

**SCREENING OF *Apis mellifera* L. COLONIES FOR
Varroa TOLERANCE AND EVALUATION OF
COLONY PERFORMANCE OF SELECTED STOCK**

Thesis

by

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CERTIFICATE - I

This is to certify that the thesis entitled, “**Screening of *Apis mellifera* L. colonies for *Varroa tolerance* and evaluation of colony performance of selected stock**”, submitted in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **ENTOMOLOGY AND APICULTURE** to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a bonafide record of research work carried out by **Mr Ritesh Sharma (H-2005-06-D)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations has been fully acknowledged.

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This is to certify that the thesis entitled, “**Screening of *Apis mellifera* L. colonies for *Varroa tolerance* and evaluation of colony performance of selected stock**”, submitted by **Mr Ritesh Sharma (H-2005-06-D)** to Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY in ENTOMOLOGY AND APICULTURE** has been approved by the Student’s Advisory Committee after an oral examination of the same in collaboration with the external examiner.

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Chapter-1

INTRODUCTION

Honey bees are of great economic importance to mankind. These bees not only provide products like, honey, pollen, bees wax, propolis, bee venom, royal jelly but also play an important role in pollination. The presence of honey, bees wax, brood and favourable environment conditions available inside the hive invite a number of enemies. Some feed on honey, others on wax, brood or/and bees. Protection of honey bees from the enemies is essential to attain maximum yield of various hive products and increase crop productivity.

Among the various enemies, mites constitute one of the major group of parasites on honey bees causing varying degree of damage (De jong *et. al.*, 1982; Naim and Bisht, 1987). The brood mites were little known until man brought *Apis mellifera* for honey production into areas of Asia that previously practised beekeeping with other honey bee species. Unfortunately, *A. mellifera* proved highly susceptible host species to *Varroa* (Acarina: Varroidae). The parasitic mite was discovered in 1904 on eastern honey bees (*Apis cerana*) in Java by Edward Jacobson and further classified and named as *Varroa jacobsoni* by Dr. A.C. Oudemans (Oudemans, 1904). The first infestation of mite on *A. mellifera* occurred in 1970^s in Germany and since then the mite has spread throughout Europe to most of continents in the World (Boecking and Genersch, 2008).

Up to the end of last century the mite infesting both *A. cerana* and *A. mellifera* was thought to belong to only one species i.e. *Varroa jacobsoni* but recent studies made on the genotypic, phenotypic, and reproductive variations showed that *Varroa* forms a complex of 18 different genetic variants belonging to two different species; viz *Varroa jacobsoni* infesting *A. cerana* and *Varroa destructor* infesting *A. mellifera* (Anderson and Trueman, 2000)

The mite *V. destructor* has become a major problem for beekeeping with western honey bees almost world wide (De Jong, 1997). These mites have killed

tens of thousands of honey bee colonies in different parts of the world in recent years. The Korean genotype of *V. destructor* is the most wide spread and common. It is found in *A. mellifera* in U.K., Europe, Middle east, Africa, Asia, Canada, North and South America and New Zealand. The Japan/ Thailand genotype has only been reported from *A. mellifera* from Japan and Thailand. In India *Varroa* was first reported from Himachal Pradesh on *A. mellifera* (Kumar *et. al.*, 1988) yet the virulent haplotype of *V. destructor* causing serious damage to colonies was reported only in 2004 (Gatoria *et. al.*, 2004) in Punjab and later in 2005 in Himachal Pradesh (Rana *et. al.*, 2005). With the attack of the mite on *A. mellifera*, honey yield has declined and the number of both apiary and feral honey bee colonies has declined considerably which resulted in reduced crop pollination and agricultural yields (Boecking and Genersch, 2008). The mites develop and reproduce in the sealed brood cells of honey bees where they feed on the haemolymph of bee pupae. It is an obligate parasite of bees and feeds externally on the immature stages of workers and drones. The parasitized individuals may die or develop into weakened, crippled adults which are incapable of functioning normally. Under high infestation the colony can be lost within one season.

