entire period the three months. Bhopale *et al*. (2013) found that neem seed kernel extract cause 75.00% larval mortality under laboratory conditions as compared to 73.33% mortality in neem oil treatment. Telles *et al.* (2020) investigated that neem and *Eucalyptus* oil caused wax moth mortality at low concentrations, but did not affect colony population growth.

The management preventive practices *viz.,* strengthening honey bee colonies via feeding, reducing the supers and unoccupied combs, the combination of these practices and trapping wax moths that are intended to enter beehives were evaluated for their effectiveness to prevent wax moth infestation and compared with control (Gele *et al.,* 2017). The pollen area measurement was significantly higher for wax moth trap than the rest of the treatments. The number of infected combs were found to decrease during the application of these control methods when compared with the control. Our results found support from the findings of Vijaykumar *et al.* 2019) who reported that old brood combs of *A. dorsata* were found to be very attractive to wax moths than *A. cerana* and *A. florae* in wax moth traps. The results revealed that the delta trap fitted with old brood combs of *A. dorsata* attracted the females of greater wax moth. It was also observed that the growth of larvae was quick on old or darker combs containing brood and pollen, but very slow and hindered on white or fresh combs. Madhu (2013) revealed that the delta trap with *A. dorsata* combs attracted 2.1 times more mated females of greater wax moth as compared to *A. cerana* combs. She also reported that *A. dorsata* combs attracted 1.9 times more to unmated females of greater wax moth when compared to *A. cerana* combs. The present results were also in agreement with findings of Noel (1934) who mentioned that under laboratory conditions the fertilized females of wax moth were attracted to wax present in hives, whereas male moths were not. Earp (1925) observed the moths emerged during dusk were attracted to wax present in the hive. Dusk period (1800 hrs onwards into scotophase) was found to be the most active period for both female and male moths of greater wax moth.

Murray *et al.* (1988) identified oxygenated compound, decanal in bee wax which was nearly 50% of the total oxygenated volatiles in the wax and is accompanied by octanal, nonanol, furfural, benzaldehyde and 1-decanol. The possible role of aldehydes and alcohols in attracting the moths could not be ruled out. The unmated females also were attracted to the combs to some extent which may be attributed to the fact that comb also contains certain sex pheromonal components of greater wax moth.

4.4.2 Management of Greater wax moth (*G. mellonella*) in *A. mellifera* colonies

a) During 2020

During the year 2020, perusal of data in Table 73 showed that initial infestation in the colonies varied non-significantly from 7.67 to 11.67 larvae per colony. One month after first treatment, minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with *Bt* (6g) which was statistically at par with *Bt* (3g) treatment (0.67 larvae per hive) and neem seed kernel extract (1.00 larvae/hive) followed by neem oil treatment were (2.67 larvae/hive) statistically at par with bottom board cleaning of the hives (3.33larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done. Significantly maximum number oflarvae/hive were found in untreated colonies (11.33 larvae/hive).

One month after second treatment, no wax moth larval infestation was found in *Bt* (9g), *Bt* (6g), lime sulphur paste treatment and the colonies in which integrated management was done. Minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with *Bt* (3g) which was statistically at par with neem seed kernel extract (0.67 larvae/hive) followed by neem oil treatment (2.00 larvae/hive) which was statistically at par with bottom board cleaning of the hives (2.33 larvae/hive). Significantly maximum number of larvae/hive were found in untreated colonies (12.67 larvae/hive).

One month after third treatment, minimum number of larvae (0.33 larvae/hive) were found in the colonies treated with neem seed kernel extract which was statistically at par with neem oil treatment (1.33 larvae/hive) followed by bottom board cleaning of the hive (2.00 larvae/hive). Significantly maximum number of larvae/hive were found in untreated colonies (12.00 larvae/hive). No wax moth larval infestation was found in all *Bt* (3g, 6g and 9g) treatments, lime sulphur paste application and the colonies in which integrated management was done.

Table 73. Effect of different management practices on the incidence of greater wax moth (*G. mellonella*) in *A. mellifera* colonies during 2020

Control methods	Mean number of larvae / hive			
	Pre-count	1MAFT*	1MAST**	1MATT***
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	8.00 (3.00)#	0.67 (1.29)	0.33 (1.15)	0.00 (1.00)
Bt var. *kurstaki* @ 6g/l	11.67 (3.56)	0.33 (1.15)	0.00 (1.00)	0.00 (1.00)
Bt var. *kurstaki* @ 9g/l	10.00 (3.32)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non-chemical method				
Neem oil(3%)	9.67 (3.27)	2.67 (1.91)	2.00 (1.73)	1.33 (1.53)
Neem seed kernel extract (5%)	9.00 (3.16)	1.00 (1.41)	0.67 (1.29)	0.33 (1.15)
Sealing cracks and crevices with lime sulphur paste	9.67 (3.27)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	7.67 (2.94)	3.33 (2.08)	2.33 (1.83)	2.00 (1.73)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	9.00 (3.16)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	10.33 (3.37)	11.33 (3.51)	12.67 (3.70)	12.00 (3.61)
C.D.	NS	(0.48)	(0.43)	(0.40)
Mechanical method	**Moth catches in the trap**			
	June	**July**	**August**	
1. Delta trap with *A. dorsata* comb	17.25 (4.27)	15.50 (4.06)	14.00 (3.87)	
2. Delta trap with *A. cerana* comb	14.50 (3.94)	12.75 (3.71)	9.50 (3.24)	
3. Delta trap with *A. mellifera* comb	9.75 (3.28)	6.00 (2.65)	7.25 (2.87)	
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	28.25 (5.41)	28.50 (5.43)	21.00 (4.69)	
5.Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	
C.D.(0.05)	(0.59)	(0.39)	(0.49)	

#Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

Maximum number of adults one month after first treatment were trapped in wax moth trap (28.25 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (17.25 females/trap) followed by delta trap with *A. cerana* comb (14.50 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (9.75 females/trap). One month after second treatment, significantly maximum number of adults were trapped in wax moth trap (28.50 adults/trap) followed by delta trap with *A. dorsata* comb (15.50 females/trap) which was statistically at par with delta trap with *A.*

cerana comb (12.75 females/trap). Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (6.00 females/trap). One month after third treatment, significantly maximum number of adults were trapped in wax moth trap (21.00 adults/trap) followed by delta trap with *A. dorsata* comb (14.00 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (7.25 females/trap) which was statistically at par with delta trap with *A. cerana* comb (9.50 females/trap).

b) During 2021

Persual of data in Table 74, revealed that the initial infestation in the colonies varied from 6.67 to 10.67 larvae per colony. One month after first treatment, minimum number of larvae (0.67 larvae/hive) were found in the colonies treated with *Bt* (6g) which was statistically at par with *Bt* (3g) treatment (1.00 larvae/hive) and neem seed kernel extract treatment (1.33 larvae/hive) followed by neem oil treatment were (2.33 larvae/hive) and bottom board cleaning of the hives (4.00 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done. Significantly maximum number oflarvae/hive were found in untreated colonies (12.00 larvae/hive).

One month after second treatment, minimum number of larvae (0.67 larvae/hive) were found in the colonies treated with *Bt* (3g) which was statistically at par with *Bt* (6g) (1.00 larvae/hive) and neem seed kernel extract treatment (1.67 larvae/hive) followed by neem oil treatment (2.00 larvae/hive) which was statistically at par with bottom board cleaning of the hives (3.33 larvae/hive). Significantly maximum number oflarvae/hive were found in untreated colonies (13.33 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done.

One month after third treatment, minimum number of larvae (0.33 larvae/hive) were found in *Bt* (6g) treatment which was statistically at par with neem seed kernel extract treatment (0.67 larvae/hive) and *Bt* (3g) treatment (1.00 larvae/hive) followed by neem oil treatment (1.67 larvae/hive) which was statistically at par with Bottom board cleaning of the hives (1.67 larvae/hive). Significantly maximum number oflarvae/hive were found in untreated colonies (11.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g)

treatment, lime sulphur paste treatment and the colonies in which integrated management was done.

Table 74. Effect of different management practices on the incidence of greater wax moth (*G. mellonella*) in *A. mellifera* colonies during 2021

Control methods	Mean number of larvae / hive			
	Pre-count	1MAFT*	1MAST**	1MATT***
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	9.33 (3.21)#	1.00 (1.41)	0.67 (1.29)	1.00 (1.41)
Bt var. *kurstaki* @ 6g/l	7.00 (2.83)	0.67 (1.29)	1.00 (1.41)	0.33 (1.15)
Bt var. *kurstaki* @ 9g/l	10.67 (3.42)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non- chemical method				
Neem oil (3%)	9.00 (3.16)	2.33 (1.83)	2.00 (1.73)	1.67 (1.63)
Neem seed kernel extract(5%)	9.00 (3.16)	1.33 (1.53)	1.67 (1.63)	0.67 (1.29)
Sealing cracks and crevices with lime sulphur paste	8.00 (3.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	8.67 (3.11)	4.00 (2.24)	3.33 (2.08)	2.33 (1.83)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	6.67 (2.77)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	8.00 (3.00)	12.00 (3.61)	13.33 (3.79)	11.67 (3.56)
C.D.	NS	(0.40)	(0.38)	(0.29)

Mechanical method	**Moth catches in the trap/month**		
	June	**July**	**August**
1. Delta trap with *A. dorsata* comb	21.50 (4.74)	16.50 (4.18)	11.25 (3.50)
2. Delta trap with *A. cerana* comb	13.25 (3.77)	11.00 (3.46)	7.50 (2.92)
3. Delta trap with *A. mellifera* comb	6.75 (2.78)	8.25 (3.04)	4.00 (2.24)
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	24.50 (5.05)	20.00 (4.58)	17.25 (4.27)
5.Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
C.D.(0.05)	(0.85)	(0.57)	(0.47)

#Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

One month after first treatment, maximum adults were trapped in wax moth trap (24.50 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (21.50 females/trap) followed by delta trap with *A. cerana* comb (13.25 females/trap). Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb

(6.75 females/trap). One month after second treatment, maximum number of adults were trapped in wax moth trap (20.00 adults/trap) which was statistically at par with delta trap with *A. dorsata* comb (16.50 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (8.25 females/trap) which was statistically at par with delta trap with *A. cerana* comb. One month after third treatment, significantly maximum number of adults were trapped in wax moth trap (17.25 adults/trap) followed by delta trap with *A. dorsata* comb (11.25 females/trap) which was statistically at par with delta trap with *A. cerana* comb (7.50 females/trap). Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (4.00 females/trap).

c) Pooled data (2020-2021)

Pooled data on management of greater wax moth (*G. mellonella*) during 2020 and 2021 is presented in Table 75 and Fig. 26 revealed that the initial infestation in the colonies varies from 7.83 to 10.33 larvae per colony. One month after first treatment, minimum number of larvae (0.50 larvae/hive) were found in the colonies treated with *Bt* (6g) which was statistically at par with *Bt* (3g) treatment (0.83 larvae/hive) followed by neem seed kernel extract (1.17 larvae/hive), neem oil treatment (2.50 larvae/hive) and bottom board cleaning of the hives (3.67 larvae/hive). Significantly maximum number oflarvae/hive were found in untreated colonies (11.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done.

One month after second treatment, minimum number of larvae (0.34 larvae/hive) were found in the colonies treated with *Bt* (6g) which was statistically at par with *Bt* (3g) (0.50 larvae/hive), neem seed kernel extract (1.17 larvae/hive) and neem oil treatment (2.00 larvae/hive) followed by bottom board cleaning (2.83 larvae/hive). Significantly maximum number oflarvae/hive were found in untreated colonies (13.00 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done.

One month after third treatment, minimum number of larvae (0.17 larvae/hive) were found in *Bt* (6g) treatment which was statistically at par with neem seed kernel extract (0.50larvae/hive) and *Bt* (3g) treatment (0.50 larvae/hive) followed by neem oil treatment (1.50 larvae/hive) which was statistically at par with bottom board cleaning of the hives (2.17 larvae/hive). Significantly maximum number of larvae/hive were found in untreated colonies

(11.83 larvae/hive). No wax moth larval infestation was found in *Bt* (9g) treatment, lime sulphur paste treatment and the colonies in which integrated management was done.

Table 75. Pooled data on effect of different management practices on the incidence of greater wax moth (*G. mellonella*) in *A. mellifera* colonies during 2020-2021

Control methods	Mean number of larvae / hive			
	Pre-count	1MAFT*	1MAST**	1MATT***
Biological method				
Bacillus thuringiensis var. *kurstaki* @ 3g/l	8.67 (3.11)#	0.83 (1.35)	0.50 (1.22)	0.50 (1.22)
Bt var. *kurstaki* @ 6g/l	9.33 (3.21)	0.50 (1.22)	0.34 (1.16)	0.17 (1.08)
Bt var. *kurstaki* @ 9g/l	10.33 (3.37)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Chemical and non- chemical method				
Neem oil (3%)	9.33 (3.21)	2.50 (1.87)	2.00 (1.73)	1.50 (1.58)
Neem seed kernel extract (5%)	9.00 (3.16)	1.17 (1.47)	1.17 (1.47)	0.50 (1.22)
Sealing cracks and crevices with lime sulphur paste	8.83 (3.14)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Cultural method				
Periodic bottom board cleaning	8.17 (3.03)	3.67 (2.16)	2.83 (1.96)	2.17 (1.78)
Integrated management				
Integrated management (periodic cleaning + delta trap installation+ lime Sulphur application+ *Bt* spray)	7.83 (2.97)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Control	9.17 (3.19)	11.67 (3.56)	13.00 (3.74)	11.83 (3.58)
C.D.	NS	(0.16)	(0.29)	(0.24)
Mechanical method	**Moth catches in the trap/month**			
	June	**July**	**August**	
1. Delta trap with *A. dorsata* comb	19.38 (4.51)	16.00 (4.12)	12.63 (3.69)	
2. Delta trap with *A. cerana* comb	13.88 (3.86)	11.88 (3.59)	8.50 (3.08)	
3. Delta trap with *A. mellifera* comb	8.25 (3.04)	7.13 (2.85)	5.63 (2.57)	
4. Wax moth trap (Cup of water+ 1 cup of sugar+1/2 cup of vineger+1 peeled banana)	26.38 (5.23)	24.25 (5.02)	19.13 (4.49)	
5.Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	
C.D.(0.05)	(0.27)	(0.93)	(0.45)	

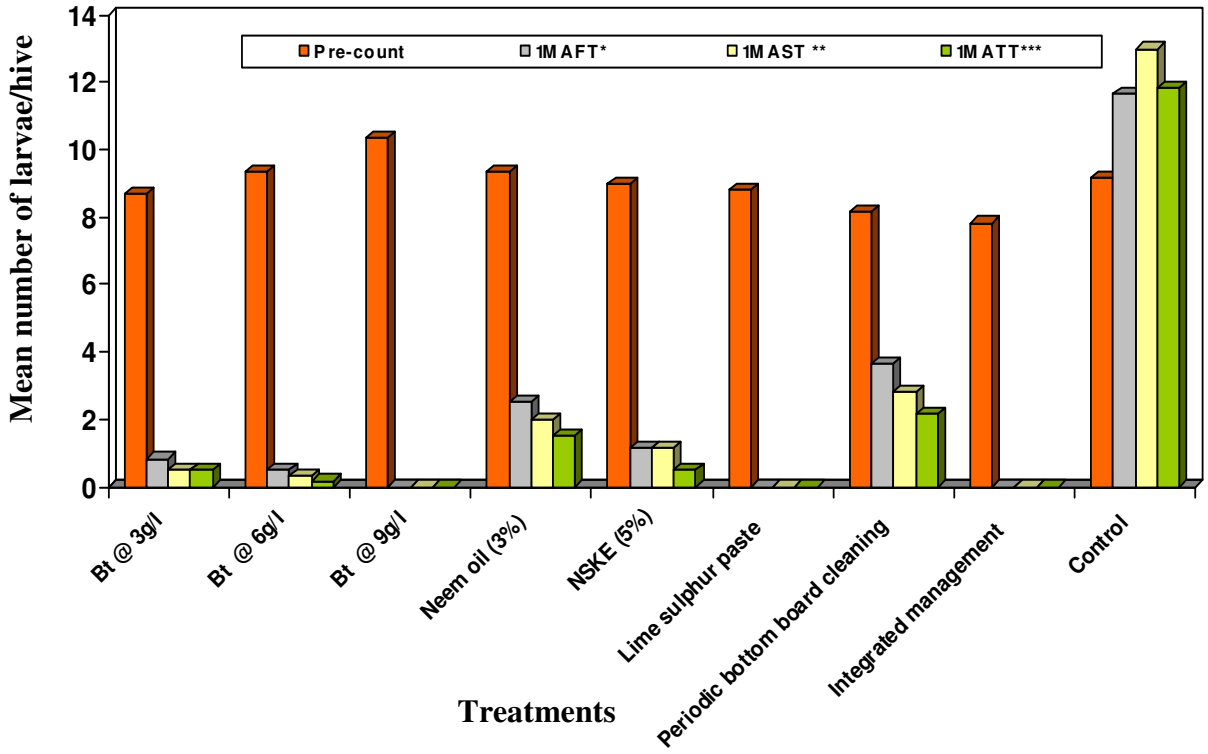
#Figures in parentheses are square root (x+1) transformed values

*One month after first treatment, **One month after second treatment and ***One month after third treatment

Persual of Table 75 and Fig. 27, showed that maximum number of adults one month after first treatment were trapped in wax moth trap (26.38 adults/trap) followed by delta trap with *A. dorsata* comb (19.38 females/trap) and delta trap with *A. cerana* comb (13.88

females/trap). Significantly, minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (8.25 females/trap). One month after second treatment, maximum number of adults were trapped in wax moth trap (24.25 adults/trap) which was statistically at par to delta trap with *A. dorsata* comb (16.00 females/trap). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (7.13 females/trap) which was statistically at par to delta trap with *A. cerana* comb (11.88 females/trap). One month after third treatment, significantly maximum number of adults were trapped in wax moth trap (19.13 adults/trap) followed by delta trap with *A. dorsata* comb (12.63females/trap) and delta trap with *A. cerana* comb (8.50 females/trap). Significantly minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (5.63 females/trap) (Plate31).

As described earlier, Madhu (2013) tested *Bt* formulations @ 3, 6 and 9 g/l in live colonies by spraying on *A. dorsata* combs for a period of three months and showed that spraying V-*Bt* @ 9 g/l thrice at 30 days interval protected the combs from infestation by greater wax moth. However the hives with no spray had larval infestation of 17.33, 37.33 and 49.06 larvae per hive at the end of first, second and third month of study. Negi *et al.* (2019) reported that when *Bt* (8 g/lt *Bt* var. *kurstaki* 0.5% WP) was sprayed on the combs with brood then maximum average wax moth mortality (40.89%) was observed without any mortality in the brood. Honey bee colonies sprayed with a suspension of Dipel (10%) (*Bt* var. *kurstaki*) caused 98.72% mortality of wax moth and protected the colonies for period of 5.5 months (Verma, 1995). Wax combs sprayed with Certan, a *Bt* var *kurstaki* product, recorded less damage by greater wax moth up-to three months in storage (McKillinf and Brown, 1991). Rodriguez and Sandovan (1991) reported that the application of 3 g/l spore crystal complex of *Bt* subspecies *thuringiensis* as spray inside bee hive gave protection against the wax moth for minimum period of 52 days. Krieg (1959) observed the spores of *B. thuringiensis* (Berliner) dusted on wax combs @ 5 mg/dm^2 caused 80-100 per cent mortality of greater wax moth larvae. Goodwin (1985) reported that the formulation of *B. thuringiensis* sub sp. *aizawai* spores (B 401 containing 10^6 viable spores/mg) when sprayed on combs or wax foundations gave protection from greater wax moth infestation up to 12 months. Metwally *et al.* (1982) found that smearing the hives with lime sulphur paste against wax moth infestation resulted in 90% protection of bee colonies. He further reported that lime sulphur paste was safe for bees as no mortality of bees was observed in any of the treated hives. Babarinde *et al*. (2010) observed that treating top bars of hive with lime was effective against apicultural pests



*one month after first treatment, ** one month after second treatment and *** one month after third treatment

Fig 26. Mean number of larvae per hive in *A. mellifera* treated colonies (2020-2021)

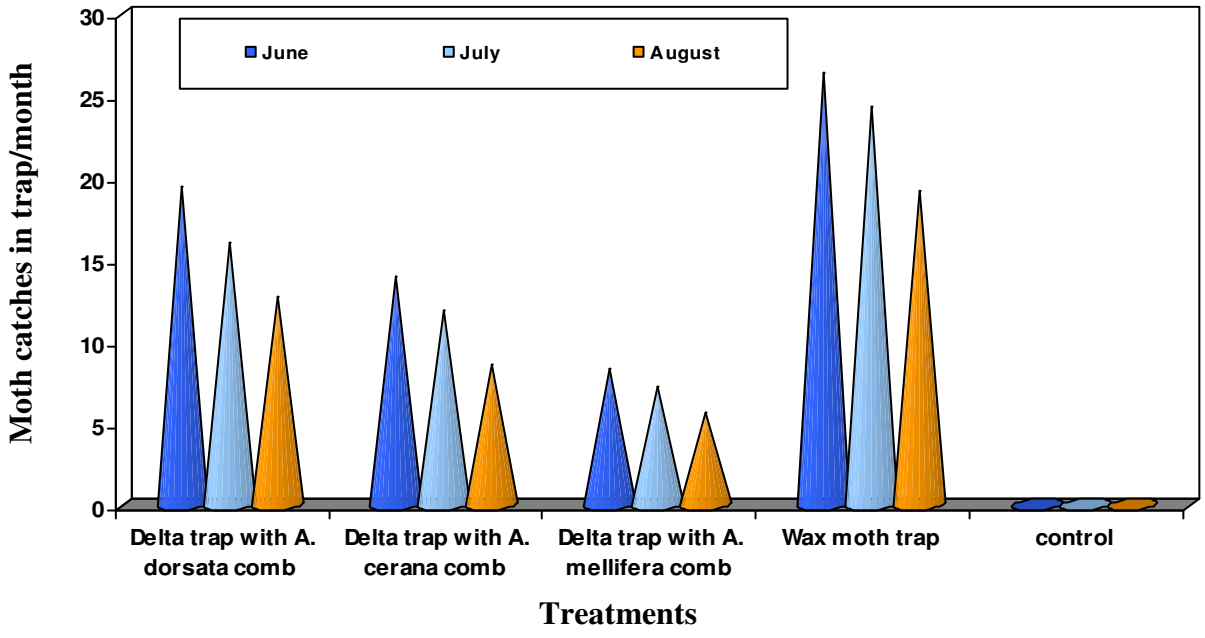


Fig 27. Mean number of moths catches per trap during June, July and August months in *A. mellifera* colonies (2020-2021)

Plate 31. a) Greater wax moth adults trapped in delta trap with *A. dorsata* comb, b) Greater wax moth adults trapped in delta trap with *A. cerana* comb, c) Greater wax moth adults trapped in delta trap with *A. mellifera* comb and d) Greater wax moth adults trapped in wax moth trap (*A. mellifera* apiary)

and harmless to honeybees. Treating cracks and crevices of hive with lime sulphur paste protected the colony up to two months from wax moth infestation (Shylesh, 1987). For protection of the colonies from greater wax moth (*G. mellonella*), If the cracks and crevices of the honey bee colonies were sealed with lime sulphur paste and integrated management was done, no infestation in the colonies was observed for at least three months (Madhu, 2013). Rahman *et al*. (2017) reported that after *Bt*, neem oil (3.00%) cause 79.99% mortality of wax moth larvae. Bhopale *et al*. (2013) found that neem seed kernel extract cause 75.00% larval mortality under laboratory conditions as compared to 73.33% mortality in neem oil treatment. Telles *et al.* (2020) found that concentrations of neem and eucalyptus oil caused wax moth mortality without affecting colony population growth. Several studies have revealed the role of regular cleaning to prevent infestation of hive with greater wax moth (Adamson, 1943). Cherian and Ramchandran (1943) stated that removal of old combs, plugging of cracks and sanitation are the major measures to be adopted to keep the apiary free from wax moths. Whitcomb (1936) and Kannagara (1940) advocated the removal of propolis, bur combs and refuse on the bottom board, as these attracted the moths for oviposition and also a shelter for the larvae. The above studies corroborated our findings.