Several methods have been tried to control *Varroa* which include restricting brood rearing and by removing and destroying newly sealed combs (Ruttner *et. al.*, 1980) or by using drone brood as a trap to capture and then destruction of major percentage of mites (Mel'nik and Mauravskaya, 1980; Schulz *et. al.*, 1983; and Oliver, 2007). However, these methods are costly in labour, increase work for bees and often mites succeed in reproducing in the worker cells and the colonies eventually become severely infested despite the control efforts (Rosenkranz and Engels, 1985; Marletto *et. al.*, 1990). Mites can also be controlled chemically by the use of aerosols (Amitraz), fumigants (Formic acid), systemics (Perizin, Apitol) and contact acaricides (Fluvalinate sold as Apistan and Flumethrin sold as Bayvarol strips). However, the use of chemicals is controversial because of the residual effects of chemicals on the bee products (Slabezki *et. al.*, 1991; Liu, 1992; Boecking and Ritter, 1994; Wallner, 1999, 2000, 2004; Bogdanov, 2006) and development of acaricide resistant

strains of *Varroa* (Loglio, 1993; Boecking and Ritter, 1994; Lodesani *et. al.*, 1995; Milani, 1995; Mozes-Koch *et. al.*, 2000; Floris *et. al.*, 2001; Spreafico *et. al.*, 2001).

One possible solution of this crisis in apiculture may be to promote natural tolerance in honey bees to *Varroa* infestation and search for *Varroa* resistant honey bee colonies. Increased resistance to *Varroa* mites has already been observed in Eastern and Africanized honey bees with some degree of resistance in the European honey bees (Moritz and Hanel, 1984; Moritz, 1985; Moritz and Mautz, 1990; Thrybom and Fries, 1991). *Varroa destructor* is recent parasite of Western honey bee *A. mellifera* in terms of co-evaluation with its host. In contrast to the original host *A. cerana*, *A. mellifera* is not well adapted to the mite and infested colony suffers severe damage (Oldroyd, 1999). Never the less, several mechanism to decrease the virulence of *V. destructor* have been well documented in *A. mellifera* (Harbo, 1992, Buchler, 1994; Boecking and Ritter, 1993; Fries *et. al.*, 1994; Calis *et. al.*, 1999). Some of the characters involved in *Varroa* tolerance include; non- reproduction of female mites, hygienic behaviour, grooming behaviour, short post capping duration and larval attractiveness (Buchler, 1994). All these mechanisms affect either the reproduction or causes death of mites or some effectively decrease the infestation within the colonies. These mechanisms have been used to select *Varroa* tolerant honey bee colonies by several workers (Siuda and Wilde, 1995; Spivak, 1996; Wallner, 1997; Spivak and Reuter, 1998, 2001; Szabo, 1998, 1999; Szabo and Szabo, 2000, 2001, 2002, 2003; Harbo and Harris, 2003; Abdullah and Spivak, 2006) with encouraging results. Spivak and Reuter (1998) selected hygienic honey bee colonies by using freeze killed brood assay and found that the hygienic colonies had significantly fewer mites ($0.6 \pm 0.86/100$ bees) than commercial colonies (1.0 ± 1.0) not selected for hygienic behaviour. Szabo and Szabo (2003) by selecting and breeding colonies (based on the lowest number of fallen mites within 24 hours, the best hygienic behaviour and the highest bee population for six years) reduced the number of naturally fallen *Varroa* per colony per day from a mean of 60.7 in 1999 to 2.0 in 2002.

Varroosis is the most destructive disease of honey bees world wide, inflicting much greater damage than the other known parasitic disease caused by the arthropods (Boecking and Genersch, 2008). Disadvantage of using chemical treatment due to contamination of bee products and acaricide resistant in mites have provoked the need to select *Varroa* tolerant colonies from the locally available bee stock. Keeping in view the important role played by the colony traits in sorting out tolerant lines, the present studies were undertaken to evaluate *A. mellifera* stock for mite tolerance. However, selection for any one character may result in loss or reduction in frequency of some other desirable characters (Greenleaf, 1988). Hence, in addition to tolerance to *Varroa*, some other colony parameters like colony population and food stores were also considered while selecting the colonies for mite tolerance. The present studies were, therefore, undertaken with the following objectives:

- i) To estimate the infestation level of *Varroa* in *A. mellifera* colonies in different apiaries and to select the colonies for tolerance against the mite.
- ii) To test selected stock for different performance parameters.

Chapter-2

REVIEW OF LITERATURE

Apiculture is threatened worldwide by various factors, especially diseases and parasites of honey bees. Parasitic mites (*Varroa* and *Acarapis*), foul brood, nosema and others lead to tremendous economic damage (Dustmann, 1993). Among all these factors *Varroa* is widely recognized as the most important problem for apiculture throughout the world (De Jong, 1997; Fries *et al.*, 1994). The beekeeping with *A. mellifera* is endangered worldwide due to *Varroa* (Broadbear, 1988; Matheson, 1996). This mite was originally restricted to its natural host *Apis cerana* in Asia but is now found throughout the world as serious pest of *A. mellifera*.

Varroa jacobsoni was first discovered in 1904 on eastern honey bees in Java by Edward Jacobson and sent for classification and description to famous scientist Dr A.C. Oudemans. Recent studies showed that *Varroa jacobsoni* is a species complex containing 18 different genetic variants that belong to 2 to 5 different species of *Varroa*. Anderson and Truemen (2000) established that the mite present on western honey bee belongs to a larger but closely related species which was named as *Varroa destructor*. The Korean and Japan/Thailand genotype of *Varroa destructor* are fatal for beekeeping with *A. mellifera*, however, the *Varroa* mite does not show notable disturbance in Asiatic hive bee, *Apis cerana*. The host parasite relation of *A. cerana* and *Varroa* seems to be balanced because the bee has higher resistance to the mite (Koeniger, 1987; Peng, *et al.*, 1987a; Rath and Drescher, 1990). Mechanisms which constitute resistance in *A. cerana* towards *Varroa* include the removal behaviour of mite infested worker brood and the resulting dependency of mite on *A. cerana* drone brood for reproduction, seasonal occurrence of drone brood, the restriction of *Varroa* to low parasitisation rates of single drone cell (the drone pupae infested with more than one mite are not able to open the cell capping and both the mite and drone die inside the cell) and the grooming behaviour (Rath, 1991). *A. mellifera* also

removes mite infested pupae from capped brood cells and grooms away the mites but to a limited extent compared to *A. cerena* (Peng *et al.*, 1987b; Boecking and Drescher, 1990, 1991; Boecking, 1992; Boecking and Ritter, 1993)

2.1 MITE MORPHOLOGY AND BIOLOGY

Adult female *Varroa* is large, hard, reddish brown, flattened and also phoretic external parasite of worker and drone bees. The body length and width of female *Varroa destructor* has been reported to be 1167.3 ± 26 and 1708.9 ± 41.2 μm respectively, however, the body length and width of *Varroa jacobsoni* has been reported to be 1063.0 ± 26.4 and 1506.8 ± 36.0 μm , respectively (Anderson and Trueman, 2000). Adult males have a more or less spherical body and are very lightly tanned, with, slightly darker legs. The male chelicerae, instead of being pointed as in the female, are highly modified and have an open hollow tip that is used to transfer sperm packets from the genital opening in the ventrally located sternogenital shield of the male to genital opening of the female (Akrantanakul, 1976). The male feeds on bee pupa at the feeding site prepared by the mother mite (Donze and Guerin, 1994).

The mites feed on the haemolymph of the prepupae, however, the phoretic mites tear the integument of the adult bees with alternate movements of their saw toothed chelicerae, labrum and hypostomum together with the hollow style forming a preoral region (Cromroy and Kloft, 1980). The phoretic mites leave the adults bees to enter the brood cells of last stage worker or drone larvae normally within about 20 or 40 hours, respectively, before the cell is sealed (Boot *et al.*, 1992). About 60 or 70 hours after the brood cell is sealed, the first egg is laid (Ifantidis, 1983; Steiner *et al.*, 1994). Eggs are laid singly, usually on the cell wall, at intervals of about 30 hours (26-32). Eggs are oval and white, about 0.30 mm long and 0.23 mm wide. The first egg is generally male, and the rest female. Male develops from egg to adult in about 5.5 to 6.2 days and female in 6.5 to 6.9 days. Female deposits five or six eggs, of which four (one male and three females) have time to reach maturity, however, when numerous original females invade the same worker brood cell, reproduction is reduced and resorption of developing eggs occurs (Steiner *et al.*, 1995). Eight legged protonymph hatches

within half a day of egg being laid. The protonymph feeds, grows and then moults to a deutonymph stage. The final moult is to the adult stage (Steiner, 1988, Steiner *et al.*, 1995).