Gele *et al.* (2017) reported that feeding, reducing the supers and unoccupied combs, the combination of these practices and trapping wax moths in wax moth minimize the damage done by wax moth to honey bee colonies. Also the number of infected combs were decreased. Our results are in agreement with the findings of Vijaykumar *et al.* (2019) who compared the efficacy of old brood combs of *A. dorsata*, *A. cerana* and *A. florea* for attracting the greater wax moth female adults and concluded that old brood combs of *A. dorsata* were found to be very attractive to wax moth adults than *A. cerana* and *A. florea*.

Kwadha *et al.* (2017) revealed that seven compounds present in bee wax viz., ethyl propanoate; 2- methyl, ethyl propanoate; ethyl 2- methyl butanoate; 3- methyl butyl acetate, nonanal, decanal and sylvestrene elicited antenna response in mated female of greater wax moth. These results demonstrate that honey bee hive related semiochemicals play crucial role in chemical communication of *G. mellonella* both at larval and adult stage. Madhu (2013) found that the delta trap with *A. dorsata* combs attracted mated and unmated females of greater wax moth 2.1 and 1.9 times more when compared to *A. cerana* combs. The present results were also in agreement with findings of Noel (1934) who mentioned that in laboratory

the fertilized females of wax moth were attracted to wax present in hives, whereas male moths were not.

4.5 Efficacy of plant and fungal extracts against sacbrood virus infecting *A. mellifera* colonies applied at an interval of four days

a) During 2020

1) Effects of plant and fungal extracts on sacbrood virus infected *A. mellifera* colonies

Different herbal and fungal extracts were given at selected/recommended dose to the *A. mellifera* colonies and brood development was studied in these colonies on 1, 15, 30 and 60 day after three treatments of the colonies.

Data on antiviral effect of plant and fungal extracts on *A. mellifera* sacbrood virus infected colonies is presented in Table 76. It was observed that number of infected larvae/1000 brood cells before application of treatments ranged from 47.25 to 58.50 and was statistically non-significant.

Four days after first treatment, the number of infected larvae/1000 brood cells were minimum in *G. lucidum* (3ml/250ml of sugar solution) (32.00 larvae/1000 brood cells) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (35.25 infected larvae/ 1000 brood cells) followed by *P. niruri* (41.75 larvae/1000 brood cells) which was statistically at par with *C. longa* (46.50 larvae/1000 brood cells). Number of infected larvae in *C. papaya* were 53.00 larvae/1000 brood cells which was statistically at par with *A. indica* (56.50larvae/hive) which was further at par with control in which maximum number of infected larvae were found (62.25 larvae/ 1000 brood cells).

Four days after second treatment, minimum number of infected larvae were present in *G. lucidum* (3ml/250ml of sugar solution) i.e.25.00 larvae/1000 brood cells which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (29.50 larvae/ 1000 brood cells) followed by *P. niruri* (38.75 larvae/1000 brood cells), *C. longa* (45.75 larvae/1000 brood cells) which was statistically at par with *C. papaya* (51.00 larvae/1000 brood cells).The number of infected larvae in *A. indica* were 57.25 larvae/1000 brood cells. Significantly maximum number of infected larvae were found control (67.00 larvae/ 1000 brood cells).

Similar trend was obtained four days after third treatment in which the minimum number of infected larvae were observed in *G. lucidum* (3ml/250ml of sugar solution) (17.50 larvae/1000 brood cells) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (22.25 larvae/ 1000 brood cells) followed by *P. niruri* (32.00 larvae/1000 brood cells) which was at par with *C. longa* (38.25 larvae/1000 brood cells). The number of sacbrood infected larvae in *C. papaya* treatment were 45.50 larva/1000 brood cells followed by *A. indica* 58.50 larvae/1000 brood cells. Maximum number of infected larvae were found control (71.25 larvae/ 1000 brood cells).

Table 76. Effectiveness of plant and fungal extracts against sacbrood virus in *A. mellifera* colonies applied at an interval of four days during 2020

Treatment	Dose /250ml	Number of cells with infected larvae per 1000 brood cells			
		Initial infestation	4 DAFT *	4 DAST**	4 DATT ***
G. lucidum	3.00ml	52.25 (7.30)[#]	32.00 (5.74)	25.00 (5.10)	17.50 (4.30)
G. lucidum	1.00ml	47.25 (6.95)	35.25 (6.02)	29.50 (5.52)	22.25 (4.82)
P. niruri	2.00g	48.75 (7.05)	41.75 (6.54)	38.75 (6.30)	32.00 (5.74)
A .indica	2.00g	54.50 (7.45)	56.50 (7.58)	57.25 (7.63)	58.50 (7.71)
C. papaya	2.00g	55.75 (7.53)	53.00 (7.35)	51.00 (7.21)	45.50 (6.82)
C. longa	2.00g	50.50 (7.18)	46.50 (6.89)	45.75 (6.84)	38.25 (6.26)
Untreated diseased check	-	58.50 (7.71)	62.25 (7.95)	67.00 (8.25)	71.25 (8.50)
C.D (0.05)		NS	(0.58)	(0.51)	(0.70)

[#]Figures in parentheses are square root (x+1) transformed values
*Days after first treatment, **Days after second treatment and ***Days after third treatment

Data recorded on per cent reduction in infected larvae/1000 brood cells in Table 77 revealed that four days after first, second and third treatment per cent decrease in diseased larvae were maximum in *G. lucidum* (3ml/250ml of sugar solution) (42.45%, 58.22% and 72.50%, respectively) followed *G. lucidum* (1ml/250ml of sugar solution) (29.89%, 45.49% and 61.34% ,respectively) and *P. niruri* (19.52%, 30.60% and 46.11% respectively), *C. longa* (13.47%, 20.90% and 37.81%, respectively) and *C. papaya* (10.66%, 15.98% and 30.04%,

respectively). Per cent decrease in infected larvae was minimum in *A. indica* (6.02%, 11.53% and 14.99%, respectively).

Table 77. Per cent reduction in the diseased larvae per thousand brood cells over control

Treatment	Dose /250ml	Per cent decrease in infected larvae/1000 brood cells		
		4 DAFT *	4 DAST **	4 DATT ***
G. lucidum	3.00ml	42.45	58.22	72.50
G. lucidum	1.00ml	29.89	45.49	61.34
P. niruri	2.00g	19.52	30.60	46.11
A. indica	2.00g	6.02	11.53	14.99
C. papaya	2.00g	10.66	15.98	30.04
C. longa	2.00g	13.47	20.90	37.81

*Days after first treatment, **Days after second treatment and ***Days after third treatment

2) Effects of plant and fungal extracts on brood area of sac brood virus infected treated *A. mellifera* colonies

Brood area one day after third treatment in Table 78 was recorded maximum in *G. lucidum* (3ml/250ml of sugar solution) (2073.68 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1791.49 cm^2) and *P. niruri* (1607.66 cm^2) followed by *C. longa* (1365.79 cm^2) which was statistically at par with *C. papaya* treated colonies (1098.11cm^2) which was further at par with *A. indica* (927.19 cm^2). Significantly minimum brood area was observed in the diseased colonies in which no treatment was given (361.20 cm^2).

After Fifteen days, brood area was maximum in *G. lucidum* (3ml/250ml of sugar solution) treatment (2133.34 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1899.53 cm^2) followed by *P. niruri* treated colonies (1693.13 cm^2) which was at par with *C. longa* treatment (1427.06 cm^2). The brood area in *C. papaya* treatment was 1130.36cm^2 which was statistically at par with *A. indica* (927.19 cm^2). Significantly minimum brood area was observed in untreated diseased check (266.06 cm^2).

Thirty days after third treatment, brood area was maximum in *G. lucidum* (3ml/250ml of sugar solution) treated colonies (2784.79 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (2388.11 cm^2) followed by *P. niruri* (2146.24 cm^2) which was statistically at par with *C. longa* (1775.36 cm^2). The brood area in *C. papaya* treatment was 1448.03 cm^2 which was statistically at par with *A. indica* (1146.49 cm^2).

Significantly minimum brood area was observed in the colonies in which no treatment was given (174.15 cm^2).

Sixty days after treatment of the colonies, significantly maximum brood area was obtained in *G. lucidum* (3ml/250ml of sugar solution) (3781.31 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (3233.06 cm^2) which was statistically at par with *P. niruri* (2981.51 cm^2). The brood area in *C. longa* treatment was 2575.16 cm^2 followed by *C. papaya* (2125.28 cm^2) and *A. indica* (1323.86 cm^2). Significantly minimum brood area was observed in the colonies with no treatment (138.67 cm^2).

Table 78. Cumulative effect of three treatments of plant and fungal extracts applied at 4 days interval on brood area against sacbrood virus in *A. mellifera* colonies during 2020

Treatment	Dose /250ml	Brood area (cm^2)after three treatment of plant and fungal extracts			
		1 DATT *	15 DATT	30 DATT	60 DATT
G. lucidum	3.00ml	2073.68 (45.55)#	2133.34 (46.20)	2784.79 (52.78)	3781.31 (61.50)
G. lucidum	1.00ml	1791.49 (42.34)	1899.53 (43.60)	2388.11 (48.88)	3233.06 (56.87)
P. niruri	2.00g	1607.66 (40.11)	1693.13 (41.16)	2146.24 (46.34)	2981.51 (54.61)
A. indica	2.00g	906.23 (30.12)	927.19 (30.47)	1146.49 (33.87)	1323.86 (36.40)
C. papaya	2.00g	1098.11 (33.15)	1130.36 (33.64)	1448.03 (38.07)	2125.28 (46.11)
C. longa	2.00g	1365.79 (36.97)	1427.06 (37.79)	1775.36 (42.15)	2575.16 (50.76)
Untreated diseased check	-	361.20 (19.03)	266.06 (16.34)	174.15 (13.23)	138.67 (11.18)
C.D (0.05)		(5.44)	(4.76)	(4.43)	(3.39)

#Figures in parentheses are square root (x+1) transformed values
*Days after third treatment

b) During 2021

1) Effects of plant and fungal extracts on sacbrood virus infected *A. mellifera* colonies

Data on antiviral effect of plant and fungal extracts on *A. mellifera* sacbrood virus infected colonies during 2021 is presented in Table 79. It was observed that number of infected larvae/1000 brood cells before application of treatments ranged from 44.50 to 58.25 and was statistically non-significant.

Table 79. Effectiveness of plant and fungal extracts against sacbrood virus in *A. mellifera* colonies applied at an interval of four days during 2021

Treatment	Dose /250ml	Number of cells with infected larvae per 1000 brood cells			
		Initial infestation	4 DAFT *	4 DAST **	4 DATT ***
G. lucidum	3.00ml	49.25 (7.09)#	30.50 (5.61)	23.75 (4.97)	18.25 (4.39)
G. lucidum	1.00ml	44.50 (6.75)	33.00 (5.83)	27.00 (5.29)	24.50 (5.05)
P. niruri	2.00g	45.75 (6.84)	42.00 (6.56)	37.50 (6.20)	30.00 (5.57)
A. indica	2.00g	53.50 (7.38)	56.00 (7.55)	58.00 (7.68)	60.00 (7.81)
C. papaya	2.00g	58.25 (7.70)	55.25 (7.50)	52.75 (7.33)	51.25 (7.23)
C. longa	2.00g	50.75 (7.19)	47.50 (6.96)	44.25 (6.73)	41.75 (6.54)
Untreated diseased check	-	55.50 (7.52)	60.00 (7.81)	65.25 (8.14)	69.50 (8.40)
C.D (0.05)		NS	(0.43)	(0.78)	(0.63)

#Figures in parentheses are square root (x+1) transformed values
*Days after first treatment, **Days after second treatment and ***Days after third treatment

Four days after first treatment, the number of infected larvae/1000 brood cells were minimum in *G. lucidum* (3ml/250ml of sugar solution) treatment (30.50 larvae/1000 brood cells) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (33.00 larvae/ 1000 brood cells) followed by *P. niruri* (42.00 larvae/1000 brood cells) which was statistically at par with *C. longa* (47.50 larvae/1000 brood cells). Maximum number of infected larvae were found in control (60.00 larvae/ 1000 brood cells) which was statistically at par with *A. indica* (56.00 larvae/ 1000 brood cells) and *C. papaya* treatment (55.25 larvae/1000 brood cells).

Four days after second treatment, minimum number of infected larvae were present in *G. lucidum* (3ml/250ml of sugar solution) (23.75 larvae/1000 brood cells) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (27.00 larvae/ 1000 brood cells) followed by *P. niruri* (37.50 larvae/1000 brood cells) was at par with *C. longa* (44.25 larvae/1000 brood cells), *C. papaya* (52.75 larvae/1000 brood cells) further at par with *A. indica* (58.00 larvae/ 1000 brood cells). Maximum number of infected larvae were found control (65.25 larvae/ 1000 brood cells) which was also statistically at par with *A. indica* (58.00larvae/hive).

Four days after third treatment, significantly minimum number of infected larvae were present in *G. lucidum* (3ml/250ml of sugar solution) (18.25 larvae/1000 brood cells) followed *G. lucidum* (1ml/250ml of sugar solution) (24.50 larvae/ 1000 brood cells) which was statistically at par with *P. niruri* treatment (30.00 larvae/1000 brood cells) followed by *C. longa* (41.75 larvae/1000 brood cells), *C. papaya* (51.25 larvae/1000 brood cells) which was statistically at par with *A. indica* (58.00 larvae/hive) which was further at par with control. Maximum number of infected larvae were found control (69.50 larvae/ 1000 brood cells).

Data in Table 80 demonstrated that four days after first, second and third treatments per cent decrease in diseased larvae were maximum in *G. lucidum* (3ml/250ml of sugar solution) (42.72%, 58.98% and 70.41%, respectively) followed *G. lucidum* (1ml/250ml of sugar solution) (31.40%, 48.39% and 56.03%, respectively), *P. niruri* (15.08%, 30.28% and 47.64%, respectively), *C. longa* (13.42%, 25.84% and 34.31%, respectively) and *C. papaya* (12.26%, 22.97% and 29.44%, respectively). Per cent decrease in infected larvae was minimum in *A. indica* (3.18%, 7.79% and 10.44%, respectively).

Table 80. Per cent reduction in the diseased larvae per thousand brood cells over control

Treatment	Dose /250ml	Per cent decrease in infected larvae/1000 brood cells		
		4 DAFT *	4 DAST **	4 DATT***
G. lucidum	3.00ml	42.72	58.98	70.41
G. lucidum	1.00ml	31.40	48.39	56.03
P. niruri	2.00g	15.08	30.28	47.64
A. indica	2.00g	3.18	7.79	10.44
C. papaya	2.00g	12.26	22.97	29.44
C. longa	2.00g	13.42	25.84	34.31

*Days after first treatment, **Days after second treatment and ***Days after third treatment

b) Effects of plant and fungal extracts on brood area of sac brood virus infected treated *A. mellifera* colonies

Table 81 revealed that brood area one day after third treatment of the infected colonies with the plant and fungal extracts, was found maximum in *G. lucidum* (3ml/250ml of sugar solution) treatment (1918.88 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1757.63 cm^2) and *P. niruri* (1622.18 cm^2) followed by *C.*

longa (1473.83 cm^2) which was at par with *C. papaya* (1338.38cm^2) which was further at par with *A. indica* (1057.80 cm^2). Significantly minimum brood area was observed in the colonies without any treatment (404.73 cm^2).

Table 81. Cumulative effect of plant and fungal extracts applied at 4 days interval on brood area against sacbrood virus in *A. mellifera* colonies during 2021

Treatment	Dose /250 ml	Brood area (cm^2) after third treatment of plant and fungal extract			
		1 DATT *	15 DATT	30 DATT	60 DATT
G. lucidum	3.00ml	1918.88 (43.82)#	2123.66 (46.09)	2352.64 (48.51)	3374.96 (58.10)
G. lucidum	1.00ml	1757.63 (41.94)	1785.04 (42.26)	2184.94 (46.75)	3125.03 (55.91)
P. niruri	2.00g	1622.18 (40.29)	1709.25 (41.36)	2125.28 (46.11)	2978.29 (54.58)
A .indica	2.00g	1057.80 (32.54)	1154.55 (33.99)	1804.39 (42.49)	1852.76 (43.06)
C. papaya	2.00g	1338.38 (36.60)	1422.23 (37.73)	2026.91 (45.03)	2480.03 (49.81)
C. longa	2.00g	1473.83 (38.40)	1507.69 (38.84)	2109.15 (45.94)	2949.26 (54.32)
Untreated diseased check	-	404.73 (20.14)	296.70 (17.25)	225.75 (15.06)	162.86 (12.80)
C.D (0.05)		(4.66)	(6.42)	(3.07)	(5.76)

#Figures in parentheses are square root (x+1) transformed values
***** Days after third treatment

After Fifteen days, brood area was maximum in *G. lucidum* (3ml/250ml of sugar solution) (2123.66 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1785.04 cm^2) and *P. niruri* (1709.25 cm^2) followed by *C. longa* (1507.69 cm^2) which was at par with *C. papaya* treated colonies (1422.23 cm^2) and *A. indica* (1154.55 cm^2). Significantly minimum brood area was observed in the colonies in which no treatment was given (296.70 cm^2).

Maximum brood area after 30 days was found in *G. lucidum* (3ml/250ml of sugar solution) (2352.64 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (2184.94 cm^2), *P. niruri* (2125.28 cm^2) and *C. longa* (2109.15 cm^2) followed by *C. papaya* treatment (2026.91 cm^2) which was statistically at par with *A. indica* treated colonies (1804.39 cm^2). Significantly minimum brood area was observed in untreated colonies (225.75 cm^2).

After 60 days of third treatment, maximum brood area was obtained in *G. lucidum* (3ml/250ml of sugar solution) treatment (3374.96 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (3125.03 cm^2), *P. niruri* (2978.29 cm^2) and *C. longa* (2949.26 cm^2) which was further at par with *C. papaya* (2949.26 cm^2) followed by *A. indica* (1852.76 cm^2). Significantly minimum brood area was observed in the colonies with no treatment (162.86 cm^2).

c) Pooled data (2020- 2021)

1) Pooled data on effects of plant and fungal extracts on sacbrood virus infected *A. mellifera* colonies

Pooled data on antiviral effect of plant and fungal extracts on *A. mellifera* sacbrood virus infected colonies is presented in Table 82 and Fig. 28. It was observed that number of infected larvae/1000 brood cells before application of treatments ranged from 45.25 to 57.00 and was non-significant.

Four days after first treatment, the number of infected larvae/1000 brood cells were minimum in *G. lucidum* (3ml/250ml of sugar solution) (31.25 larvae/1000 brood cells) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) treatment (34.13 larvae/ 1000 brood cells) followed by *P. niruri* (41.88 larvae/1000 brood cells), *C. longa* (47.00 larvae/1000 brood cells), *C. papaya* (54.13 larvae/1000 brood cells) which was statistically at par with *A. indica* (56.25 larvae/hive). Significantly maximum number of infected larvae were found control (61.13 larvae/ 1000 brood cells).

Four days after second treatment, significantly minimum number of infected larvae were present in *G. lucidum* (3ml/250ml of sugar solution) (24.38 larvae/1000 brood cells) followed by *G. lucidum* (1ml/250ml of sugar solution) (28.25 larvae/ 1000 brood cells), *P. niruri* (38.13 larvae/1000 brood cells), *C. longa* (45.00 larvae/1000 brood cells), *C. papaya* (51.88 larvae/1000 brood cells) and *A. indica* (57.63larvae/hive). Significantly maximum number of infected larvae were found in control (66.15 infected larvae/ 1000 brood cells).

Similarly four days after third treatment, minimum number of infected larvae were present in *G. lucidum* (3ml/250ml of sugar solution) (17.88 larvae/1000 brood cells) followed by *G. lucidum* (1ml/250ml of sugar solution) (23.38 larvae/ 1000 brood cells), *P. niruri* (31.00 larvae/1000 brood cells), *C. longa* (40.00 infected larvae/1000 brood cells), *C.*

papaya (48.38 infected larvae/1000 brood cells) and *A. indica* (59.25larvae/hive). Significantly maximum number of infected larvae were found in control (70.38 larvae/ 1000 brood cells).

Table 82 Pooled data on effectiveness of plant and fungal extracts against sacbrood virus in *A. mellifera* colonies applied at an interval of four days during 2020-2021

Treatment	Dose /250ml	Number of cells with infected larvae per 1000 brood cells			
		Initial infestation	4 DAFT *	4 DAST**	4 DATT ***
G. lucidum	3.00ml	50.75 (7.19)#	31.25 (5.68)	24.38 (5.04)	17.88 (4.34)
G. lucidum	1.00ml	45.88 (6.85)	34.13 (5.93)	28.25 (5.41)	23.38 (4.94)
P. niruri	2.00g	45.25 (6.95)	41.88 (6.55)	38.13 (6.25)	31.00 (5.66)
A. indica	2.00g	54.00 (7.42)	56.25 (7.57)	57.63 (7.66)	59.25 (7.76)
C. papaya	2.00g	57.00 (7.62)	54.13 (7.42)	51.88 (7.27)	48.38 (7.03)
C. longa	2.00g	50.63 (7.19)	47.00 (6.93)	45.00 (6.78)	40.00 (6.40)
Untreated diseased check	-	57.00 (7.62)	61.13 (7.88)	66.15 (8.19)	70.38 (8.45)
C.D (0.05)		NS	(0.25)	(0.23)	(0.40)

#Figures in parentheses are square root (x+1) transformed values
*Days after first treatment, **Days after second treatment and ***Days after third treatment

Maximum reduction in percentage of diseased larvae in treated colonies (Table 83) over the diseased control were recorded in *G. lucidum* (3ml/250ml of sugar solution) (42.58%, 58.60% and 71.45%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (30.64%, 46.94% and 58.68%, respectively) and *P. niruri* (17.30%, 30.44% and 46.87%, respectively), *C. longa* (13.44%, 23.37% and 36.06%, respectively) and *C. papaya* (11.46%, 19.47% and 29.74%, respectively). Per cent decrease in infected larvae was minimum in *A. indica* (4.60%, 9.66% and 12.71%, respectively).

Our findings got support from the findings of Stamets *et al.* (2018) who tested mycelia extracts of multiple polypore fungal species amadou (*Fomes formentarius*) and reishi (*Ganoderma resinaceum*) against honey bee deformed wing virus (DWV) and Lake Sinai virus (LSV). They reported that colonies fed with *Ganoderma* extract exhibited a 79-fold reduction in DWV and a 45,000-fold reduction in LSV compared to control colonies in field

trials. These findings indicated honeybees may gain health benefits from fungi and their antimicrobial compounds. Aruna *et al.* (2017) have tested different plant extracts against Thai sacbrood infected *A. cerana* colonies and reported that *P. niruri* L. extract in 250 ml sugar solution combined with modified shook swarm method recorded the lowest number of infected larvae per thousand brood cells and highest per cent decrease from diseased control after third round of treatment at 4 days interval and prevented absconding of colonies and minimum decrease from diseased control was observed from *A. indica*. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, identified from various parts of *P. niruri* and its extracts have been proved to have therapeutic effects in many clinical studies (Paithankar *et al.,* 2011). The phytochemicals present in the herbal extracts can be the reason for the effectiveness against the virus. Many plants contain ribosome-inactivating proteins (RIPs) that alter ribosomal function in the infected cell and inhibit viral protein synthesis (Olevieri *et al.,* 1996). Gomez *et al.* (2016) examined the effect of neem (*Azadirachta indica*) oil on mortality and development of honey bee worker brood, queen oviposition, colony performance, and *Varroa destructor* mortality. It was indicated that adequate concentrations of neem oil may control *V. destructor* without affecting bee colonies. Neem oil at concentrations of 0.33–21.10%, with 7.26–464.64 mg l^{-1} azadirachtin, was sprayed on bee (*Apis mellifera*) combs. Their effects on mortality and developmental time of the brood, worker bee response on feeding and capping the larvae, and number of eggs laid by the queen were quantified. Strachecka *et al.* (2015) observed the influence of curcumin supplemented feeding on worker life span and Nosema resistance. The bees that consumed curcumin lived longer and were less infested with *Nosema* spp. Curcumin decreased global DNA methylation levels especially in older bees in which the natural, age-related level increase was observed. Curcumin appeared to be an unexpectedly effective natural bio-stimulator, improving honey bees health and vitality.

Table 83 Per cent reduction in the diseased larvae per thousand brood cells over control

Treatment	Dose /250ml	Per cent decrease in diseased larvae/1000 brood cells		
		4 DAFT *	4 DAST **	4 DATT***
G. lucidum	3.00ml	42.58	58.60	71.45
G. lucidum	1.00ml	30.64	46.94	58.68
P. niruri	2.00g	17.30	30.44	46.87
A. indica	2.00g	4.60	9.66	12.71
C. papaya	2.00g	11.46	19.47	29.74
C. longa	2.00g	13.44	23.37	36.06

*Days after first treatment, **Days after second treatment and ***Days after third treatment

2) Pooled data on effects of plant and fungal extracts on development of brood area in sacbrood virus infected treated *A. mellifera* colonies

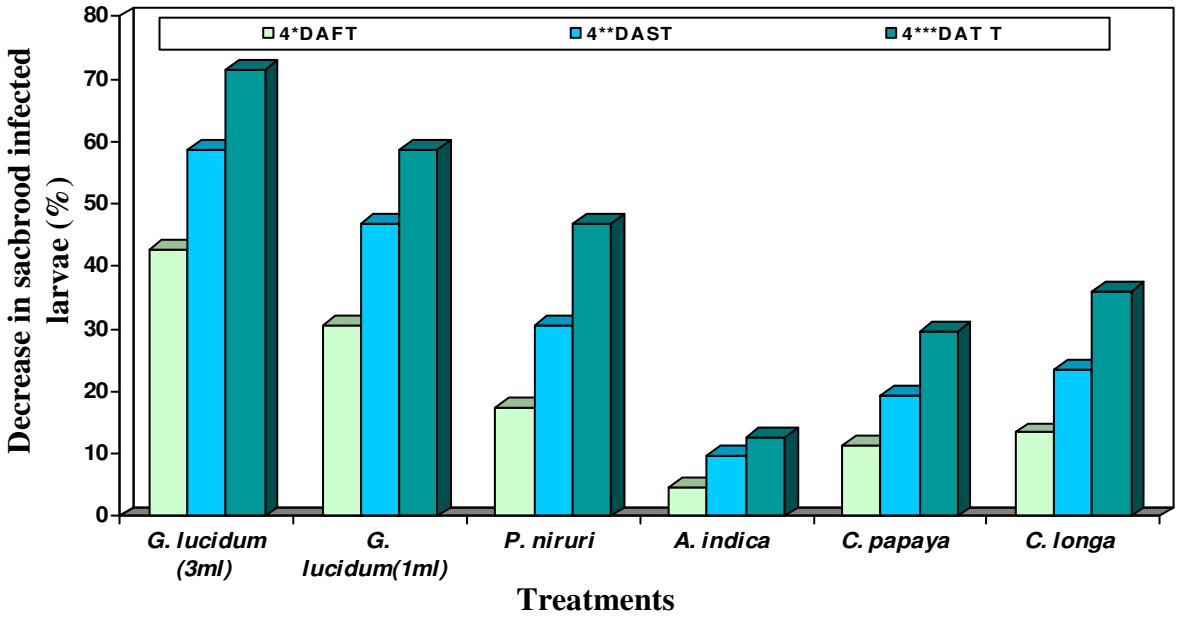
Data in Table 84 and Fig. 29 revealed that one day after three rounds of treatments, maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (1996.28 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1774.56 cm^2) followed by *P. niruri* (1614.92 cm^2) which was statistically at par with *C. longa* (1419.81 cm^2) which was at par with *C. papaya* (1218.24 cm^2). Significantly minimum brood area was observed in the colonies in which no treatment was given (382.96 cm^2).

Fifteen days after third treatment, brood area was maximum in *G. lucidum* (3ml/250ml of sugar solution) treatment (2128.50 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1842.28 cm^2) followed by *P. niruri* (1701.19 cm^2) which was statistically at par with *C. longa* (1467.38 cm^2). The brood area in *C. papaya* was 1276.29cm^2 which was statistically at par with *A. indica* (1040.87 cm^2). Significantly minimum brood area was observed in untreated colonies (281.38 cm^2).

After thirty days, brood area was significantly maximum in *G. lucidum* (3ml/250ml of sugar solution) (2568.71 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (2286.53 cm^2) which was statistically at par with *P. niruri* (2135.76 cm^2) followed by *C. longa* (1942.26 cm^2), *C. papaya* (1737.47 cm^2) and *A. indica* (1475.44 cm^2). Significantly minimum brood area was observed in untreated colonies (199.95 cm^2).

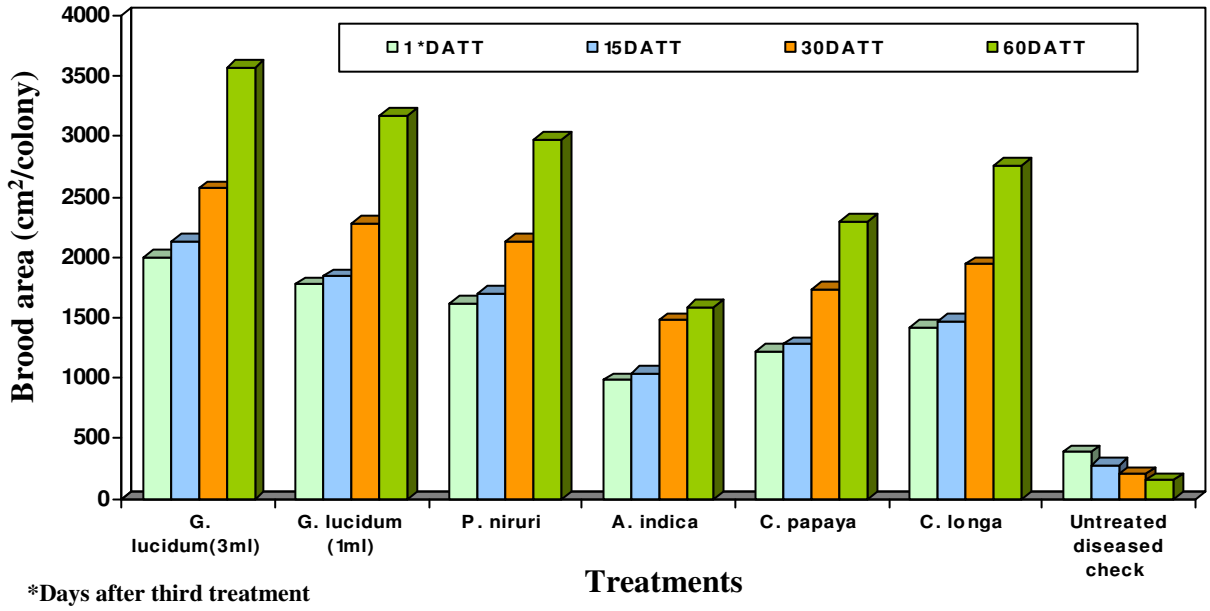
Sixty days after third treatment, significantly maximum brood area was obtained in *G. lucidum* (3ml/250ml of sugar solution) (3578.14 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (3179.04 cm^2) which was statistically at par with *P. niruri* (2979.90 cm^2) which was further at par with *C. longa* ((2762.21 cm^2). The brood area in *C. papaya* was 2302.65 cm^2 followed by *A. indica* (1588.31 cm^2). Significantly minimum brood area was observed in the colonies with no treatment (150.76 cm^2).

Patruica *et al.* (2017) examined the effect of using the medicinal plant extracts upon the health of bee colonies and reported that plant and fungal extracts increase the number of brood cells and reduced the number of bacteria (*Bacillus, Pseudomonas* and *Staphylococcus*) in worker bee intestines. The honey bee colonies fed with *Ganoderma* extract produced 2385 more brood cells as compared to the control group. The feeding of the colonies with protein-enriched candy in which extracts of plants and fungus (*Ganoderma*) was present also stimulate queen egg laying. Aruna *et al.* (2017) have tested different plant extracts against



*Days after first treatment, **Days after second treatment and ***Days after third treatment

Fig 28. Effect of plant and fungal extracts on SBV infected *A. mellifera* colonies (2020-2021)



*Days after third treatment

Fig 29. Effect of plant and fungal extracts on brood area in treated SBV infected *A. mellifera* colonies (2020-2021)

Thai sacbrood infected *A. cerana* colonies and demonstrated that the adult population as well as the total brood cells on 60 days after third treatment which are indicators of colony growth, was highest in the colonies managed by modified shook swarm method combined with provision of 2 g of *P. niruri* extract in sugar solution. The least effective treatment was *A. indica* treatment.

Table 84 Pooled data on cumulative effect of plant and fungal extracts applied at 4 days interval on brood area against sacbrood virus in *A. mellifera* colonies during 2020-2021

Treatment	Dose /250ml	Brood area (cm^2) after third treatment of plant and fungal extract			
		1 DATT *	15 DATT	30 DATT	60 DATT
G. lucidum	3.00ml	1996.28 (44.69)#	2128.5 (46.15)	2568.71 (50.69)	3578.14 (59.83)
G. lucidum	1.00ml	1774.56 (42.14)	1842.28 (42.93)	2286.53 (47.83)	3179.04 (56.39)
P. niruri	2.00g	1614.92 (40.20)	1701.19 (41.26)	2135.76 (46.23)	2979.9 (54.60)
A. indica	2.00g	982.01 (31.35)	1040.87 (32.28)	1475.44 (38.42)	1588.31 (39.87)
C. papaya	2.00g	1218.24 (34.92)	1276.29 (35.74)	1737.47 (41.69)	2302.65 (48.00)
C. longa	2.00g	1419.81 (37.69)	1467.38 (38.32)	1942.26 (44.08)	2762.21 (52.57)
Untreated diseased check	-	382.96 (19.59)	281.38 (16.80)	199.95 (14.17)	150.76 (12.31)
C.D (0.05)		(3.50)	(4.29)	(2.28)	(3.33)

#Figures in parentheses are square root (x+1) transformed values
*-Days after third treatment

4.6 Effects of fungal, plant extracts and bee products on European foulbrood infected *A. mellifera* colonies

a) During 2020

Effect of different fungal, plant extracts and bee products on *A. mellifera* European foulbrood infected colonies is presented in Table 85. European foulbrood incidence before application of treatments ranged between 34.33% to 45.33% and was statistically non-significant.

Seven days after first treatment of the colonies, minimum percentage of European foulbrood infection was observed in the *G. lucidum* (3ml/250ml of sugar solution) treatment (28.00%) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (30.33%) followed by Clove oil (*S. aromaticum)* 36.67%) which was statistically at par with Cinnamon (*C. zeylanicum*) (44.00%) and Thymol oil (*T. vulgaris*) (43.67%) treatment. The per cent infected cells in bee propolis treatment was 45.00 per cent which was statistically at par with honey treatment (46.00%). Significantly maximum European foulbrood infected cells (56.00%) were found in untreated colony. Per cent reduction from the diseased control was maximum in *G. lucidum* (3ml/250ml of sugar solution) (41.42%) followed by *G. lucidum* (1ml/250ml of sugar solution) (32.68%), Clove oil (*S. aromaticum)* treatment (29.57%), Cinnamon (*C. zeylanicum*) treatment (26.04%), Thymol oil (*T. vulgaris*) treatment (22.62%) and bee propolis (14.28%). Per cent reduction in infected cells over the diseased control was minimum in Honey treatment (6.11%).

Seven days after second treatment, minimum percentage of diseased cells were observed in the *G. lucidum* (3ml/250ml of sugar solution) (16.67%) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (21.00%) followed by Clove oil (*S. aromaticum)* treatment (31.00%) which was statistically at par with Cinnamon (*C. zeylanicum*) treatment (39.00%). The number of infected cells in Thymol oil treatment (*T. vulgaris*) were 42.00 per cent which was statistically at par with bee propolis (47.00%) and honey treatment (51.33%). Significantly maximum European foulbrood infected cells were found in control (68.33%) in which no treatment was applied. Per cent reduction in diseased cells over the diseased control was maximum in *G. lucidum* (3ml/250ml of sugar solution) (71.61%) followed by *G. lucidum* (1ml/250ml of sugar solution) (61.80%), Clove oil (*S. aromaticum)* treatment (51.20%), Cinnamon (*C. zeylanicum*) (46.27%), Thymol oil (*T. vulgaris*) (38.53%), bee propolis (22.76%) treatment and minimum per cent reduction from the diseased control was observed in honey treatment (14.13%).

One day after two treatments, maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (993.30 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (866.45 cm^2), Clove oil (*S. aromaticum)* treatment (851.40 cm^2), Cinnamon (*C. zeylanicum*) (829.90 cm^2) and Thymol (*T. vulgaris*) oil treatment (797.65 cm^2) (Table 85).

Table 85. Effectiveness of fungal, plant extracts and bee products on European foulbrood infected *A. mellifera* colonies during 2020

Treatments	Dose	Initial infestation	% Infestation 7days after 1st treatment	% Reduction in disease over control	% Infestation 7day]s after 2nd treatment	% Reduction in disease over control	Brood area one day after two treatments	Brood area 30 days after two treatments
G. lucidum	3.00ml/250ml of sugar solution	36.67 (6.14)*	28.00 (5.39)	41.42	16.67 (4.20)	71.61	993.30 (31.53)	3298.10 (57.44)
G. lucidum	1.00ml/250ml of sugar solution	34.33 (5.94)	30.33 (5.60)	32.68	21.00 (4.69)	61.80	866.45 (29.45)	2504.75 (50.06)
Honey	1.00ml/ 100 ml of water	37.33 (6.19)	46.00 (6.86)	6.11	51.33 (7.23)	14.13	543.95 (23.34)	634.25 (25.20)
Propolis	1.00g/ 100 ml of water	38.00 (6.24)	45.00 (6.78)	14.28	47.00 (6.93)	22.76	602.00 (24.56)	700.90 (26.49)
Cinnamon powder	1.00g/ 100 ml of water	45.33 (6.81)	44.00 (6.71)	26.04	39.00 (6.32)	46.27	829.90 (28.83)	1040.60 (32.27)
Clove	1.00ml/ 100 ml of water	39.67 (6.38)	36.67 (6.14)	29.57	31.00 (5.66)	51.20	851.40 (29.20)	1182.50 (34.40)
Thymol	1.00ml/ 100 ml of water	43.00 (6.63)	43.67 (6.68)	22.62	42.33 (6.58)	38.53	797.65 (28.26)	943.85 (30.74)
Control	-	42.67 (6.61)	56.00 (7.55)		68.33 (8.33)		460.10 (21.47)	219.30 (14.84)
C.D.(0.05)		NS	(0.58)		(0.67)		(4.32)	(3.93)

*Figures in parentheses are square root (x+1) transformed values

Minimum brood area was observed in control in which no treatment was applied (460.10 cm^2) which was statistically at par with honey (543.95 cm^2) and bee propolis (602.00 cm^2) treatment. Thirty days after two treatments, significantly maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (3298.10 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (2504.75 cm^2), Clove oil (*S. aromaticum)* treatment (1182.50 cm^2), which was statistically at par with Cinnamon (*C. zeylanicum*) (1040.60 cm^2) and Thymol oil (*T. vulgaris*) treatment (943.85 cm^2). The brood area in propolis treatment was 700.90 cm^2 which was statistically at par with honey treatment (634.25 cm^2). Significantly minimum brood area was observed in diseased control colony in which no treatment was applied (219.30 cm^2).

b) During 2021

Data in Table 86 revealed that initially European foulbrood incidence ranged from 39.00 to 52.33% in *A. mellifera* experimental colonies and was non-significant.

Seven Days after first treatment, minimum per cent infection was observed in the *G. lucidum* (3ml/250ml of sugar solution) treatment (24.67%) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (29.33%) followed by Clove oil (*S. aromaticum)* treatment (36.33%) which was statistically at par with Cinnamon (*C. zeylanicum*) treatment (37.67%), Thymol oil (*T. vulgaris*) treatment (43.00%) and bee propolis (46.00%). Maximum per cent infected cells were observed in control in which no treatment was given (58.33%) which was statistically at par with honey treatment (55.33%). Per cent reduction in diseased cells over the diseased control was maximum in *G. lucidum* (3ml/250ml of sugar solution) (45.78%) followed by *G. lucidum* (1ml/250ml of sugar solution) (39.67%), Clove oil (*S. aromaticum)* treatment (34.20%), Cinnamon (*C. zeylanicum*) (22.72%), Thymol oil (*T. vulgaris*) treatment (19.87 %), bee propolis (11.73%). The per cent reduction from the diseased control was minimum in honey treatment (9.37%).

Seven Days after second treatment of colonies, significantly minimum per cent infection was observed in the *G. lucidum* (3ml/250ml of sugar solution) (14.00 %) followed by *G. lucidum* (1ml/250ml of sugar solution) (21.00%), Clove oil (*S. aromaticum)* treatment (28.00%) which was statistically at par with Cinnamon (*C. zeylanicum*) treatment (34.67%).

Table 86. Effectiveness of fungal, plant extracts and bee products on European foulbrood infected *A. mellifera* colonies during 2021

Treatments	Dose	Initial infestation	% Infestation 7days after 1st treatment	% Reduction in disease over control	% Infestation 7days after 2nd treatment	% Reduction in disease over control	Brood area one day after two treatments	Brood area 30 days after two treatments
G. lucidum	3.00 ml/250 ml of sugar solution	39.00 (6.32)*	24.67 (5.07)	45.78	14.00 (3.87)	74.60	1141.65 (33.80)	3349.7 (57.89)
G. lucidum	1.00 ml/250 ml of sugar solution	41.67 (6.53)	29.33 (5.51)	39.67	21.00 (4.69)	64.34	1085.75 (32.97)	2685.35 (51.83)
Honey	1.00 ml/ 100 ml of water	52.33 (7.30)	55.33 (7.51)	9.37	60.33 (7.87)	18.43	425.70 (20.66)	397.75 (19.97)
Propolis	1.00 g/ 100 ml of water	44.67 (6.76)	46.00 (6.86)	11.73	49.67 (7.12)	21.33	763.25 (27.65)	780.45 (27.95)
Cinnamon	1.00 g/ 100 ml of water	46.00 (6.86)	37.67 (6.22)	22.72	34.67 (5.97)	42.05	877.20 (29.63)	1109.40 (33.32)
Clove	1.00 ml/ 100 ml of water	47.33 (6.95)	36.33 (6.11)	34.20	28.00 (5.39)	58.14	935.25 (30.60)	1313.65 (36.26)
Thymol	1.00 ml/ 100 ml of water	46.00 (6.86)	43.00 (6.63)	19.87	41.00 (6.48)	36.94	819.15 (28.64)	965.35 (31.09)
Control	-	50.00 (7.14)	58.33 (7.70)		70.67 (8.47)		307.45 (17.56)	298.85 (17.32)
C.D.(0.05)		N/S	(0.78)		(0.69)		(5.62)	(4.17)

*Figures in parentheses are square root (x+1) transformed values

The percentage of European foulbrood infected cells in Thymol oil (*T. vulgaris*) treatment was 41.00 per cent which was statistically at par with bee propolis treatment (49.67%). Maximum per cent infestation 70.67% was observed in diseased control without any treatment which was statistically at par with honey treatment (60.33%). Per cent reduction in diseased cells over the diseased control was maximum in *G. lucidum* (3ml/250ml of sugar solution) (74.60%) followed by *G. lucidum* (1ml/250ml of sugar solution) (64.34%), Clove oil (*S. aromaticum)* treatment (58.14%), Cinnamon (*C. zeylanicum*) treatment (42.05%), Thymol oil (*T. vulgaris*) treatment (36.94%), bee propolis (21.33%). Minimum per cent reduction over the diseased control was observed in honey treatment (18.43%).