The mature female mite mates within the capped cells with the males which die soon afterwards. All immature mites die after the emerging bee opens the cell, while the young adult female mite and the mature (gravid) females move on to the passing bees. When the adult bee emerges from its cell, all surviving adult female mites enter a phoretic phase, during which they hitchhike on adult bees, feeding on their haemolymph until they are ready to enter a brood cell and lay eggs. *V. destructor* prefers younger nurse bees to older foragers (Sammataro *et al.*, 2000). Phoretic periods can last for 4-11 days, the duration of which largely depends upon brood availability (Martin 1998, Sammataro *et al.*, 2000).

Mites enter larval cells when the larvae are large enough to cover the entire cell bottom; past this point, the distance between the larva and the rim of the brood cell decreases (Boot *et al.*, 1992). The mites invade cells based on several different factors, especially olfactory and mechanical cues. Aumeier and Rosenkranz (2001) found stage-specific odour compounds found in larval cuticles including five methyl esters and 14 hydrocarbons (kairomones) such as methyl palmitate, ethyl palmitate, and heneicosane, all of which are thought to be attractive to female mites. Nazzi *et al.* (2001) suggested that semiochemicals in larval food also attract varroa mites prior to cell capping, since ether and acetone extracts of larval food elicited the same response from the mites as did the actual food itself. The rate of cell invasion by mites is affected by the amount of brood present, such that mites enter worker brood at a higher rate when there is more brood present (Boot *et al.*, 1992).

Female mite can go through more than one reproductive cycle. As many as seven cycles, producing up to 30 eggs have been demonstrated by artificial transfer of mites (De Ruijter, 1987). Fries and Rosenkranz (1993) found that 13 per cent of introduced mother mites reproduced at least three times. Adult female *Varroa* preferentially invade drone brood cells. In original host *A. cerana*, nearly all reproduction is accomplished in drone brood cells (De Jong, 1988). Mites have much greater reproductive success in drone brood cells. Fuchs and Langenbach (1989) reported an average of 1.4 new female mites per original

mother mite in worker cells and 2.2 in drone cells. Generally eight to ten times more mites are found per cell in drone cells than in workers cells of European honey bees (Fuchs, 1990; Fries *et al.*, 1994; Boot, 1995), though this ratio varies considerably depending on the ratio between drone and worker brood cells, the season of the year, the race of the bees infested, the type of climate and the origin of mite (Otten and Fuchs, 1988). Mites selectively prefer drone brood because it increases their reproductive output; because drones have a longer post-capping duration than workers. Drone brood enables mites to produce more offspring (Calderone and Kuenen 2001).

The mites being tiny and blind, apparently recognize nurse bees by odour and ride around on them until they smell a drone larva of the right age. Since nurse bees spend much more time feeding drone larvae than worker larvae (Calderone and Kuenen, 2001), the mites have ample time to come in contact with drone larvae. The high attractiveness of drone brood can partly be explained by a 2-3 times longer attraction period of drone brood to mites and by the larger surface of drone cells compared to worker cells (Boot *et al.*, 1995a). Higher reproductive success of the *Varroa* mite in drone cells has probably resulted in a selection for the high preference of mites to invade drone brood. Fuchs (1992) predicted that if the searching time of mites for drone cells is prolonged, mites enter worker cells to minimize a cost of delay in reproduction. Also, high mite infestation levels or a small amount of drone brood results in a high number of mites per drone cell, which leads to a lower number of viable offspring per mother mite. Consequently, a mixed strategy of infesting both drone and worker cells should be preferred by the mite (Fuchs, 1992). Using an olfactometer, Le Conte *et al.* (1989) confirmed that the *Varroa* mite is attracted preferentially to the odour of drones. Further chemical analysis of the kairomone showed that this attraction was caused by the methyl ethyl esters of straight chain fatty acids. Longer emission of the kairomone and stronger kairomonal signals in drone compared to worker larvae possibly explain the preferences of *V. jacobsoni* to drone brood (Trouiller *et al.*, 1992). The mites are also attracted to cuticular hydrocarbons of eight day old worker larvae (Rickli *et al.*, 1994). The distance between the larva and the rim of the cell is also critical in the attraction of *V.*

jacobsoni to the honey bee larvae. Worker larvae in artificially lengthened cells are attractive to mites later in the larval development because the critical distance between larvae and rim of the cell is reached later in the larval development (Goetz and Koeniger, 1993; Boot *et al.*, 1995b).