One day after two treatments, maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (1141.65 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (1085.75 cm^2), Clove oil (*S. aromaticum)* treatment (935.25 cm^2), Cinnamon (*C. zeylanicum*) treatment (877.20 cm^2) and Thymol oil (*T. vulgaris*) treatment (819.15 cm^2) followed by bee propolis treatment (763.25 cm^2). Minimum brood area was observed in control (307.45 cm^2) which was statistically at par with honey treatment (425.70 cm^2). After thirty days of second treatment, significantly maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (3349.70 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (2685.35 cm^2), clove oil (*S. aromaticum)* treatment (1313.65 cm^2), which was statistically at par with Cinnamon (*C. zeylanicum*) treatment (1109.40 cm^2). The brood area in Thymol oil (*T. vulgaris*) treatment (965.35 cm^2) which was statistically at par with bee propolis treated colonies (780.45 cm^2). Minimum brood area was observed in control in which no treatment was applied (298.85 cm^2) and statistically at par with honey treated colonies (397.75 cm^2).

c) Pooled data (2020-2021)

Pooled data on effect of fungal, plant extracts and bee products presented in Table 87 and Fig. 30 revealed that European foulbrood incidence before application of treatments ranged from 37.83% to 46.33% non-significantly.

Seven Days after first treatment of the colonies, minimum per cent infection was observed in the *G. lucidum* (3ml/250ml of sugar solution) (26.33%) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (29.83%) followed by Clove oil treatment

(*S. aromaticum)* (36.50%) which was statistically at par with Cinnamon (*C. zeylanicum*) (40.83%), Thymol oil (*T. vulgaris*) (43.33%) and bee propolis (45.50%) treatment. Maximum percentage of European foulbrood infection was observed in control (57.17%) which was statistically at par with honey (50.67%). Per cent reduction in infected cells over the diseased control was maximum in *G. lucidum* (3ml/250ml of sugar solution) (43.60%) followed by *G. lucidum* (1ml/250ml of sugar solution) (36.18%), Clove oil (*S. aromaticum)* (31.89%), Cinnamon (*C. zeylanicum*) (24.38%), Thymol oil (*T. vulgaris*) (21.25 %) and bee propolis (13.01%) treatment. Minimum per cent reduction in infected cells was in honey treatment (7.74 %).

Seven Days after second treatment of colonies, minimum per cent diseased cells were observed in the *G. lucidum* (3 ml/250 ml of sugar solution) (15.33%) which was statistically at par with *G. lucidum* (1ml/250 ml of sugar solution) (21.00%) followed by Clove oil (*S. aromaticum)* treatment (29.50%) which was statistically at par with Cinnamon (*C. zeylanicum*) treatment (36.83%). Per cent infestation in Thymol oil (*T. vulgaris*) treatment was 41.67 % which was statistically at par with bee propolis (48.33%) which was further at par with honey treatment (55.83%). Significantly maximum percentage of European foulbrood infected cells (69.50%) was observed in diseased colony without any treatment (control). Per cent reduction in infected cells over diseased control was maximum in *G. lucidum* (3 ml/250 ml of sugar solution) i.e. 73.11% followed by *G. lucidum* (1ml/250ml of sugar solution) (64.07%), Clove oil (*S. aromaticum)* treatment (54.67%), Cinnamon (*C. zeylanicum*) (44.16%), Thymol oil (*T. vulgaris*) treatment (33.74%) and bee propolis (22.05%) treatment. The per cent reduction in the infected cells from the diseased control was minimum in honey treatment (16.28%).

Feeding of the bee colonies with protein enriched candy in extracts of plants and fungus (*Ganoderma lucidium*) had shown to stimulate queen egg laying and lead to a reduction in the number of bee intestinal bacteria, *Bacillus, Pseudomonas* and *Staphylococcus* (Patruica *et al.,* 2017). Yoon *et al.* (1994) demonstrated that *Ganoderma lucidium* has antimicrobial activity against both gram positive and gram negative bacteria.

Table 87. Pooled data on effectiveness of fungal, plant and bee products on *A. mellifera* European foulbrood infected colonies during 2020-2021

Treatments	Dose	Initial infestation	% Infestation 7days after 1st treatment	% Reduction in disease over control	% Infestation 7 days after 2nd treatment	% Reduction in disease over control	Brood area one day after two treatments	Brood area 30 days after two treatments
G. lucidum	3.00 ml/250 ml of sugar solution	37.83 (6.23)*	26.33 (5.23)	43.60	15.33 (4.04)	73.11	1067.48 (32.69)	3323.90 (57.66)
G. lucidum	1.00 ml/250 ml of sugar solution	38.00 (6.24)	29.83 (5.55)	36.18	21.00 (4.69)	64.07	976.10 (31.26)	2595.10 (50.95)
Honey	1.00 ml/ 100 ml of water	44.83 (6.77)	50.67 (7.19)	7.74	55.83 (7.54)	16.28	484.83 (22.04)	516.10 (22.74)
Propolis	1.00 g/ 100 ml of water	41.33 (6.51)	45.50 (6.82)	13.01	48.33 (7.02)	22.05	682.63 (26.15)	740.68 (27.23)
Cinnamon	1.00 g/ 100 ml of water	43.83 (6.70)	40.83 (6.47)	24.38	36.83 (6.15)	44.16	853.55 (29.23)	1075.00 (32.80)
Clove	1.00 ml/ 100 ml of water	43.50 (6.67)	36.50 (6.12)	31.89	29.50 (5.52)	54.67	893.33 (29.91)	1248.10 (35.34)
Thymol	1.00 ml/ 100 ml of water	44.50 (6.75)	43.33 (6.66)	21.25	41.67 (6.53)	33.74	808.40 (28.45)	954.60 (30.91)
Control	-	46.33 (6.88)	57.17 (7.63)		69.50 (8.40)		383.78 (19.62)	259.08 (16.13)
C.D.(0.05)		NS	(0.77)		(0.67)		(5.40)	(4.85)

*Figures in parentheses are square root (x+1) transformed values

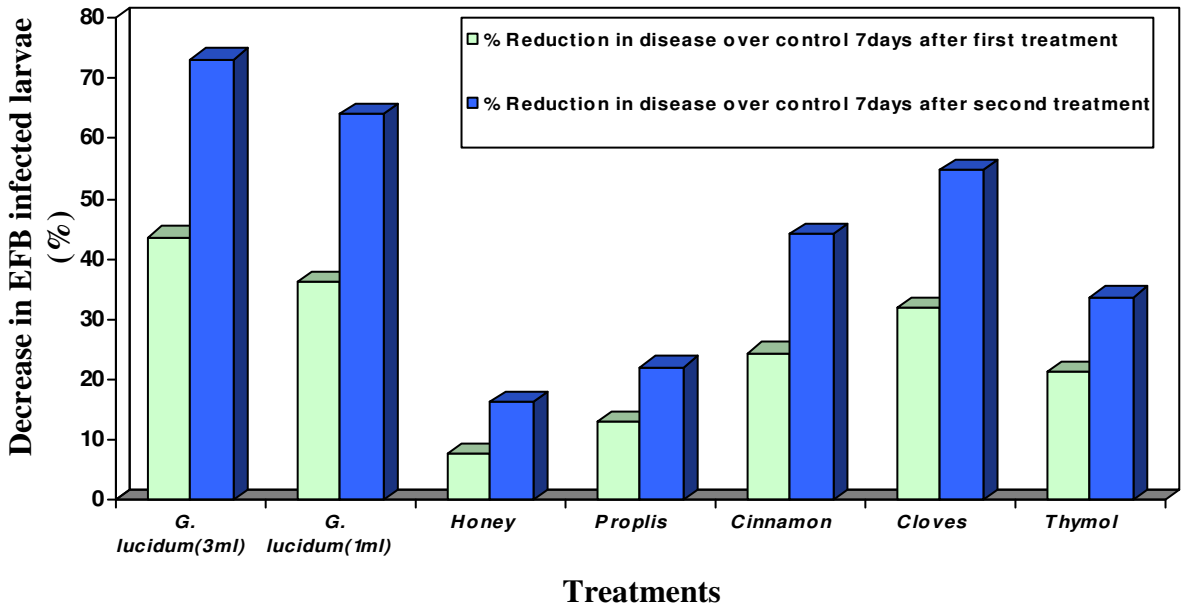


Fig 30. Effects of fungal, plant extracts and bee products on EFB infected *A. mellifera* colonies (2020-2021)

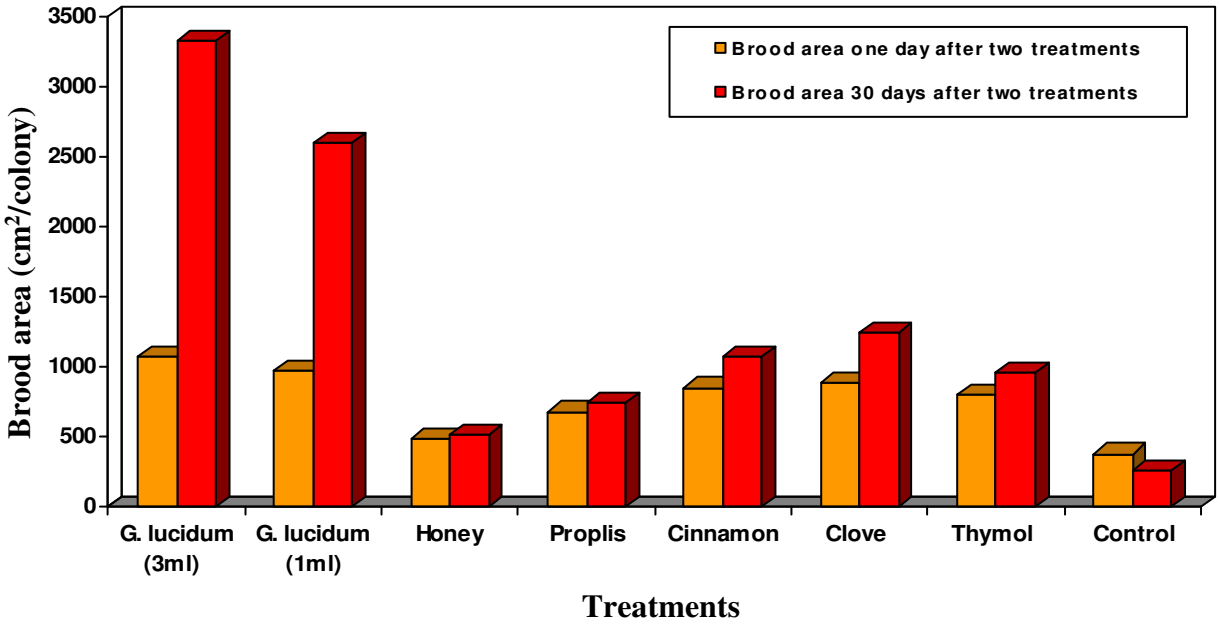


Fig 31. Effect of fungal, plant extracts and bee products on brood area of treated EFB infected *A. mellifera* colonies (2020-2021)

Ganoderma contains a variety of bioactive compounds including a range of triterpenoids, and other lipids, proteins, lysozyme, polysaccharides and nucleotides. It contains elements including calcium, magnesium, potassium and germanium and different compounds such as flavonoids, alkaloids, coumarins. Most of these compounds show various therapeutic effects. Hassona (2018) reported the effect of using natural products (Honey, Cinnamon, Cloves, Propolis and Thymol) against the *Paenibacillus larvae* (SH33) (AFB) and *Melissococcus plutonius* (EFB). She reported clove oil (*S. aromaticum)* at first position against European foulbrood among Honey, Cinnamon, Cloves, Propolis and Thymol treatments. Similar results were reported by Gende *et al.* (2008) who evaluated the antimicrobial activity of cinnamon essential oil (*Cinnamomum zeylanicum*) against *Paenibacillus* larvae. Cinnamaldehyde and eugenol proved to have antibacterial effects against *Paenibacillus* larvae.

Antunez *et al.* (2008) reported the effects of ethanolic extracts of propolis against *Paenibacillus* larvae and its potential to control AFB. Field assays showed that 21 and 42 days after the application of the treatments, the number of *Paenibacillus* larval spores/g of honey was significantly lower in colonies treated with Propolis ethanolic extract compared to the colonies in which no treatment was done. Collin *et al.* (2019) examined the effect of Thymol on bee hygienic behaviours that prevent the spread *Varroa* mite and diseases.They measured the efficiency of colonies at removing dead adult bees, uncapping dead pupal cells and removing dead brood. Thymol increased the uncapping and removal of dead brood by 24 to 36% after 48 h. The natural therapies have a good sound of attention in recent years (Kuzysinova *et al.,* 2016). Therefore most of the researchers trying to use the natural products to solve all the Problems especially the diseases in honey bee colonies.

Data in Table 87 and Fig. 31 further revealed that one day after two treatments of colonies, maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (1067.48 cm^2) which was statistically at par with *G. lucidum* (1ml/250ml of sugar solution) (976.10 cm^2), Clove oil (*S. aromaticum)* treatment (893.33 cm^2), Cinnamon (*C. zeylanicum*) (853.55 cm^2) and Thymol oil (*T. vulgaris*) treatment (808.40 cm^2) followed by bee propolis (682.63 cm^2) which was at par with honey treatment (484.83 cm^2) which was further at par with control (484.83 cm^2). Minimum brood area was observed in control (383.78 cm^2). Thirty days after second treatment, significantly maximum brood area was observed in *G. lucidum*

treatment (3ml/250ml of sugar solution) (3323.90 cm^2) followed by *G. lucidum* (1ml/250ml of sugar solution) (2595.10 cm^2), clove oil (*S. aromaticum)* treatment (1248.10 cm^2) which was statistically at par with Cinnamon (*C. zeylanicum*) (1075.10 cm^2) and Thymol oil (*T. vulgaris*) treatment (954.60 cm^2). The brood area in propolis treatment was 740.68 cm^2 which was at par with brood area in the colonies treated with honey (516.10 cm^2) which was statistically at par with brood area in untreated colonies. Significantly minimum brood area was observed in control (259.08 cm^2).

Patruica *et al.* (2017) reported that the honey bee colonies fed with *Ganoderma* extract produce 2385 more brood cells as compared to the control group. The feeding of the colonies with protein-enriched candy in which extracts of plants and fungus *Ganoderma* were present also stimulate queen brood rearing. Our findings further got support from the findings of Hassona (2018) who revealed that clove oil has biggest change in the amount of capped brood through all the treatment period and increased the capped brood area by 25.30% and mean weight of healthy pupae were also increased by 1.747±0.172 mg.

Chapter-5

SUMMARY AND CONCLUSION

The present investigations entitled **"Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees"** were carried out during January, 2019 to August, 2021. The salient findings of studies are summarized below.

5.1 SEASONAL INCIDENCE OF BROOD DISEASES, MITES AND GREATER WAX MOTH (*G. mellonella*) IN *A. cerana* COLONIES

The observations on the colony records in *A. cerana* colonies under stationary conditions (Nauni, Solan) have been recorded at one month interval during January 2019 to December 2020.

5.1.1 Pooled data on colony records of *A. cerana* during 2019 – 2020

Average colony strength was minimum during November, December and January when the temperature was low. With the onset of spring the experimental colonies gained strength during February (4.73 bee frames) and March (4.48 bee frames) when build up flora was in bloom at Nauni, Solan. Thereafter, colonies increased in their average strength to 5.38 bee frames during April. Average brood area was maximum during April (1815.00 cm^2) followed by March (1444.80 cm^2) and February (1166.16 cm^2). Maximum pollen area (238.65 cm^2) was recorded in the month of May followed by pollen area in October (179.96 cm^2) which was statistically at par with April (176.73 cm^2). The minimum average pollen area was observed in January (58.70 cm^2) which was at par with December (66.44 cm^2) and September (69.02 cm^2). Average honey stores in *A. cerana* kept at Nauni remained poor during both the years being maximum in September (1213 g) which was statistically at par with July (975 g) and November (944 g). The lowest average honey stores were found in February (48.50 g).

5.1.2 Pooled data on Thai sacbrood disease incidence in *A. cerana* during 2019–2020

The average incidence of Thai sacbrood disease in *A. cerana* colonies varied from 0.10 to 6.90 per cent during 2019 to 2020. Significantly maximum incidence was observed in the month of May (6.90%) and least incidence of Thai sacbrood disease was recorded in the month of October (0.10%) which was statistically at par with March (0.60%) and November

(0.70%). No incidence of Thai sacbrood disease was observed in the month of December in both the years.

5.1.3 Pooled data on European foulbrood incidence in *A. cerana* during 2019- 2020

The average incidence of European foulbrood disease in *A. cerana* during 2019-2020 both the years showed that European foulbrood incidence was maximum in the month of July (17.50%) which was statistically at par with 15.80 per cent in the month of May and June (14.30%) followed by August (6.50%) which was further at par with September (6.30%) and April (6.10%). However the incidence of European foulbrood did not show significant reduction in colony strength and brood area. Pooled data further showed that minimum incidence (0.80%) of European foulbrood was observed in the month of March which was statistically at par with February (1.10%), November (1.50%) and January (1.60%). No incidence of European foulbrood disease was observed in the month of December in *A. cerana* colonies.

5.1.4 Pooled data on incidence of ectoparasitic mite (*T. clareae*) in *A. cerana* colonies during 2019- 2020

The average incidence of *T. clareae* in *A. cerana* colonies was recorded maximum in the month of June (7.80%) during both the years of study. No incidence of mite was observed in the month of January, February, August, September, October, November and December. Minimum incidence of *T. clareae* was observed in the month of March (1.80%) which was statistically at par with July (2.00%) and April (2.20%).

5.1.5 Pooled data on incidence of greater wax moth (*G. mellonella)* in *A. cerana* colonies during January, 2019- 2020

The mean population of the greater wax moth in *A. cerana* colonies during 2019-2020 arrayed from 0.83 to 8.97. The incidence of greater wax moth was firstly observed in the month of February. The highest average population of larvae, pupae and adults were recorded in the month of July (13.10, 9.10 and 4.70, respectively) when the temperature relative humidity and rainfall were high and after that population declined till November. Minimum average population of wax moth was observed in the month of February (1.20, 1.30, 0.00) and no incidence was found in the month of December and January when the temperature and rainfall were low. It was observed that weather conditions during summer and rainy season (May to September) were more favorable for the development of greater wax moth

development in *A. cerana* colonies. During the whole two years of study, the mean population of all the stages of wax moth was found to be highest (8.97) in the month of July followed by August (7.60), June (6.80), May (6.00), September (5.53), April (4.73), March (3.63), October (3.30) and November (1.50). Minimum population of wax moth (*G. mellonella*) larvae, pupae and adults were found in the month of February (0.83).

5.2 SEASONAL INCIDENCE OF BROOD DISEASES (SACBROOD AND EUROPEAN FOULBROOD), MITES (*V. destructor* AND *T. clarae*) AND GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* COLONIES

5.2.1 Colony records

5.2.1.1 Pooled data under stationary conditions during 2019- 2020

The average colony strength during both the years recorded maximum in May (6.01 bee frames) and minimum in the month of January (2.23 bee frames) which was statistically at par with December, November and February when the temperature was low. With the onset of spring the experimental colonies gained strength during March (3.96 bee frames) and April (4.82 bee frames) when build up flora was in bloom at Nauni, Solan. Average brood area was significantly maximum during May (3308.85 cm^2) followed by April (2844.45 cm^2) and June (1905.98 cm^2). Maximum average pollen area (199.31 cm^2) was recorded in the month of April followed by pollen area in May (158.67) which was statistically at par in June (137.39 cm^2). The minimum average pollen area was observed in December (22.58 cm^2). Average honey stores in *A. mellifera* colonies recorded maximum in September (518g) which was statistically at par with August (446.50 g) and July (407.50 g) though remained statistically low under stationary conditions during period of study. The low average honey stores were found in February (74g) which was statistically at par in January (84.50 g). The bees were fed with sugar syrup before and after the winter months.

5.2.1.1 Pooled data under stationary and migratory conditions during 2019- 2020

The average colony strength in *A. mellifera* in 2019-2020 under migratory conditions ranged from 4.76 to 6.49 bee frames during winter months (November to January). The average colony strength increased in February (7.33 bee frames) when honey bees were foraging for nectar and pollen on mustard crop at Hisar. Maximum average brood area (4040.93 cm^2) was recorded during February followed by brood area in January (2741.25 cm^2). Maximum average pollen area (432.15 cm^2) was recorded in the month of February which was statistically at par with January (374.10 cm^2), December (345.14 cm^2) and March

(225.75 cm^2). The minimum average pollen area (22.58 cm^2) was observed in April. Maximum average honey stores were recorded in spring season under migratory conditions i.e. February (1108 g) followed by honey stores in January (490 g).

5.2.2 European foulbrood disease

5.2.2.1 Pooled data under stationary conditions during 2019- 2020

The average incidence of European foulbrood disease recorded maximum in September (37.10%) followed by disease incidence in June (28.60) and May (22.30). Minimum incidence of European foulbrood disease was recorded in the month of December (1.90%) which was statistically at par with November (3.50%) and January (3.90%). The high incidence of European foulbrood in September showed reduction in colony strength and brood area.

5.2.2.1 Pooled data under stationary and migratory conditions during 2019- 2020

The average incidence of European foulbrood was high in July, August and September (38.30%, 30.00% and 23.20 %, respectively) which reduced subsequently in the month of October (10.20%). The European foulbrood disease incidence was observed in *A. mellifera* colonies in different winter months which was statistically low being 3.10, 2.30, 1.70 and 2.40 per cent, respectively during November, December, January and February. However, the disease increased again in the month of March (34.80%).

5.2.3 Sacbrood disease

5.2.3.1 Pooled data under stationary conditions during 2019- 2020

The average incidence of sacbrood disease varied from 0.40 to 6.60 per cent. Maximum incidence was observed in the month of May (6.60%) which was statistically at par with June (5.30%) followed by April (3.30%) which was further statistically at par with August (2.90%) and July (2.30%). Whereas, minimum disease incidence was recorded in the month of October (0.40%) which was statistically at par with January (0.70%) and September (0.90%). No incidence of Thai sacbrood disease was observed in the month of November and December.

5.2.3.2 Pooled data under migratory conditions during 2019- 2020

Under migratory conditions sacbrood disease incidence was maximum in the month of May (5.80%) which was statistically at par with June (4.70%) followed by August (4.40%) which was further statistically at par with April (3.60%). During migratory months low disease incidence was recorded as compared to stationary conditions. Whereas, minimum incidence was recorded in the month of December (0.50%) which was statistically at par with disease incidence in November (1.00%).

5.2.4 *Tropilaelaps clareae* mite

5.2.4.1 Pooled data under stationary conditions during 2019- 2020

The average minimum population of *T. clareae* was observed in *A. mellifera* colonies in the month of February (2.80 mites /colony) at Nauni. Thereafter *T. clareae* population increased gradually in March (4.90 mites/colony), April (6.40 mites) and May (7.10 mites). Maximum population of *T. clareae* was recorded in the month of June (10.40 mites). The mite population was increased significantly in the month of May (7.10 mites) and June (10.40 mites). Perusal of data further revealed that brood infestation of mites was observed initially in the month of February (1.80%). In general, maximum infestation was observed in the month of June in both the methods of estimation of mite population. Experimental colonies were found free from *T. clareae* from September to January on adult bees and on brood.

5.2.4.2 Pooled data under migratory conditions during 2019- 2020

The colonies of *A. mellifera* were noticed free from *T. clareae* incidence in January, September, October, November and December. When colonies were shifted to Hisar the mites were only found in February in both per hundred bees method and brood infestation. *T. clareae* incidence was noticed minimum in February (0.90 mites/colony). *T. clareae* population was significantly more in June (13.70 mites) in per hundred bees method. Similar trend of *T. clareae* infestation in respective months was observed in per cent brood infestation method.

5.2.5 *Varroa destructor*

5.2.5.1 Pooled data under stationary conditions during 2019- 2020

During both the years, *Varroa* mite appeared in February (3.30 mites/ colony) which increased in March (7.80 mites/ colony), April (8.80 mites/ colony), May (12.40 mites/

colony) and reached its peak during June (14.90 mites/ colony). The data on mite population recorded on per hundred bees method showed that the incidence of *Varroa* mite in *A. mellifera* colonies was maximum in summer months at Nauni, Solan. Similar trend in mite population has been observed in per cent brood infestation when estimated by examining visually in the open and sealed brood cells. Brood also found free from mite infestation in January, August, September, October, November and December.

5.2.5.2 Pooled data under migratory conditions during 2019- 2020

There was no *Varroa* mite population when colonies were kept at Nauni, Solan from September to December and on migration the colonies of *A. mellifera* were also remained free from mite infestation during November to February. When the colonies of *A. mellifera* were shifted back to Nauni, the incidence of *Varroa* mite was detected in the month of March and was found maximum (16.30 mites/ colony) during June in per 100 bees method of mite estimation similar trends of brood infestation was observed in per cent brood infestation method.

5.2.5 Greater wax moth *(Galleria mellonella)*

5.2.5.1 Pooled data under stationary conditions during 2019- 2020

Mean population of the greater wax moth during both the years arrayed from 0.50 to 7.47. Seasonal incidence of greater wax moth started in the month of February and the average or mean highest number of larvae, pupae and adults were recorded in the month of July (11.60, 7.10 and 3.70, respectively) when the temperature and relative humidity were high and rainfall was maximum After that population declined till November and the average minimum population of all stages of wax moth was observed in the month of February (0.90 larvae, 0.60 pupae and 0.00 adults) and no incidence were found in the month of December and January when the temperature and rainfall were low. It was observed that weather conditions during summer and rainy period (May to September) were more favorable for the development of greater wax moth in *A. mellifera* colonies.

The population of wax moth (larval, pupal as well as adults) varied during different months during 2019-2020. During both the years, mean population of all stages of greater wax moth started increasing from March and increased till July; thereafter from August the population started declining and declined till November. During the two years of study period, the mean population of all stages of wax moth was found highest (7.47) in the month

of July followed by August (6.67), June (6.17), May(4.80), September (4.00), April(3.90), March (2.60), October (2.30), November (1.03). Minimum number of wax moth (*G. mellonella*) larvae, pupae and adults were found in the month of February (0.50).

5.2.5.2 Pooled data under migratory conditions during 2019- 2020

Seasonal incidence of greater wax moth started in the month of February and the average highest number of larvae, pupae and adults were recorded in the month of July (10.30, 6.10 and 3.20, respectively) when the temperature and relative humidity were high and rainfall was maximum. It was observed that weather conditions during summer and rainy period (May to September) were more favorable for greater wax moth development in *A. mellifera* colonies. After that population declined till December and average minimum incidence of wax moth population was observed in the month of January (0.90, 0.50 and 0.00) when the temperature and rainfall were low. During 2019-2020 under migratory conditions, the mean population of all stages of wax moth was found highest (6.53) in the month of July followed by August (5.50), June (5.13), May (4.03), September (4.00), April (2.87), March (2.03), October (1.63), February (1.23), November (0.97) and January (0.47). Lowest mean population of wax moth was found in the month of December (0.33).

5.3 MANAGEMENT OF GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* AND *A. cerana* COMBS UNDER LABORATORY CONDITIONS

5.3.1 Pooled data on area damaged and per cent area infestation of greater wax moth (*G. mellonella*) in *A. mellifera* wax combs during 2020-2021

Four plant extracts viz., neem seed kernal extract (5%), neem leaf powder (8.33g), alsi seed extract (20g/100 ml acetone), Pumpkin seed extract (20g/ 100 ml acetone); two essential oils like neem oil (3ml/100 ml water), karanj oil (3ml/100 ml water); three fungi like *T. viridae* (2 ml/l water)**,** *B. bassiana* (2 ml/l water), *M. anisopliae* (2 ml/l water) and two organic acids viz., acetic acid (2ml/ l water) and formic acid (0.8ml/l water) were evaluated by spraying or dusting on *A. mellifera* combs against third instar larvae of *G. mellonella* under laboratory conditions. Similarly *B. thuringiensis* var. *kurstaki* @ 5g/ l water was sprayed in the wax combs; mechanical practices such as keeping the comb in deep freezer at -8°C to -10°C for 5 hours along with sulphur fumigation of combs were evaluated. Deep freezing treatment was the best treatment resulting in 100% wax moth mortality within 5 hours of treatment. So the area damaged and per cent area infestation was nil in this treatment. After 7 days of treatment, the minimum area damaged and per cent damaged comb

area was recorded in *Bt* (5.03 cm^2 and 2.19 %, respectively) followed by neem seed kernel extract (8.87 cm^2 and 3.29 %, respectively), neem oil treatment (11.77 cm^2 and 4.17 %, respectively), sulphur fumigation (11.14 cm^2 and 4.23 %, respectively), neem leaf powder (16.62 cm^2 and 5.54 %, respectively), acetic acid (24.90 cm^2 and 8.43 %, respectively), karanj oil (23.17 cm^2 and 9.28 %, respectively), formic acid (30.23 cm^2 and 11.21 %, respectively), *T. viridae* (47.27 cm^2 and 17.05 %, respectively), *B. bassiana* (51.88 cm^2 and 20.05 %, respectively), *M. anisopliae* (60.97 cm^2 and 20.97 %, respectively), alsi seed extract (65.09 cm^2 and 25.67 %, respectively) and pumpkin seed extract (75.99 cm^2 and 28.83 %, respectively). Fourteen days after treatment again *Bt* was the best among all treatments with 8.81 cm^2 area damaged and 3.24 % per cent area infestation followed by neem seed kernel extract (14.21 cm^2 and 5.23 %, respectively), neem oil treatment (17.64 cm^2 and 6.48 %, respectively), sulphur fumigation (11.63 cm^2 and 5.64 %, respectively), neem leaf powder (22.49 cm^2 and 8.27 %, respectively), acetic acid (27.15 cm^2 and 11.34 %, respectively), formic acid (35.35 cm^2 and 14.36 %, respectively), karanj oil (31.66 cm^2 and 11.64 %, respectively), *T. viridae* (79.44 cm^2 and 29.21 %, respectively), *B. bassiana* (86.86 cm^2 and 31.93 %, respectively), *M. anisopliae* (92.51 cm^2 and 34.01 %, respectively), alsi seed extract (114.89 cm^2 and 42.24 %, respectively) and pumpkin seed extract (134.65 cm^2 and 49.50 %, respectively). After 21 days of treatment area damaged and per cent area infestation was minimum in *Bt* (12.27 cm^2 and 3.95 %, respectively) followed by neem seed kernel extract (15.90 cm^2 and 5.95 %, respectively), sulphur fumigation (20.44 cm^2 and 8.58 %, respectively), neem oil (20.39 cm^2 and 8.20 %, respectively), neem leaf powder (28.74 cm^2 and 10.68 %, respectively), acetic acid (35.52 cm^2 and 14.19 %, respectively), karanj oil (32.13 cm^2 and 13.23 %, respectively), formic acid (46.73 cm^2 and 16.59 %, respectively), *T. viridae* (98.59 cm^2 and 36.39 %, respectively), *B. bassiana* (114.47 cm^2 and 41.25 %, respectively), *M. anisopliae* (110.13 cm^2 and 41.50 %, respectively), alsi seed extract (164.03 cm^2 and 61.53 %, respectively) and pumpkin seed extract (180.87 cm^2 and 67.61 %, respectively). Maximum area damaged and per cent area infestation was recorded in control after 7, 14 and 21 days of treatment (122.02 cm^2 and 46.17 %, 172.45 cm^2 and 63.40 % and 232.37 cm^2 and 85.80 %, respectively).

5.3.2 Pooled data on area damaged and per cent infestation of greater wax moth (*G. mellonella*) in *A. cerana* wax combs during 2020-2021

Among all treatments used for the control of greater wax moth *G. mellonella* infesting *A. cerana* wax combs under laboratory conditions, deep freezing was the best treatment

followed by *Bt,* NSKE and Neem oil treatment. Least effective treatments were plant extracts (alsi seed extraxt and pumpkin seed extract) and biopesticides, *T. viridae*, *B. bassiana*, *M. anisopliae*. After 7 days of treatment the minimum area damaged and per cent damaged comb area was recorded in *Bt* (7.61 cm^2 and 2.80 %, respectively) followed by neem seed kernel extract (13.13 cm^2 and 4.83 %, respectively), sulphur fumigation (16.47 cm^2 and 6.06 %, neem oil treatment (16.79 cm^2 and 6.17 %, respectively), respectively), neem leaf powder (22.85 cm^2 and 8.40 %, respectively), acetic acid (23.85 cm^2 and 8.77 %, respectively), formic acid (35.86 cm^2 and 13.19 %, respectively), karanj oil (39.29 cm^2 and 14.15 %, respectively), *T. viridae* (43.37 cm^2 and 15.94 %, respectively), *B. bassiana* (50.94 cm^2 and 18.73 %, respectively), *M. anisopliae* (61.51 cm^2 and 22.62 %, respectively), alsi seed extract (65.48 cm^2 and 24.08 %, respectively) and pumpkin seed extract (79.75 cm^2 and 29.32 %, respectively). Fourteen days after treatment minimum area damaged and per cent area infestation was recorded in *Bt* (10.75 cm^2 and 3.95 %, respectively) followed by neem seed kernel extract (16.62 cm^2 and 6.11 %, respectively), neem oil treatment (20.24 cm^2 and 7.44 %, respectively), sulphur fumigation (20.40 cm^2 and 7.50 %, respectively), neem leaf powder (27.71 cm^2 and 9.72 %, respectively), acetic acid (27.96 cm^2 and 10.28 %, respectively), karanj oil (36.97 cm^2 and 13.59 %, respectively), formic acid (37.22 cm^2 and 13.69 %, respectively), *T. viridae* (90.15 cm^2 and 33.14 %, respectively), *B. bassiana* (99.35 cm^2 and 36.53 %, respectively), *M. anisopliae* (111.21 cm^2 and 40.89 %, respectively), alsi seed extract (131.09 cm^2 and 48.19 %, respectively) and pumpkin seed extract (171.71 cm^2 and 63.16 %, respectively). After 21 days of treatment area damaged and per cent area infestation was minimum in *Bt* (13.81 cm^2 and 5.17 %, respectively) followed by neem seed kernel extract (22.05 cm^2 and 8.06 %, respectively), sulphur fumigation (24.32 cm^2 and 8.51 %, respectively), neem oil (29.32 cm^2 and 10.03 %, respectively), neem leaf powder (31.87 cm^2 and 11.80 %, respectively), karanj oil (42.74 cm^2 and 15.46 %, respectively), acetic acid (43.00 cm^2 and 16.83 %, respectively), formic acid (71.05 cm^2 and 20.88 %, respectively), *T. viridae* (111.58 cm^2 and 42.40 %, respectively), *B. bassiana* (122.91 cm^2 and 47.60 %, respectively), *M. anisopliae* (141.40 cm^2 and 51.87 %, respectively), alsi seed extract (150.51 cm^2 and 56.39 %, respectively) and pumpkin seed extract (185.68 cm^2 and 69.99 %, respectively). Maximum area damaged and per cent area infestation was recorded in control after 7, 14 and 21 days of treatment (129.58 cm^2 and 47.64 %, 186.28 cm^2 and 68.49 % and 245.37 cm^2 and 91.08 %, respectively). All treatments showed similar effectiveness for the control of *G. mellonella* in *A. cerana* combs and *A. mellifera* combs. However the per cent

infested area was maximum in almost all treatments in *A. cerana* combs as compared to the *A. mellifera* combs. This showed high preference of *G. mellonella* for *A. cerana* combs.

5.3.3 Pooled data on effects of larval feeding on weight of treated *A. mellifera* combs under laboratory conditions during 2020-2021

After 7, 14 and 21 days of treatment deep freezing treatment showed no loss in weight of treated *A. mellifera* combs because all the third instar larvae got killed in five hours after treatment. The next most effective treatment was *Bt* which showed 1.92 % loss in weight of combs followed by neem seed kernel extract (2.73%), sulphur fumigation (2.82%), neem oil treatment (3.85%), neem leaf powder (4.55%), acetic acid (6.14%), karanj oil (7.84%), formic acid (8.73%), *T. viridae* (14.66%), *B. bassiana* (16.16%), *M. anisopliae* (18.55%), alsi seed extract (21.03%) and pumpkin seed extract (26.57%). After fourteen days of treatment minimum loss in weight of *A. mellifera* combs were recorded in *Bt* (3.38 %) followed by neem seed kernel extract (3.98%), sulphur fumigation (5.23%), neem oil treatment (6.12%), neem leaf powder (7.10%), karanj oil (10.25%), acetic acid (10.27%), formic acid (12.97%), *T. viridae* (27.61%), *B. bassiana* (31.11%), *M. anisopliae* (33.61%), alsi seed extract (30.81%) and pumpkin seed extract (39.86%). After twenty one days of treatment *Bt* showed minimum reduction in weight of comb (3.99 %) followed by neem seed kernel extract (3.98%), sulphur fumigation (6.81%), neem oil (8.65%), neem leaf powder (10.17%), acetic acid (12.36%), karanj oil (13.01%), formic acid (15.57%), *T. viridae* (36.09%), *B. bassiana* (40.96%), *M. anisopliae* (41.09%), alsi seed extract (44.25%) and pumpkin seed extract (61.16%). Among all treatments excluding deep freezing treatment, *Bt* treatment resulted in minimum reduction in weight of treated *A. mellifera* combs due to larval feeding of *G. mellonella* under storage conditions.

NSKE and sulphur fumigation treatments were equally effective as *Bt* treatment in per cent reduction in weight of treated combs. Among all neem formulations used NSKE was most effective showing less reduction in weight of treated combs. Biopesticides (*T. viridae, B. bassiana and M. anisopliae*) showed higher weight loss in treated combs as compared to plant products and organic acids and out of these biopesticides *T. viridae* was more effective. Alsi and pumpkin seed extracts were least effective resulting in highest weight loss of combs. The highest reduction in weight of combs was observed in control after 7, 14 and 21 days of treatment (37.80%, 60.99% and 80.50%, respectively).

5.3.4 Pooled data on effects of larval feeding on weight of treated *A. cerana* combs under laboratory conditions during 2020-2021

The efficacy of various non-chemical and chemical treatments against larval feeding of greater wax moth on treated *A. cerana* combs showed similar trend of effectiveness on weight reduction of combs. No reduction in weight of combs was observed in deep freezing treatment due to mortality of all larvae in first five hours of treatment. Minimum weight loss in treated combs was recorded in *Bt* (2.49 %) after seven days of treatment followed by neem seed kernel extract (3.15%), sulphur fumigation (3.82%), neem oil treatment (4.09%), neem leaf powder (5.08%), acetic acid (7.52%), karanj oil (9.59%), formic acid (10.11%), *T. viridae* (15.24%), *B. bassiana* (16.75%), *M. anisopliae* (18.86%), alsi seed extract (19.65%) and pumpkin seed extract (26.34%). After fourteen days of treatment again minimum weigh loss of the comb was observed in *Bt* (3.52 %) followed by neem seed kernel extract (4.54%), sulphur fumigation (5.90%), neem oil (6.18%), neem leaf powder (8.31%), acetic acid (12.25%), karanj oil (12.61%), formic acid (15.33%), alsi seed extract (29.95%), *T. viridae* (29.97%), *B. bassiana* (33.47%), *M. anisopliae* (35.97%), and pumpkin seed extract (42.08%). Twenty one days after treatment *Bt* showed minimum weight loss of combs (4.77 %) followed by neem seed kernel extract (6.39%), sulphur fumigation (6.65%), neem oil treatment (9.33%), neem leaf powder (6.39%), karanj oil (14.30%), acetic acid (15.04 %), formic acid (17.15 %), *T. viridae* (36.21%), *B. bassiana* (40.00 %), *M. anisopliae* (42.02%), alsi seed extract (44.70%) and pumpkin seed extract (64.99%). Maximum weight loss was recorded in untreated combs (control) (34.79%, 63.07% and 83.43%, respectively).

5.3.5 Pooled data on effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) by using *A. mellifera* combs during 2020- 2021

Highest larval mortality (100%) was recorded in deep freezing treatment as all ten larvae were found dead in first five hours. Next best treatment was *Bt* resulting in 86.25% larval mortality followed by neem seed kernel extract (76.25%), sulphur fumigation (73.25 %), neem oil treatment (66.25 %), acetic acid (63.75%), neem leaf powder (58.75%), karanj oil (55.00%), formic acid (50.00%), *T. viridae* (33.75%), *B. bassiana* (31.25 %), *M. anisopliae* (28.75 %), alsi seed extract (26.25 %) and pumpkin seed extract (13.75%). Highest pupal (86.11%) mortality and lowest adult emergence (12.50%) was recorded in *Bt* followed by neem seed kernel extract (78.13%, 21.88%, respectively), sulphur fumigation (76.04 %, 23.96%, respectively), neem oil treatment (65.63 %, 34.38, respectively), acetic

acid (58.75%,41.25%, respectively), neem leaf powder (58.13%, 37.50, respectively), karanj oil (51.07%,48.93%, respectively), formic acid (49.64%,50.36%, respectively), *T. viridae* (33.81%, 66.19%, respectively), alsi seed extract (32.84 %,67.16%, respectively), *B. bassiana* (25.95 %,74.05%, respectively), *M. anisopliae* (23.40 %,76.60%, respectively), and pumpkin seed extract (14.86 %, 85.14%, respectively). Lowest larval, pupal mortality and highest adult emergence was observed in control (3.75%, 2.50% and 97.50%, respectively).

5.3.6 Pooled data on effects of different treatments on larval, pupal mortality and adult emergence of greater wax moth (*G. mellonella*) by using *A. cerana* combs during 2020-2021

The effectiveness of different treatments on larval, pupal mortality and adult emergence of *A. cerana* combs was similar as recorded in treated combs of *A. mellifera*. *Bt* accounted second highest larval mortality (86.25%) after deep freezing treatment (100%) followed by neem seed kernel extract (72.50 %), sulphur fumigation (70.00 %), neem oil treatment (63.75 %), neem leaf powder (63.75 %), acetic acid (57.50 %), formic acid (50.00 %), karanj oil (48.75%), *T. viridae* (30.00%), *B. bassiana* (27.50 %), *M. anisopliae* (25.00 %), alsi seed extract (20.00 %) and pumpkin seed extract (11.25%). *Bt* also resulted in pupal mortality (87.50%) followed by neem seed kernel extract (78.75 %), sulphur fumigation (73.54 %), neem oil treatment (67.71 %), neem leaf powder (63.49 %), acetic acid (60.00 %), karanj oil (56.25%), formic acid (47.41 %), *T. viridae* (33.15%), *B. bassiana* (28.45 %), *M. anisopliae* (25.12 %), alsi seed extract (24.55 %) and pumpkin seed extract (3.75%). Minimum adult emergence was recorded in *Bt* (12.50%) followed by neem seed kernel extract (21.25 %), sulphur fumigation (26.46 %), neem oil treatment (32.29 %), neem leaf powder (36.51 %), acetic acid (40.00 %), karanj oil (43.75%), formic acid (52.29 %), *T. viridae* (66.85%), *B. bassiana* (71.55 %), *M. anisopliae* (74.88 %), alsi seed extract (75.45 %) and pumpkin seed extract (88.08 %). Lowest larval (1.25%), pupal mortality (3.75%) and highest adult emergence (96.25%) was recorded in untreated combs.

5.4 POOLED DATA ON MANAGEMENT OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING 2020-2021

Among all treatments evaluated for the management of *G. mellonella* under laboratory conditions, the treatments performed better like spray of *Bt* @ 3,6,9g/l , chemical and non-chemical treatments viz., spray of 3% neem oil, 5% NSKE and lime sulphur paste along with periodical bottom board cleaning (cultural method) and integration of *Bt* spray,

periodical bottom board cleaning, delta trap, lime sulphur paste application were evaluated for the control of *G. mellonella* in *A. cerana* and *A. mellifera* colonies under field conditions. All treatments were given three times at an interval of 30 days.

Initial infestation in *A. cerana* colonies varied from 6.00 to 10.00 larvae per colony and was statistically non-significant. One month after first treatment, minimum number of larvae (0.17 larvae/ hive) were found in the colonies treated with lime sulphur paste followed by *Bt* (6 g) treatment (1.00 larvae/ hive), neem seed kernel extract (1.83 larvae/hive), *Bt* (3g) (2.17 larvae/ hive), neem oil treatment (2.83 larvae/ hive) and bottom board cleaning of the hives (4.50 larvae/ hive). No wax moth larval infestation was found in *Bt* (9g) treatment and the colonies in which integrated management was done. Significantly maximum number of larvae/ hive were found in control without any treatment (12.83 larvae/hive).

One month after second treatment, minimum number of larvae (0.50 larvae/ hive) were found in the colonies treated with *Bt* (6 g) followed by neem seed kernel extract treatment (0.67 larvae/hive), *Bt* (3g) treatment (1.50 larvae/ hive), neem oil (1.83 larvae/ hive) and bottom board cleaning of the hive (4.67 larvae/ hive). Significantly maximum number of larvae/ hive were found in control (14.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g) treatment, lime sulphur paste treatment and the colonies with integrated management.

One month after third treatment, minimum number of larvae were found in the colonies treated with neem seed kernel extract and *Bt* (3g) treatments (0.50 larvae/ hive) and neem oil treatment (1.33 larvae/ hive).Significantly maximum number of larvae/ hive were found in control colonies (13.83 larvae/hive). No wax moth larval infestation was found in *Bt* (6g and 9g), lime sulphur paste treatment and the colonies with integrated treatments.

Wax moth trap and delta traps with *A. dorsata*, *A. cerana* and *A. mellifera* trap were installed and evaluated for the control of wax moth *A. cerana* colonies under field conditions. The traps were also replicated three times. One month after first, second and third installation maximum mean number of adults were trapped in wax moth trap (29.00 adults/trap, 20.50 adults/trap and19.88 adults/trap, respectively) followed by delta trap with *A. dorsata* comb (21.75 females/trap, 19.13 females/trap and 16.00 females/trap, respectively), delta trap with *A. cerana* comb (14.88 females/ trap, 10.88 females/trap and 9.88 females/trap, respectively).

Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (10.25 females/trap), 9.50 females/trap and 6.00 females/trap, respectively).

5.5 POOLED DATA ON MANAGEMENT OF GREATER WAX MOTH (*G. mellonella)* IN *A. mellifera* COLONIES DURING 2020-2021

One month after first treatment initial infestation of *G. mellonella* in *A. mellifera* colonies the colonies varies from 7.83 to 10.33 non- significantly. Minimum number of larvae (0.50 larvae/ hive) were found in the colonies treated with *Bt* (6g) followed by *Bt* (3g) treatment (0.83 larvae/ hive), neem seed kernel extract (1.17 larvae/ hive), neem oil (2.50 larvae/hive), bottom board cleaning of the hives (3.67 larvae/hive). Significantly maximum number of larvae/ hive were found in untreated colonies (11.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies in which integrated management was done. 100% larval mortality of *G. mellonella* was observed with *Bt* 9g/l (spray) on infested combs of *A. mellifera,* lime sulphur paste treatment and integrated management treatments one month after first treatment.

One month after second treatment minimum number of larvae (0.34 larvae/ hive) was observed in *Bt* (6g) followed by *Bt* (3g) (0.50 larvae/ hive) treatment, neem seed kernel extract (1.17 larvae/ hive), neem oil treatment (2.00 larvae/hive) and periodical bottom board cleaning of the hives (2.83 larvae/ hive). Significantly maximum number of larvae/ hive were found in untreated colonies (13.00 larvae/hive). Initial infestation in the colonies varies from 7.83 to 10.33 non- significantly. One month after first treatment minimum number of larvae (0.50 larvae/ hive) were found in the colonies treated with *Bt* (6g) followed by *Bt* (3g) treatment (0.83 larvae/ hive), neem seed kernel extract (1.17 larvae/ hive), neem oil (2.50 larvae/hive), bottom board cleaning of the hives (3.67 larvae/hive). Significantly maximum number of larvae/ hive were found in untreated colonies (11.67 larvae/hive). No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste treatment and the colonies with integrated management.

One month after third treatment, minimum number of larvae (0.17 larvae/ hive) were found in *Bt* (6g) treatment followed by neem seed kernel extract (0.50 larvae/ hive), *Bt* (3g) (0.50 larvae/ hive), neem oil treatment (1.50 larvae/hive) and bottom board cleaning of the hives (2.17 larvae/hive). Significantly maximum number of larvae/ hive were found in untreated colonies (11.83 larvae/hive. No wax moth larval infestation was found in *Bt* (9g), lime sulphur paste and integrated management.

One month after first, second and third treatment maximum number of adults were trapped in wax moth trap (26.38 adults/trap, 24.25 adults/trap and19.13 adults/trap, respectively) followed by delta trap with *A. dorsata* comb (19.38 females/trap, 16.00 females/trap and 12.63females/trap, respectively) and delta trap with *A. cerana* comb (13.88 females/ trap, 11.88 females/trap and 8.50 females/trap, respectively). Minimum number of wax moth females were trapped in delta trap with *A. mellifera* comb (8.25 females/trap), 7.13 females/trap and 5.63 females/trap, respectively). Among all traps installed the best was wax moth trap whereas delta trap with *A. mellifera* comb was least effective for trapping adults and females of *G. mellonella* respectively.

5.6 POOLED DATA ON ANTIVIRAL EFFECT OF PLANT AND FUNGAL EXTRACTS ON SACBROOD VIRUS INFECTED *A. mellifera* COLONIES DURING 2020-2021

One fungal extract (*G. lucidum*) and four plant extracts (*P. niruri, A .indica, C. papaya* and *C. longa*) were evaluated by feeding to *A. mellifera* colonies for their antiviral effects on sacbrood virus disease. Before application of treatments the number of infected larvae per thousand brood cells varied and varied non-significant between 45.25 to 57.00 larvae/ hive.

Four days after first treatment, the minimum number of infected larvae/1000 brood cells and maximum per cent reduction in disease over control was recorded in recorded in *G. lucidum* (3ml/250ml of sugar solution) (31.25 larvae/1000 brood cells, 42.58%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (34.13 larvae/ 1000 brood cells and 30.64% respectively), *P. niruri* (41.88 larvae/1000 brood cells and 17.30, respectively), *C. longa* (47.00 larvae/1000 brood cells and 13.44% respectively), *C. papaya* (54.13 larvae/1000 brood cells and 11.46%, respectively) and *A. indica* (56.25 larvae/ hive and 4.60, respectively). Significantly maximum number of infected larvae were found in control (61.13 larvae/ 1000 brood cells).

Four days after second treatment, significantly minimum number of infected larvae and maximum per cent reduction in disease was recorded in *G. lucidum* (3ml/250ml of sugar solution) (24.38 larvae/1000 brood cells and 58.60%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (28.25 larvae/ 1000 brood cells and 46.94 %, respectively), *P. niruri* (38.13 larvae/1000 brood cells and 30.44%, respectively), *C. longa* (45.00 larvae/1000 brood cells and 23.37%, respectively), *C. papaya* (51.88 larvae/1000 brood cells and

19.47%, respectively) and *A. indica* (57.63 larvae/ hive and 9.66%, respectively). Significantly maximum number of infected larvae were found in control (66.15 infected larvae/ 1000 brood cells).

Similarly four days after third treatment, minimum number of infected larvae and maximum per cent reduction in sacbrood disease was recorded in *G. lucidum* (3ml/250ml of sugar solution) treatment (17.88 larvae/1000 brood cells and 71.45%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (23.38 larvae/ 1000 brood cells and 58.68%, respectively), *P. niruri* (31.00 larvae/1000 brood cells and 46.87%, respectively), *C. longa* (40.00 infected larvae/1000 brood cells and 36.06%, respectively), *C. papaya* (48.38 infected larvae/1000 brood cells and 29.74 %, respectively) and *A. indica* (59.25 larvae/ hive and 12.71 %, respectively). Significantly maximum number of infected larvae were found in control (70.38 larvae/ 1000 brood cells). Among all plant and fungal extracts evaluated for sacbrood virus disease in *A. mellifera* colonies *G. lucidum* (3ml/250ml of sugar solution) was the best treatment resulting in 70.41% reduction in disease after four days of third treatment. *A. indica* 2g/250 ml of sugar solution was least effective for the management of sacbrood disease with (10.44%) disease reduction.

One, fifteen, thirty and sixty days after third treatment of plant and fungal extracts, maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (1996.28 cm^2, 2128.50 cm^2, 2568.71 cm^2 and 3578.14 cm^2, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (1774.56 cm^2, 1842.28 cm^2, 2286.53 cm^2 and 3179.04 cm^2, respectively), *C. longa* (1419.81 cm^2, 1467.38 cm^2, 1942.26 cm^2 and 2762.21 cm^2, respectively), *C. papaya* (1218.24 cm^2, 1276.29 cm^2, 1737.47 cm^2 and 2302.65 cm^2, respectively) and *A. indica* (982.01 cm^2, 1040.87 cm^2, 1475.44 cm^2 and 1588.31 cm^2, respectively). Significantly minimum brood area was observed in untreated colonies (382.96 cm^2, 281.38 cm^2, 199.95 $cm^2$and 150.76 cm^2, respectively).

5.7 EFFECTIVENESS OF FUNGAL, PLANT EXTRACTS AND BEE PRODUCTS ON EUROPEAN FOULBROOD INFECTED *A. mellifera* COLONIES (2020-2021)

Different plant (cinnamon, thymol and clove), fungal (*G. lucidum*) and bee products (honey and propolis) were evaluated for the control of EFB disease by spraying / feeding in sugar solution. Before application of treatments disease incidence was non- significant ranged from 37.83% to 46.33%.

Seven days after first treatment of colonies minimum per cent infection and maximum per cent reduction in disease was observed in the *G. lucidum* (3ml/250ml of sugar solution) (26.33% and 43.60%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (29.83%, 36.18%, respectively), clove oil (*S. aromaticum)* (36.50% and 31.89%, respectively), Cinnamon (*C. zeylanicum*) (40.83%, 24.38%, respectively), Thymol oil (*T. vulgaris*) treatment (43.33%, 21.25 %, respectively), bee propolis (45.50% and 13.01%, respectively) and honey treatment (50.67% and 7.74 %, respectively). Maximum percentage of European foulbrood infection was observed in control (57.17%).

Seven days after second treatment of colonies, minimum per cent infection and maximum per cent reduction in disease were observed in the *G. lucidum* (3ml/250ml of sugar solution) (15.33% and 73.11%, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (21.00% and 64.07%, respectively), clove oil (*S. aromaticum)* (29.50% and 54.67%, respectively), Cinnamon (*C. zeylanicum*) (36.83% and 44.16%, respectively), Thymol oil (*T. vulgaris*) (41.67 % and 33.74 %, respectively), bee propolis (48.33% and 22.05%, respectively) and honey treatment (55.83% and 16.28%, respectively). Significantly maximum percentage of European foulbrood infected cells (69.50%) was observed in diseased colony without any treatment (control).

One and thirty days after second treatment maximum brood area was observed in *G. lucidum* (3ml/250ml of sugar solution) (1067.48 cm^2 and 3323.90 cm^2, respectively) followed by *G. lucidum* (1ml/250ml of sugar solution) (976.10 cm^2 and 2595.10 cm^2, respectively), clove oil (*S. aromaticum)* (893.33 cm^2 and 1248.10 cm^2, respectively), Cinnamon (*C. zeylanicum*) (853.55 cm^2 and 1075.10 cm^2, respectively), Thymol (*T. vulgaris*) (808.40 cm^2 and 954.60 cm^2, respectively),bee propolis (682.63 cm^2 and 740.68 cm^2, respectively) and honey (484.83 cm^2 and 516.10 cm^2, respectively). Minimum brood area was observed in control (383.78 cm^2 and 259.08 cm^2, respectively). In general, brood area increased and per cent disease infection decreased in diseased colonies of *A. mellifera* after treatments over untreated diseased check.

Among all plant and fungal extracts and bee products evaluated for EFB disease in *A. mellifera* colonies *G. lucidum* (3ml/250ml of sugar solution) was the best treatment resulting in 73.11 % disease in EFB disease after seven days of second treatment.

CONCLUSION:

- ✓ Bee diseases, European foulbrood, Thai sacbrood and sacbrood were found associated with developmental stages of *A. cerana* and *A. mellifera* during the study period. Seasonal incidence of European foulbrood in *A. cerana* colonies was maximum in July whereas, incidence of Thai sacbrood disease was maximum in May.
- ✓ EFB disease in *A. mellifera* colonies under stationary conditions firstly appeared in March and found maximum in the month of September when the colony strength, brood area were low, temperature was high, relative humidity and rainfall were moderate. However under stationary and migratory conditions maximum incidence was in July.
- ✓ Under stationary (Nauni, Solan) and migratory conditions (Hisar, Haryana), the incidence of SBV disease in *A. mellifera* was maximum in May when brood area and colony strength were maximum, temperature was high, relative humidity and rainfall were moderate.
- ✓ Ectoparasitic mite *T. clareae* was found associated with *A. cerana* and *A. mellifera* whereas the *V. destructo*r was observed in *A. mellifera* only. Seasonal incidence of ectoparasitic mites were found maximum in the month of June when the temperature was high, relative humidity and rainfall were moderate. High mite population coincides with increased brood rearing activity in *A. cerana* and *A. mellifera* colonies.
- ✓ Summer and rainy season were observed more suitable for the development and multiplication of greater wax moth (*G. mellonella* L.) in honey bee colonies. In both *A. cerana* and *A. mellifera* colonies under stationary conditions the infestation of GWM was first spotted in February then increased rapidly in the subsequent months and found maximum in July when the temperature was high. However under migratory months in Hisar (Haryana) the incidence was low. Therefore management measures should be taken according to seasonal incidence of wax moth, diseases and mites to rescue the bee colonies.
- ✓ Deep freezing (-8°C to -10°C) treatment was the most effective however, *Bacillus thuringiensis* (5g/l), neem products [(neem oil (3%) and NSKE (5%)] and sulphur fumigation (5g) were found effective for the management of wax moth in stored combs. Treatments viz., acetic acid, formic acid and karanj oil were moderately effective.

- ✓ Under field conditions sealing cracks and crevices of the hives with lime sulphur paste and spraying *Bt* (9g/l) on infested frames in honey bee colonies resulted in 100% mortality of GWM larvae. Installation of wax moth trap and delta trap with *A. dorsata* comb were the most effective traps for management of *G. mellonella*.
- ✓ Integration of management measures comprising of cultural (periodical bottom board cleaning), mechanical (delta trap with *A. dorsata* comb), chemical (lime sulphur paste) and biological (spray of *Bt* 3g/l) can be used to prevent damages to honey bee colonies caused by GWM.
- ✓ In *A. mellifera* colonies for the management of SBV, *G. lucidum* (3ml / 250 ml of 50% sugar solution) (71.45%) was most effective while, *G. lucidum* (1ml / 250 ml of 50% sugar solution) (58.68%) and *P. niruri* (46.87%) were moderately effective and *A. indica* (12.71%) was least effective in per cent reduction of the disease.
- ✓ Among different plant, fungal extracts and bee products, fungal extract of *Ganoderma lucidum* (3ml/ 250 ml of 50% sugar solution) (73.11%) and *G. lucidum* (1ml/ 250 ml of 50% sugar solution) (64.07%) were found to be most effective for the management of EFB disease while, Clove oil (54.67%) and Cinnamon oil (44.16%) were moderately effective and Honey (16.28%) and propolis (22.05%) were least effective in per cent decrease of the diseased larvae.

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APPENDIX I

ANNOVA FOR COLONY RECORDS OF *A. cerana* DURING JANUARY TO DECEMBER, 2019 AT NAUNI (TABLE 1)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3.59	0.33	4.19
Error	48	3.74	0.08	
Total	59	7.33		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3,139.58	285.42	17.27
Error	48	793.53	16.53	
Total	59	3,933.12		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	363.96	33.09	22.09
Error	48	71.89	1.50	
Total	59	435.85		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3,996.90	363.36	23.30
Error	48	748.40	15.59	
Total	59	4,745.30		

ANNOVA FOR COLONY RECORDS OF *A. cerana* DURING JANUARY TO DECEMBER, 2020 AT NAUNI (TABLE 2)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3.35	0.30	2.57
Error	48	5.69	0.12	
Total	59	9.04		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	2,594.57	235.87	11.42
Error	48	991.45	20.66	
Total	59	3,586.01		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	298.38	27.13	31.98
Error	48	40.713	0.85	
Total	59	339.09		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3,186.10	289.65	15.97
Error	48	870.50	18.14	
Total	59	4,056.60		

ANNOVA FOR POOLED DATA ON COLONY RECORDS OF *A. cerana* DURING JANUARY, 2019 TO DECEMBER, 2020 AT NAUNI (TABLE 3)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	2.26	2.26	1.74
Replication within Year	8	10.37	1.29	
Treatment	11	97.71	8.88	5.30
Year X Treatment	11	18.44	1.68	1.03
Pooled Error	88	143.64	1.63	
Total	119	272.40		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	28.22	28.22	1.77
Replication within Year	8	127.93	15.99	
Treatment	11	5,694.45	517.68	58.22
Year X Treatment	11	97.80	8.89	0.64
Pooled Error	88	1,222.79	13.89	
Total	119	7,171.18		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.95	0.95	1.09
Replication within Year	8	6.99	0.87	
Treatment	11	655.68	59.61	95.21
Year X Treatment	11	6.89	0.63	0.52
Pooled Error	88	105.68	1.20	
Total	119	776.18		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	62.08	62.08	5.38
Replication within Year	8	92.38	11.55	
Treatment	11	7,042.05	640.19	49.86
Year X Treatment	11	141.23	12.84	0.74
Pooled Error	88	1,526.62	17.35	
Total	119	8,864.37		

ANNOVA FOR INCIDENCE OF THAI SACBROOD DISEASE IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2019 (TABLE 4)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	13.5	1.23	4.45
Error	48	13.24	0.28	
Total	59	26.74		

ANNOVA FOR INCIDENCE OF THAI SACBROOD DISEASE IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2020 (TABLE 5)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	18.92	1.72	15.19
Error	48	5.43	0.11	
Total	59	24.35		

ANNOVA FOR POOLED DATA ON INCIDENCE OF THAI SACBROOD DISEASE IN *A. cerana* COLONIES DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 6)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.02	0.02	0.13
Replication within Year	8	1.02	0.13	
Treatment	11	32.87	2.99	46.92
Year X Treatment	11	0.70	0.06	0.44
Pooled Error	88	12.85	0.15	
Total	119	47.46		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2019 (TABLE 7)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	61.38	5.58	31.62
Error	48	8.47	0.18	
Total	59	69.86		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. Cerana* COLONIES DURING JANUARY TO DECEMBER, 2020 (TABLE 8)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	71.17	6.47	25.15
Error	48	12.35	0.26	
Total	59	83.52		

ANNOVA FOR POOLED DATA ON INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. cerana* COLONIES DURING JANUARY, 2019 TO DECEMBER, 2020 TABLE 9)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.16	0.16	1.03
Replication within Year	8	1.24	0.16	
Treatment	11	104.61	9.51	54.91
Year X Treatment	11	1.91	0.17	0.89
Pooled Error	88	17.02	0.19	
Total	119	124.92		

ANNOVA FOR INCIDENCE OF ECTOPARASITIC MITE (*T. clareae*) IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2019 (TABLE 10)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	21.49	1.95	10.37
Error	48	9.04	0.19	
Total	59	30.53		

ANNOVA FOR INCIDENCE OF ECTOPARASITIC MITE (*T. clareae*) IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2020 (TABLE 11)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	21.69	1.97	14.89
Error	48	6.36	0.13	
Total	59	28.05		

ANNOVA FOR POOLED DATA INCIDENCE OF ECTOPARASITIC MITE (*T. clareae*) IN *A. cerana* COLONIES DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 12)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.03	0.03	0.12
Replication within Year	8	2.21	0.28	
Treatment	11	40.83	3.71	17.04
Year X Treatment	11	2.40	0.22	1.45
Pooled Error	88	13.19	0.15	
Total	119	58.66		

ANNOVA FOR INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2019 (TABLE 13)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	50.49	4.59	22.41
Error	48	9.83	0.21	
Total	59	60.32		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	34.56	3.14	30.51
Error	48	4.94	0.10	
Total	59	39.50		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	12.19	1.11	14.14
Error	48	3.76	0.08	
Total	59	15.95		

ANNOVA FOR INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING JANUARY TO DECEMBER, 2020 (TABLE 14)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	52.31	4.76	54.33
Error	48	4.20	0.09	
Total	59	56.51		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	34.04	3.09	28.28
Error	48	5.25	0.12	
Total	59	39.29		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	11.05	1.00	7.70
Error	48	6.26	0.13	
Total	59	17.30		

ANNOVA FOR POOLED DATA ON INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 15)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.07
Replication within Year	8	0.74	0.09	
Treatment	11	97.83	8.89	200.01
Year X Treatment	11	0.49	0.04	0.31
Pooled Error	88	12.84	0.15	
Total	1	0.01	0.01	0.07

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.41	0.41	4.08
Replication within Year	8	0.80	0.10	
Treatment	11	59.87	5.44	41.39
Year X Treatment	11	1.45	0.13	1.33
Pooled Error	88	8.69	0.09	
Total	119	71.22		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.15	0.15	1.05
Replication within Year	8	1.16	0.14	
Treatment	11	23.19	2.11	30.11
Year X Treatment	11	0.77	0.07	0.71
Pooled Error	88	8.69	0.09	
Total	119	33.96		

ANNOVA FOR COLONY RECORDS OF *A. mellifera* UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 16)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	68.86	6.26	15.16
Error	48	19.82	0.41	
Total	59	88.68		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	5,658.74	514.43	47.13
Error	48	523.95	10.92	
Total	59	6,182.69		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	440.28	40.02	25.68
Error	48	74.81	1.56	
Total	59	515.09		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	960.97	87.36	12.06
Error	48	347.72	7.244	
Total	59	1,308.69		

ANNOVA FOR COLONY RECORDS OF *A. mellifera* UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 17)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	61.50	5.59	77.08
Error	48	3.48	0.07	
Total	59	64.98		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	4,519.01	410.82	26.88
Error	48	733.66	15.29	
Total	59	5,252.67		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	2,129.11	193.56	24.41
Error	48	380.57	7.93	
Total	59	2,509.68		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	1,764.61	160.42	11.56
Error	48	665.94	13.87	
Total	59	2,430.56		

ANNOVA FOR COLONY RECORDS OF *A. mellifera* UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2020 (TABLE 18)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	94.09	8.55	168.89
Error	48	2.43	0.05	
Total	59	96.52		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	6,071.16	551.92	16.31
Error	48	1,624.71	33.85	
Total	59	7,695.87		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	556.23	50.57	7.32
Error	48	331.71	6.91	
Total	59	887.94		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	1,360.53	123.69	25.70
Error	48	230.98	4.81	
Total	59	1,591.51		

ANNOVA FOR COLONY RECORDS OF *A. mellifera* UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2020 (TABLE 19)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	96.83	8.80	142.59
Error	48	2.96	0.06	
Total	59	99.79		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	3,643.77	331.25	11.45
Error	48	1,388.80	28.93	
Total	59	5,032.57		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	1,423.17	129.38	20.78
Error	48	298.92	6.23	
Total	59	1,722.10		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	1,537.25	139.75	15.62
Error	48	429.41	8.95	
Total	59	1,966.66		

ANNOVA FOR POOLED DATA ON COLONY RECORDS OF *A. mellifera* UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 20)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.10	1.10	1.780
Replication within Year	8	4.91	0.61	
Treatment	11	160.55	14.59	67.23
Year X Treatment	11	2.39	0.22	1.10
Pooled Error	88	17.34	0.19	
Total	119	186.29		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	27.04	27.04	1.82
Replication within Year	8	118.66	14.83	
Treatment	11	11,672.03	1,061.09	201.76
Year X Treatment	11	57.85	5.26	0.23
Pooled Error	88	2,030.00	23.07	
Total	119	13,905.57		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	20.14	20.14	2.73
Replication within Year	8	58.95	7.37	
Treatment	11	983.62	89.42	75.86
Year X Treatment	11	12.97	1.179	0.30
Pooled Error	88	347.42	3.95	
Total	119	1,423.10		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	4.46	4.46	1.11
Replication within Year	8	32.16	4.02	
Treatment	11	2,250.07	204.55	31.42
Year X Treatment	11	71.62	6.51	1.05
Pooled Error	88	546.73	6.21	
Total	119	2,905.04		

ANNOVA FOR POOLED DATA ON COLONY RECORDS OF *A. mellifera* UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 21)

Colony strength

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	3.77	3.77	13.00
Replication within Year	8	2.32	0.29	
Treatment	11	151.79	13.80	23.21
Year X Treatment	11	6.54	0.59	12.69
Pooled Error	88	4.12	0.04	
Total	119	168.55		

Brood area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	116.57	116.57	15.97
Replication within Year	8	58.4	7.3	
Treatment	11	8,034.91	730.45	63.20
Year X Treatment	11	127.13	11.56	0.49
Pooled Error	88	2,064.40	23.46	
Total	119	10,401.41		

Pollen area

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	68.75	68.75	22.54
Replication within Year	8	24.39	3.05	
Treatment	11	3,225.75	293.25	9.89
Year X Treatment	11	326.07	29.64	3.98
Pooled Error	88	655.04	7.44	
Total	119	4,300.00		

Honey stores

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	8.46	8.46	1.74
Replication within Year	8	38.86	4.86	
Treatment	11	3,215.98	292.36	37.55
Year X Treatment	11	85.65	7.79	0.65
Pooled Error	88	1,056.66	12.01	
Total	119	4,405.61		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 22)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	135.10	12.28	14.44
Error	48	40.82	0.85	
Total	59	175.92		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 23)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	171.5	15.59	15.98
Error	48	46.82	0.98	
Total	59	218.32		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2020 (TABLE 24)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	165.33	15.03	24.94
Error	48	28.93	0.60	
Total	59	194.26		

ANNOVA FOR INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2020 (TABLE 25)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	154.30	14.03	11.99
Error	48	56.17	1.17	
Total	59	210.47		

ANNOVA FOR POOLED DATA ON INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER, 2020 (Table 26)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.30	0.30	0.47
Replication within Year	8	5.18	0.65	
Treatment	11	346.07	31.46	93.40
Year X Treatment	11	3.705	0.34	0.69
Pooled Error	88	43.13	0.49	
Total	119	398.39		

ANNOVA FOR POOLED DATA ON INCIDENCE OF EUROPEAN FOULBROOD DISEASE IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 27)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.63	1.63	0.92
Replication within Year	8	14.15	1.77	
Treatment	11	323.31	29.39	127.72
Year X Treatment	11	2.53	0.23	0.23
Pooled Error	88	88.81	1.01	
Total	119	430.42		

ANNOVA FOR INCIDENCE OF SACBROOD DISEASE IN *A. mellifera* UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 28)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	18.53	1.69	8.63
Error	48	9.37	0.20	
Total	59	27.91		

ANNOVA FOR INCIDENCE OF SACBROOD DISEASE IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 29)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	11.99	1.09	3.96
Error	48	13.20	0.28	
Total	59	25.18		

ANNOVA FOR INCIDENCE OF SACBROOD DISEASE IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER 2020 (TABLE 30)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	16.33	1.48	5.67
Error	48	12.56	0.26	
Total	59	28.89		

ANNOVA FOR INCIDENCE OF SACBROOD DISEASE IN *A. MELLIFERA* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2020 (TABLE 31)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	9.72	0.88	2.81
Error	48	15.07	0.31	
Total	59	24.79		

ANNOVA FOR POOLED DATA ON INCIDENCE OF SACBROOD DISEASE IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER 2020 9 (TABLE 32)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.18	0.18	0.96
Replication within Year	8	1.49	0.19	
Treatment	11	34.26	3.12	49.66
Year X Treatment	11	0.69	0.06	0.27
Pooled Error	88	20.49	0.23	
Total	119	57.12		

ANNOVA FOR POOLED DATA ON INCIDENCE OF SACBROOD DISEASE IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 33)

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.05	0.05	0.26
Replication within Year	8	1.65	0.21	
Treatment	11	21.35	1.94	50.12
Year X Treatment	11	0.43	0.04	0.13
Pooled Error	88	26.68	0.30	
Total	119	50.15		

ANNOVA FOR INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 34)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	44.73	4.07	16.31
Error	48	11.97	0.25	
Total	59	56.70		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	27.51	2.50	11.53
Error	48	10.41	0.22	
Total	59	37.92		

ANNOVA FOR INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 35)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	48.08	4.37	14.11
Error	48	14.87	0.31	
Total	59	62.95		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	20.18	1.83	6.57
Error	48	13.40	0.28	
Total	59	33.59		

ANNOVA FOR INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2020 (TABLE 36)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	18.75	1.70	7.38
Error	48	11.09	0.23	
Total	59	29.83		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	18.75	1.70	7.38
Error	48	11.09	0.23	
Total	59	29.83		

ANNOVA FOR INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2020 (TABLE 37)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	37.98	3.45	9.99
Error	48	16.59	0.35	
Total	59	54.57		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	30.22	2.75	15.88
Error	48	8.31	0.17	
Total	59	38.53		

ANNOVA FOR POOLED DATA ON INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 38)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.60	1.60	9.11
Replication within Year	8	1.41	0.18	
Treatment	11	77.89	7.08	28.46
Year X Treatment	11	2.74	0.25	1.43
Pooled Error	88	15.29	0.17	
Total	119	98.93		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.91	0.91	3.02
Replication within Year	8	2.41	0.30	
Treatment	11	44.01	4.00	19.19
Year X Treatment	11	2.29	0.21	0.96
Pooled Error	88	19.13	0.22	
Total	119	68.76		

ANNOVA FOR POOLED DATA ON INCIDENCE OF *T. clareae* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 39)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.06	1.06	3.82
Replication within Year	8	2.21	0.28	
Treatment	11	83.58	7.59	33.60
Year X Treatment	11	2.49	0.23	0.68
Pooled Error	88	29.28	0.33	
Total	119	118.62		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.05	1.05	3.68
Replication within Year	8	2.27	0.28	
Treatment	11	48.16	4.38	20.84
Year X Treatment	11	2.31	0.21	0.95
Pooled Error	88	19.49	0.22	
Total	119	73.27		

ANNOVA FOR INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 40)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	68.07	6.19	29.77
Error	48	9.98	0.21	
Total	59	78.04		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	36.59	3.33	24.18
Error	48	6.60	0.14	
Total	59	43.19		

ANNOVA FOR INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 41)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	62.22	5.66	18.92
Error	48	14.35	0.30	
Total	59	76.58		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	37.80	3.44	28.65
Error	48	5.76	0.12	
Total	59	43.56		

ANNOVA FOR INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2020 (TABLE 42)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	78.71	7.16	30.06
Error	48	11.43	0.24	
Total	59	90.13		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	44.73	4.07	18.60
Error	48	10.49	0.22	
Total	59	55.22		

ANNOVA FOR INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2020 (TABLE 43)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	57.40	5.22	44.61
Error	48	5.62	0.12	
Total	59	63.02		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	43.84	3.99	50.95
Error	48	3.76	0.08	
Total	59	47.59		

ANNOVA FOR POOLED DATA ON INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER STATIONARY (NAUNI, SOLAN) CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 44)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.02
Replication within Year	8	2.18	0.27	
Treatment	11	145.18	13.20	93.42
Year X Treatment	11	1.55	0.14	0.65
Pooled Error	88	19.22	0.22	
Total	119	168.14		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.04	0.04	0.29
Replication within Year	8	1.05	0.13	
Treatment	11	79.34	7.21	39.78
Year X Treatment	11	1.99	0.18	0.99
Pooled Error	88	16.07	0.18	
Total	119	98.49		

ANNOVA FOR POOLED DATA ON INCIDENCE OF *V. destructor* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 45)

Per hundred bees method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.00
Replication within Year	8	2.02	0.25	
Treatment	11	117.56	10.69	58.75
Year X Treatment	11	2.00	0.18	0.89
Pooled Error	88	17.96	0.20	
Total	119	139.54		

Brood infestation method

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.06	0.06	0.78
Replication within Year	8	0.57	0.07	
Treatment	11	80.59	7.33	71.02
Year X Treatment	11	1.13	0.10	1.01
Pooled Error	88	8.95	0.10	
Total	119	91.30		

ANNOVA FOR INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2019 (TABLE 46)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	45.22	4.11	14.77
Error	48	13.36	0.28	
Total	59	58.58		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	28.49	2.59	11.36
Error	48	10.95	0.22	
Total	59	39.44		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	7.98	0.73	7.13
Error	48	4.88	0.10	
Total	59	12.86		

ANNOVA FOR INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY TO DECEMBER, 2019 (TABLE 47)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	31.49	2.86	17.74
Error	48	7.75	0.16	
Total	59	39.24		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	16.12	1.47	6.61
Error	48	10.64	0.22	
Total	59	26.75		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	16.44	1.49	12.94
Error	48	5.55	0.12	
Total	59	21.99		

ANNOVA FOR INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY TO DECEMBER, 2020 (TABLE 48)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	45.31	4.12	12.53
Error	48	15.78	0.33	
Total	59	61.08		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	26.25	2.39	8.85
Error	48	12.94	0.27	
Total	59	39.19		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	9.88	0.89	7.10
Error	48	6.08	0.13	
Total	59	15.96		

ANNOVA FOR INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER STATIONARY (NAUNI, SOLAN) AND MIGRATORY CONDITIONS (HISAR, HARYANA) DURING JANUARY TO DECEMBER, 2020 (TABLE 49)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	33.47	3.04	12.77
Error	48	11.44	0.24	
Total	59	44.90		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	14.33	1.30	7.48
Error	48	8.36	0.17	
Total	59	22.69		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	11	13.60	1.24	8.55
Error	48	6.94	0.15	
Total	59	20.55		
	11	13.60	1.24	

ANNOVA FOR POOLED DATA ON INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER STATIONARY CONDITIONS AT NAUNI DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 50)

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.59	0.59	2.47
Replication within Year	8	1.91	0.24	
Treatment	11	90.00	8.18	149.64
Year X Treatment	11	0.60	0.06	0.18
Pooled Error	88	27.25	0.31	
Total	119	120.35		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.23	0.23	2.54
Replication within Year	8	0.73	0.09	
Treatment	11	54.16	4.92	81.48
Year X Treatment	11	0.67	0.06	0.23
Pooled Error	88	23.20	0.26	
Total	119	78.98		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.02
Replication within Year	8	0.48	0.06	
Treatment	11	17.65	1.60	75.54
Year X Treatment	11	0.23	0.02	0.17
Pooled Error	88	10.50	0.12	
Total	119	28.85		

ANNOVA FOR POOLED DATA ON INCIDENCE OF *G. mellonella* IN *A. mellifera* COLONIES UNDER MIGRATORY (HISAR, HARYANA) AND STATIONARY (NAUNI, SOLAN) CONDITIONS DURING JANUARY, 2019 TO DECEMBER, 2020 (TABLE 51).

Number of larvae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.03	0.03	0.09
Replication within Year	8	2.20	0.28	
Treatment	11	64.68	5.88	184.54
Year X Treatment	11	0.35	0.03	0.17
Pooled Error	88	17.02	0.19	
Total	119	84.27		

Number of pupae

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.10	0.10	0.31
Replication within Year	8	2.58	0.32	
Treatment	11	30.26	2.75	98.46
Year X Treatment	11	0.31	0.03	0.15
Pooled Error	88	16.46	0.19	
Total	119	49.71		

Number of adults

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.07
Replication within Year	8	1.19	0.15	
Treatment	11	29.89	2.72	113.64
Year X Treatment	11	0.26	0.02	0.19
Pooled Error	88	11.36	0.13	
Total	119	42.71		

APPENDIX II

ANNOVA FOR AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. mellifera* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2020 (TABLE 52)

Area damaged seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	440.89	31.49	89.94
Error	45	15.75	0.35	
Total	59	456.65		

Per cent area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	148.20	10.58	91.40
Error	45	5.21	0.11	
Total	59	153.41		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	733.62	52.401	241.017
Error	45	9.784	0.217	
Total	59	743.404		

Per cent area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	253.32	18.09	239.91
Error	45	3.39	0.07	
Total	59	256.71		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	1,026.02	73.28	524.49
Error	45	6.28	0.14	
Total	59	1,032.31		

Per cent area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	358.39	25.59	518.48
Error	45	2.22	0.04	
Total	59	360.61		

ANNOVA FOR AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. mellifera* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2021 (TABLE 53).

Area damaged seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	429.64	30.68	144.79
Error	45	9.53	0.21	
Total	59	439.18		

Per cent area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	145.66	10.40	143.45
Error	45	3.26	0.07	
Total	59	148.92		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	707.12	50.50	201.28
Error	45	11.29	0.25	
Total	59	718.42		

Per cent area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	244.22	17.44	134.10
Error	45	5.85	0.13	
Total	59	250.07		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	970.81	69.34	425.58
Error	45	7.33	0.16	
Total	59	978.14		

Per cent area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	339.99	24.28	319.49
Error	45	3.42	0.07	
Total	59	343.41		

ANNOVA FOR POOLED DATA ON AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. mellifera* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2020-2021 (TABLE 54)

Area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.91	1.91	4.26
Replication within Year	6	2.69	0.44	
Treatment	14	868.40	62.02	374.49
Year X Treatment	14	2.31	0.16	0.61
Pooled Error	84	22.61	0.26	
Total	119	897.94		

Per cent area infestation seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.61	0.61	4.09
Replication within Year	6	0.89	0.15	
Treatment	14	293.15	20.94	393.36
Year X Treatment	14	0.74	0.05	0.58
Pooled Error	84	7.60	0.09	
Total	119	303.02		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.56	0.56	1.45
Replication within Year	6	2.34	0.39	
Treatment	14	1,438.40	102.74	610.22
Year X Treatment	14	2.35	0.16	0.75
Pooled Error	84	18.72	0.22	
Total	119	1,462.40		

Per cent area infestation fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.17	0.17	1.249
Replication within Year	6	0.83	0.13	
Treatment	14	496.78	35.48	618.09
Year X Treatment	14	0.80	0.05	0.57
Pooled Error	84	8.41	0.1	
Total	119	507.00		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	2.12	2.12	17.23
Replication within Year	6	0.73	0.12	
Treatment	14	1,994.05	142.43	771.6
Year X Treatment	14	2.58	0.18	1.20
Pooled Error	84	12.87	0.15	
Total	119	2,012.37		

Per cent area infestation twenty day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.701	0.701	9.447
Replication within Year	6	0.44	0.07	
Treatment	14	697.33	49.81	789.12
Year X Treatment	14	0.88	0.06	1.02
Pooled Error	84	5.19	0.06	
Total	119	704.55		

ANNOVA FOR AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. cerana* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2020 (TABLE 55)

Area damaged seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	400.77	28.62	73.05
Error	45	17.63	0.39	
Total	59	418.40		

Per cent area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	135.24	9.66	73.86
Error	45	5.88	0.13	
Total	59	141.13		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	838.02	59.85	351.82
Error	45	7.65	0.17	
Total	59	845.68		

Per cent area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	291.29	20.80	350.08
Error	45	2.67	0.05	
Total	59	293.97		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	1,022.12	73.01	311.88
Error	45	10.53	0.23	
Total	59	1,032.65		

Per cent area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	357.52	25.53	303.51
Error	45	3.78	0.08	
Total	59	361.30		

ANNOVA FOR AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. mellifera* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2021 (TABLE 56)

Area damaged seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	381.50	27.25	85.59
Error	45	14.32	0.31	
Total	59	395.82		

Per cent area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	128.73	9.19	82.85
Error	45	4.99	0.11	
Total	59	133.72		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	803.53	57.39	304.24
Error	45	8.48	0.18	
Total	59	812.02		

Per cent area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	279.93	19.99	214.34
Error	45	4.19	0.09	
Total	59	284.13		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	982.56	70.18	255.35
Error	45	12.36	0.27	
Total	59	994.93		

Per cent area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	343.40	24.52	254.23
Error	45	4.34	0.09	
Total	59	347.75		

ANNOVA FOR POOLED DATA ON AREA DAMAGED AND PER CENT AREA INFESTATION PER COMB IN *A. cerana* WAX COMBS DUE TO GREATER WAX MOTH (*G. mellonella)* DURING 2020-2021 (TABLE 57)

Area damaged seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.95	0.95	1.21
Replication within Year	6	4.72	0.78	
Treatment	14	777.44	55.53	163.95
Year X Treatment	14	4.74	0.33	1.04
Pooled Error	84	27.13	0.32	
Total	119	815.00		

Per cent area infestation seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.31	0.31	1.19
Replication within Year	6	1.58	0.26	
Treatment	14	262.36	18.74	159.13
Year X Treatment	14	1.64	0.11	1.06
Pooled Error	84	9.28	0.11	
Total	119	275.19		

Area damaged fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.74	0.74	8.55
Replication within Year	6	0.52	0.08	
Treatment	14	1,639.70	117.12	815.84
Year X Treatment	14	2.01	0.14	0.77
Pooled Error	84	15.64	0.18	
Total	119	1,658.63		

Per cent area infestation fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.20	0.20	6.73
Replication within Year	6	0.18	0.03	
Treatment	14	570.44	40.74	909.80
Year X Treatment	14	0.62	0.04	0.56
Pooled Error	84	6.68	0.08	
Total	119	578.13		

Area damaged twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.03
Replication within Year	6	1.437	0.239	
Treatment	14	2,002.49	143.03	917.94
Year X Treatment	14	2.18	0.15	0.60
Pooled Error	84	21.49	0.25	
Total	119	2,027.61		

Per cent area infestation twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.03
Replication within Year	6	0.49	0.08	
Treatment	14	700.14	50.01	911.32
Year X Treatment	14	0.76	0.05	0.60
Pooled Error	84	7.62	0.09	
Total	119	709.04		

ANNOVA FOR EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. mellifera* COMBS UNDER LABORATORY CONDITIONS DURING 2020 (TABLE 58)

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	118.05	8.43	111.07
Error	45	3.416	0.07	
Total	59	121.46		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	225.07	16.07	250.95
Error	45	2.883	0.064	
Total	59	227.96		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	305.09	21.79	384.19
Error	45	2.55	0.057	
Total	59	307.65		

ANNOVA FOR EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. mellifera* COMBS UNDER LABORATORY CONDITIONS DURING 2021 (TABLE 59).

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	100.77	7.19	132.28
Error	45	2.44	0.05	
Total	59	103.22		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	236.28	16.87	269.46
Error	45	2.819	0.063	
Total	59	239.10		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	301.98	21.57	323.92
Error	45	2.99	0.06	
Total	59	304.98		

ANNOVA FOR POOLED DATA ON EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. mellifera* COMBS UNDER LABORATORY CONDITIONS DURING 2020-2021 (TABLE 60)

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.091	0.09	1.94
Rep within Year	6	0.28	0.04	
Treatment	14	218.09	15.57	306.07
Year X Treat	14	0.71	0.05	0.76
Pooled Error	84	5.58	0.06	
Total	119	224.76		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.078	0.07	0.97
Rep within Year	6	0.477	0.08	
Treatment	14	460.63	32.90	622.46
Year X Treat	14	0.74	0.05	0.85
Pooled Error	84	5.20	0.06	
Total	119	467.13		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.001	0.001	0.042
Rep within Year	6	0.212	0.035	
Treatment	14	608.413	43.458	715.252
Year X Treat	14	0.851	0.061	0.96
Pooled Error	84	5.314	0.063	
Total	119	614.791		

ANNOVA FOR EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. cerana* COMBS UNDER LABORATORY CONDITIONS DURING 2020 (TABLE 61)

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	123.30	8.80	91.44
Error	45	4.33	0.09	
Total	59	127.63		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	227.03	16.21	217.53
Error	45	3.35	0.07	
Total	59	230.39		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	317.94	22.71	332.81
Error	45	3.07	0.06	
Total	59	321.01		

ANNOVA FOR EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. cerana* COMBS UNDER LABORATORY CONDITIONS DURING 2021 (TABLE 62)

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	107.22	7.65	223.75
Error	45	1.54	0.03	
Total	59	108.76		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	214.51	15.32	238.48
Error	45	2.89	0.06	
Total	59	217.40		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	291.63	20.83	305.22
Error	45	3.07	0.06	
Total	59	294.70		

ANNOVA FOR POOLED DATA ON EFFECTS OF LARVAL FEEDING ON WEIGHT OF TREATED *A. cerana* COMBS UNDER LABORATORY CONDITIONS DURING 2020-2021 (TABLE 63)

Per cent weight loss of comb seven day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.05	0.05	0.68
Replication within Year	6	0.48	0.08	
Treatment	14	229.42	16.38	200.93
Year X Treatment	14	1.142	0.082	1.26
Pooled Error	84	5.42	0.06	
Total	119	236.52		

Per cent weight loss of comb fourteen day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.39	0.39	3.67
Replication within Year	6	0.63	0.10	
Treatment	14	440.29	31.44	385.52
Year X Treatment	14	1.14	0.08	1.22
Pooled Error	84	5.58	0.06	
Total	119	448.05		

Per cent weight loss of comb twenty one day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.74
Replication within Year	6	0.13	0.02	
Treatment	14	607.26	43.37	260.44
Year X Treatment	14	2.33	0.16	2.32
Pooled Error	84	6.01	0.07	
Total	119	615.76		

ANNOVA FOR EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* COMBS DURING 2020 (TABLE 64)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	301.55	21.53	7.91
Error	45	122.52	2.72	
Total	59	424.07		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	95.03	6.78	2.14
Error	45	142.34	3.16	
Total	59	237.37		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	117.24	8.37	1.99
Error	45	188.48	4.18	
Total	59	305.73		

ANNOVA FOR EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* COMBS DURING 2021 (TABLE 65)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	323.52	23.10	7.19
Error	45	144.45	3.21	
Total	59	467.97		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	345.45	24.67	6.54
Error	45	169.55	3.76	
Total	59	515.00		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	426.32	30.45	8.16
Error	45	167.79	3.72	
Total	59	594.11		

ANNOVA FOR POOLED DATA ON EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. mellifera* COMBS DURING 2020-2021 (TABLE 66)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.32	0.32	0.02
Replication within Year	6	80.57	13.42	
Treatment	14	621.76	44.41	187.88
Year X Treatment	14	3.30	0.23	0.10
Pooled Error	84	186.40	2.21	
Total	119	892.38		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.13	0.13	0.02
Replication within Year	6	35.76	5.96	
Treatment	14	651.87	46.56	57.14
Year X Treatment	14	11.40	0.81	0.26
Pooled Error	84	256.15	3.04	
Total	119	955.33		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	5.50	5.50	1.30
Replication within Year	6	25.26	4.21	
Treatment	14	763.85	54.56	67.47
Year X Treatment	14	11.32	0.80	0.23
Pooled Error	84	285.14	3.39	
Total	119	1,091.09		

ANNOVA FOR EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. cerana* COMBS DURING 2020 (TABLE 67)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	348.24	24.87	7.88
Error	45	141.95	3.15	
Total	59	490.20		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	317.07	22.64	6.47
Error	45	157.43	3.49	
Total	59	474.49		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	223.27	15.94	4.84
Error	45	148.12	3.29	
Total	59	371.40		

ANNOVA FOR EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. cerana* COMBS DURING 2021 (TABLE 68)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	351.76	25.12	12.78
Error	45	88.46	1.96	
Total	59	440.23		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	323.23	23.08	5.408
Error	45	192.10	4.26	
Total	59	515.33		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	14	212.93	15.20	3.61
Error	45	189.20	4.20	
Total	59	402.13		

ANNOVA FOR POOLED DATA ON EFFECTS OF DIFFERENT TREATMENTS ON LARVAL, PUPAL MORTALITY AND ADULT EMERGENCE OF GREATER WAX MOTH (*G. mellonella*) IN *A. cerana* COMBS DURING 2020-2021 (TABLE 69)

Larval mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.07	0.07	0.00
Replication within Year	6	83.93	13.98	
Treatment	14	697.16	49.79	249.90
Year X Treatment	14	2.79	0.19	0.11
Pooled Error	84	146.50	1.74	
Total	119	930.46		

Pupal mortality

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	4.04	4.04	1.53
Replication within Year	6	15.80	2.63	
Treatment	14	605.81	43.27	17.15
Year X Treatment	14	35.30	2.52	0.70
Pooled Error	84	298.87	3.55	
Total	119	959.85		

Adult emergence

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.09	1.09	0.28
Replication within Year	6	23.38	3.89	
Treatment	14	423.18	30.22	32.08
Year X Treatment	14	13.18	0.94	0.25
Pooled Error	84	313.9	3.73	
Total	119	774.74		

ANNOVA FOR EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING 2020 (TABLE 70)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	14.78	1.84	16.90
Error	18	1.96	0.11	
Total	26	16.74		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	2	0.13		
Error	8	19.61	2.45	13.84
Total	16	2.83	0.17	

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	17.97	2.24	33.96
Error	18	1.19	0.06	
Total	26	19.17		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.37		
Error	4	48.18	12.04	93.57
Total	12	1.54	0.12	

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.50		
Error	4	36.63	9.15	39.67
Total	12	2.77	0.23	

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.48		
Error	4	30.59	7.64	134.82
Total	12	0.68	0.05	

ANNOVA FOR EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING 2021 (TABLE 71)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	21.46	2.68	27.17
Error	18	1.77	0.09	
Total	26	23.24		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	25.31	3.16	52.82
Error	18	1.07	0.06	
Total	26	26.39		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	24.13	3.01	32.99
Error	18	1.64	0.09	
Total	26	25.78		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.99		
Error	4	45.99	11.49	61.12
Total	12	2.25	0.18	

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.82		
Error	4	31.50	7.87	63.33
Total	12	1.49	0.12	

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.35		
Error	4	32.34	8.08	69.56
Total	12	1.39	0.11	

ANNOVA FOR POOLED DATA ON EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. cerana* COLONIES DURING 2020-2021 TABLE 72)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.09
Replication within Year	4	0.15	0.03	
Treatment	8	35.75	4.46	70.64
Year X Treatment	8	0.50	0.06	0.56
Pooled Error	32	3.61	0.11	
Total	53	40.02		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.34
Replication within Year	4	0.16	0.04	
Treatment	8	44.08	5.51	52.44
Year X Treatment	8	0.84	0.10	0.86
Pooled Error	32	3.88	0.12	
Total	53	48.98		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0	0	0.00
Replication within Year	4	1.16	0.29	
Treatment	8	41.65	5.20	84.31
Year X Treatment	8	0.49	0.06	1.18
Pooled Error	32	1.66	0.05	
Total	53	44.96		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.00
Replication within Year	6	1.37	0.22	
Treatment	4	93.36	23.34	108.95
Year X Treatment	4	0.85	0.21	1.35
Pooled Error	24	3.78	0.15	
Total	39	99.38		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.00
Replication within Year	6	1.33	0.22	
Treatment	4	66.82	16.70	50.67
Year X Treatment	4	1.31	0.33	1.85
Pooled Error	24	4.26	0.17	
Total	39	73.74		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.01
Replication within Year	6	0.841	0.14	
Treatment	4	62.72	15.68	365.37
Year X Treatment	4	0.17	0.04	0.49
Pooled Error	24	2.08	0.08	
Total	39	65.81		

ANNOVA FOR EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. mellifera* COLONIES DURING 2020 (TABLE 73)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	16.08	2.01	38.38
Error	18	0.94	0.05	
Total	26	17.02		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	18.32	2.29	45.88
Error	18	0.89	0.05	
Total	26	19.22		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	17.4	2.17	76.98
Error	18	0.50	0.02	
Total	26	17.90		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.59		
Error	4	42.38	10.59	71.65
Total	12	1.77	0.14	

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.63		
Error	4	43.86	10.96	172.25
Total	12	0.76	0.06	

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.13		
Error	4	30.35	7.58	76.23
Total	12	1.19	0.10	

ANNOVA FOR EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. mellifera* COLONIES DURING 2021 (TABLE 74)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	16.08	2.01	38.38
Error	18	0.94	0.05	
Total	26	17.02		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	18.32	2.29	45.88
Error	18	0.89	0.05	
Total	26	19.22		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	8	17.4	2.17	76.98
Error	18	0.50	0.02	
Total	26	17.90		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.49		
Error	4	42.22	10.55	34.81
Total	12	3.63	0.30	

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.47		
Error	4	31.00	7.75	56.85
Total	12	1.63	0.13	

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	3	0.09		
Error	4	24.87	6.22	66.03
Total	12	1.13	0.09	

ANNOVA FOR POOLED DATA ON EFFECT OF DIFFERENT MANAGEMENT PRACTICES ON THE INCIDENCE OF GREATER WAX MOTH (*G. mellonella)* IN *A. mellifera* COLONIES DURING 2020-2021 (TABLE 75)

a) Biological, chemical and non-chemical, cultural and integrated management

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.04	0.04	0.60
Replication within Year	4	0.28	0.07	
Treatment	8	32.74	4.09	392.26
Year X Treatment	8	0.08	0.01	0.15
Pooled Error	32	2.10	0.06	
Total	53	35.25		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.24	0.24	1.20
Replication within Year	4	0.80	0.20	
Treatment	8	36.66	4.58	127.73
Year X Treatment	8	0.28	0.03	0.93
Pooled Error	32	1.22	0.03	
Total	53	39.22		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.10	0.10	9.01
Replication within Year	4	0.04	0.01	
Treatment	8	33.03	4.12	168.24
Year X Treatment	8	0.19	0.02	0.53
Pooled Error	32	1.46	0.04	
Total	53	34.84		

b) Mechanical control

One month after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	51.14	51.14	57.72
Replication within Year	6	5.31	0.88	
Treatment	4	47.51	11.87	1.26
Year X Treatment	4	37.62	9.40	9.27
Pooled Error	24	24.33	1.01	
Total	39	165.92		

One month after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.14	0.14	0.77
Replication within Year	6	1.10	0.18	
Treatment	4	73.04	18.26	41.08
Year X Treatment	4	1.77	0.44	4.43
Pooled Error	24	2.40	0.10	
Total	39	78.47		

One month after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	1.23	1.23	31.73
Replication within Year	6	0.23	0.04	
Treatment	4	54.78	13.69	130.33
Year X Treatment	4	0.42	0.10	1.08
Pooled Error	24	2.32	0.09	
Total	39	58.99		

APPENDIX III

ANNOVA FOR EFFECTIVENESS OF PLANT AND FUNGAL EXTRACTS AGAINST SACBROOD VIRUS IN *A. mellifera* COLONIES APPLIED AT AN INTERVAL OF FOUR DAYS DURING 2020 (TABLE 76)

Four days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	16.00	2.66	16.91
Error	21	3.31	0.15	
Total	27	19.31		

Four day after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	32.00	5.33	43.72
Error	21	2.56	0.12	
Total	27	34.56		

Four day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	54.41	9.06	40.49
Error	21	4.70	0.22	
Total	27	59.11		

ANNOVA FOR CUMULATIVE EFFECT OF PLANT AND FUNGAL EXTRACTS APPLIED AT 4 DAYS INTERVAL ON BROOD AREA AGAINST SAC BROOD VIRUS IN *A. mellifera* COLONIES DURING 2020 (TABLE 78)

One day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	1,947.29	324.54	23.97
Error	21	284.30	13.53	
Total	27	2,231.59		

Fifteen day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	2,475.99	412.66	39.88
Error	21	217.28	10.34	
Total	27	2,693.27		

Thirty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	4,175.16	695.86	77.60
Error	21	188.31	8.96	
Total	27	4,363.47		

Sixty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	6,905.26	1,150.88	219.06
Error	21	110.32	5.25	
Total	27	7,015.59		

ANNOVA FOR EFFECTIVENESS OF PLANT AND FUNGAL EXTRACTS AGAINST SACBROOD VIRUS IN *A. mellifera* COLONIES APPLIED AT AN INTERVAL OF FOUR DAYS DURING 2021 (TABLE 79)

Four days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	18.00	3.00	34.74
Error	21	1.81	0.08	
Total	27	19.82		

Four day after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	34.57	5.76	20.53
Error	21	5.89	0.28	
Total	27	40.47		

Four day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	53.40	8.90	48.65
Error	21	3.84	0.18	
Total	27	57.24		

ANNOVA FOR CUMULATIVE EFFECT OF PLANT AND FUNGAL EXTRACTS APPLIED AT 4 DAYS INTERVALON BROOD AREA AGAINST SAC BROOD VIRUS IN *A. mellifera* COLONIES DURING 2021 (TABLE 81)

One day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	1,531.91	255.31	25.74
Error	21	208.29	9.91	
Total	27	1,740.20		

Fifteen day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	2,138.81	356.46	18.95
Error	21	394.87	18.80	
Total	27	2,533.68		

Thirty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	3,328.18	554.69	128.39
Error	21	90.72	4.32	
Total	27	3,418.91		

Sixty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	6	5,995.45	999.24	65.96
Error	21	318.11	15.14	
Total	27	6,313.57		

ANNOVA FOR POOLED DATA ON EFFECTIVENESS OF PLANT AND FUNGAL EXTRACTS AGAINST SACBROOD VIRUS IN *A. mellifera* COLONIES APPLIED AT AN INTERVAL OF FOUR DAYS DURING 2020-2021 (TABLE 82)

Four day after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.01	0.01	0.12
Replication within Year	6	0.76	0.12	
Treatment	6	33.78	5.63	161.10
Year X Treatment	6	0.21	0.03	0.28
Pooled Error	36	4.36	0.12	
Total	55	39.13		

Four day after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.09	0.09	0.28
Replication within Year	6	1.91	0.31	
Treatment	6	65.36	10.89	381.25
Year X Treatment	6	0.17	0.02	0.15
Pooled Error	36	6.55	0.18	
Total	55	74.09		

Four day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.20	0.20	0.58
Replication within Year	6	2.05	0.34	
Treatment	6	107.82	17.97	201.30
Year X Treatment	6	0.53	0.08	0.49
Pooled Error	36	6.46	0.17	
Total	55	117.07		

ANNOVA FOR POOLED DATA ON CUMULATIVE EFFECT OF PLANT AND FUNGAL EXTRACTS APPLIED AT 4 DAYS INTERVAL ON BROOD AREA AGAINST SACBROOD VIRUS IN *A. mellifera* COLONIES DURING 2020-2021 (TABLE 84)

One day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	13.55	13.55	1.90
Replication within Year	6	42.65	7.10	
Treatment	6	3,443.05	573.84	95.56
Year X Treatment	6	36.02	6.00	0.48
Pooled Error	36	450.19	12.50	
Total	55	3,985.48		

Fifteen day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	17.39	17.39	0.57
Replication within Year	6	182.35	30.39	
Treatment	6	4,575.23	762.53	116.67
Year X Treatment	6	39.21	6.53	0.54
Pooled Error	36	429.80	11.93	
Total	55	5,244.00		

Thirty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	63.51	63.51	11.66
Replication within Year	6	32.67	5.44	
Treatment	6	7,241.55	1,206.93	27.64
Year X Treatment	6	261.92	43.65	6.37
Pooled Error	36	246.43	6.84	
Total	55	7,846.10		

Sixty day after third treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	29.71	29.71	2.02
Replication within Year	6	87.90	14.65	
Treatment	6	12,759.09	2,126.52	90.37
Year X Treatment	6	141.18	23.53	2.48
Pooled Error	36	340.70	9.46	
Total	55	13,358.60		

ANNOVA FOR EFFECTIVENESS OF FUNGAL, PLANT EXTRACTS AND BEE PRODUCTS ON EUROPEAN FOULBROOD INFECTED *A. mellifera* COLONIES DURING 2020 (TABLE 85)

Seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	10.73	1.53	13.52
Error	16	1.81	0.11	
Total	23	12.55		

Seven days after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	38.58	5.51	37.10
Error	16	2.37	0.14	
Total	23	40.96		

Brood area one day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	272.97	38.99	6.35
Error	16	98.12	6.13	
Total	23	371.10		

Brood area thirty day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	3,972.35	567.47	111.69
Error	16	81.29	5.08	
Total	23	4,053.64		

ANNOVA FOR EFFECTIVENESS OF FUNGAL, PLANT EXTRACTS AND BEE PRODUCTS ON EUROPEAN FOULBROOD INFECTED *A. mellifera* COLONIES DURING 2021 (TABLE 86)

Seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	17.78	2.54	12.73
Error	16	3.19	0.19	
Total	23	20.97		

Seven days after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	51.52	7.36	46.47
Error	16	2.53	0.15	
Total	23	54.06		

Brood area one day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	743.50	106.21	10.23
Error	16	166.00	10.37	
Total	23	909.50		

Brood area thirty day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Treatment	7	4,263.48	609.06	106.41
Error	16	91.58	5.72	
Total	23	4,355.06		

ANNOVA FOR POOLED DATA ON EFFECTIVENESS OF FUNGAL, PLANT AND BEE PRODUCTS ON *A. mellifera* EUROPEAN FOULBROOD INFECTED COLONIES DURING 2020-2021 (TABLE 87)

Seven days after first treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.07
Replication within Year	4	0.22	0.05	
Treatment	7	27.27	3.89	22.01
Year X Treatment	7	1.23	0.17	1.03
Pooled Error	28	4.79	0.17	
Total	47	33.53		

Seven days after second treatment

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	0.00	0.00	0.01
Replication within Year	4	0.66	0.16	
Treatment	7	89.11	12.73	81.02
Year X Treatment	7	1.10	0.15	1.03
Pooled Error	28	4.23	0.15	
Total	47	95.11		

Brood area one day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	3.39	3.39	0.36
Replication within Year	4	37.25	9.31	
Treatment	7	936.11	133.73	11.60
Year X Treatment	7	80.65	11.52	1.42
Pooled Error	28	226.96	8.10	
Total	47	1,284.38		

Brood area thirty day after two treatments

Source of Variation	df	Sum of Squares	Mean Squares	F-Calculated
Year	1	3.06	3.06	2.61
Replication within Year	4	4.68	1.17	
Treatment	7	8,171.34	1,167.33	125.73
Year X Treatment	7	64.98	9.28	1.54
Pooled Error	28	168.26	6.01	
Total	47	8,412.34		

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Title of Thesis	**:**	**''Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees''**
Name of the Student	**:**	Sapna Devi
Admission Number	**:**	H-2017-06-D
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Major Field	**:**	Entomology
Minor Field	**:**	Plant Pathology
Degree awarded	**:**	Ph.D. Entomology
Year of Award of Degree	**:**	2021
No. of pages in Thesis	**:**	277+li
No. of words in Abstract	**:**	490

ABSTRACT

The present investigations entitled "**Seasonal incidence and management of brood diseases and greater wax moth (*Galleria mellonella* L.) in hive bees**" was conducted during the year 2019-2021 at Department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. European foulbrood disease incidence in *Apis cerana* F. colonies was maximum (17.50%) in the month of July, when temperature, relative humidity and rainfall were high. Thai sacbrood disease was recorded maximum during May (6.90%) when temperature was high and relative humidity and rainfall were low. The incidence of *Tropilaelaps clareae* was maximum in the month of June (7.20%) when temperature was high. In *Apis mellifera* L. colonies the European foulbrood disease incidence was maximum in September (37.10%) under stationary conditions when temperature was high and relative humidity and rainfall were moderate. Under migratory conditions the sacbrood disease incidence was maximum in the month of July (38.30%) when temperature was high, relative humidity was low and rainfall was maximum. Sacbrood disease incidence was maximum in May under both stationary and migratory conditions (6.60% and 5.80%) when the temperature was high and relative humidity and rainfall were moderate. Under stationary and migratory conditions incidence of *V. destructor* and *T. clareae* was observed during summer months when the temperature was high. The incidence of wax moth (larvae, pupae and adults) in *A. cerana* and *A. mellifera* colonies was maximum in the month of July under stationary and migratory conditions (7.47, 6.53) in *A. mellifera* and (8.97) in *A. cerana* colonies when the temperature and rainfall were high and relative humidity was moderate. Management studies of greater wax moth under laboratory conditions revealed that after 21 days of treatment of the combs, no infested area and reduction in weight of the combs (*A. cerana* and *A. mellifera*) was recorded in deep freezing at -8℃ to -10 ℃ followed by *Bt* (3.95%, 3.99%), NSKE, sulphur fumigation and Neem oil treatment. Maximum larval and pupal mortality was also recorded in deep freezing followed by *Bt* and minimum adult emergence was recorded in *Bt* (12.50%). Under field conditions placing wax moth trap and delta trap fitted with *A. dorsata* comb in front of the hives showed that maximum adult moths were trapped in wax moth trap and delta trap with *A. dorsata* comb. Out of three *Bt* concentrations (3, 6 and 9g/l) the highest larval mortality with less comb damage was recorded in *Bt* 9g/l. The IPM practices comprising cultural (periodic cleaning), Mechanical (delta trap installation) and chemical (lime sulphur application) when integrated helped in total protection of both bee colonies with no infestation in the colonies. For the non-chemical treatment of the brood diseases (sacbrood and European foulbrood) in *A. mellifera* and *A. cerana* colonies, different plant and fungal extracts were used and maximum reduction in percentage of diseased larvae in treated colonies over the diseased control were recorded in *Ganoderma lucidum* (3ml/250ml of sugar solution) in sacbrood and European foulbrood infected colonies.

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Signature of Major Advisor **Signature of the student**
(Dr. Kiran Rana)

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