2.2 POPULATION GROWTH OF VARROA

The population growth and virulence of varroa depend on numerous factors. The most obvious ones are race and strain of the bee (Moritz and Hanel, 1984; Buchler 1994), climate (De jong *et al.*, 1984 ; Moretto *et al.*, 1991) and possibly *Varroa* biotype (Delfinado-Baker and Houck, 1989). In temperate and cold climates, mite population increases during summer and declines in the broodless periods during winter (Fries *et al.*, 1991). The authors demonstrated that the population of *Varroa* in a cold climate can increase more than 100 times during one summer and more than 10 fold per year with a brood rearing period of less than five months and subsequent mite mortality during winter. In contrast, in Mediterranean climates where brood is present through the year, populations continue to grow throughout winter and can increase up to 300 folds during one year (Kraus and Page, 1995). Harris *et al.* (2002) found two factors to be linear predictors of *Varroa* population growth: percentage of reproducing female mites and proportion of total mites in capped brood. Conversely, mortality of mites in cells, growth of the honey bee population, area of capped brood present at the end of the test, and duration of the test were all factors not found to be predictors of population growth. According to Wilkinson and Smith (2002a), the most important factors to mite population growth are the timing of the beginning and end of foraging season and brood rearing. This is because of the associated increased mite mortality and the length of time brood is available (not, however, the amount of brood available). Likewise, according to Calis *et al.* (1999) a longer brood rearing period will drastically increase the mite population, as will increased drone brood area. Although mite population growth is exponential at the peak of brood rearing (Calis *et al.*, 1999), once mite populations reach a high level, their rate of increase declines because mites in multiple infested cells do not reach their reproductive potential (De Grandi -Hoffman and Curry, 2004).

Krauss and Page (1995) in order to find the population growth of varroa in Mediterranean climates of California artificially infested honey bee colonies

(having no mites) with 50 individuals of *Varroa* each and at the end of experiment to estimate the total mites in a colonies, each colony was treated with Apistan for 28 days and number of dead mites fallen were counted. Population growth was studied from April to October in 24 colonies and October to April in 6 colonies. The results obtained revealed that during April to October an intrinsic rate of increase per week was $r_7 = 1.16$ and from October to April an intrinsic rate of increase per week was $r_7 = 1.14$. De Guzman *et al.* (2007) also monitored the population of *Varroa destructor* in Russian and Italian honey bee from 2001 to 2003 and found lower mite growth in Russian than the Italian colonies. In 2001, 2002 and 2003 the growth rates per week (r_7) were 0.191 ± 0.011 , 0.122 ± 0.01 and 0.145 ± 0.009 , respectively, for mites in Italian colonies and 0.137 ± 0.012 , 0.061 ± 0.016 and 0.021 ± 0.011 , respectively, in Russian honey bees.

2.3 INFESTATION LEVEL AND EXTENT OF DAMAGE

The mite has caused severe damage to the European bee colonies in the infested regions in different parts of the world ranging from extensive colony loss to loss of honey production due to weakened colonies (Chun, 1965; Ritter, 1981; De Jong *et al.*, 1982; Choi, 1986). Colonies severely infested with *Varroa* appear restless and weakened, dead larvae, pupae, malformed workers and drones appear at hive entrance. Spotty brood pattern and white droppings are seen on the walls of empty cells and after some time of infestation only a handful of bees remain along with the queen (Ritter *et al.*, 1984). Shimanuki *et al.* (1994) have suggested the terminology “parasite mite syndrome” for colonies infested with *Varroa*. The symptoms are; reduced adult bee population, evacuation of the hive by crawling bees, queen supersedure, spotty brood, sick brood with symptoms resembling European foul brood and sacbrood disease may be present. Young larvae turn light brown in colour, scale resulting from the dead brood are easy to remove and the dead larvae and pupae do not have a ‘ropy’ consistency. Damage to individual bee that hatches from mite infested brood cells includes 6 to 25 per cent weight loss, depending upon the degree of infestation (De Jong *et al.*, 1982) and reduction of 34 to 68 per cent in mean adult life span (De Jong and De Jong, 1983; Schneider, 1986). Weight loss of the developing bee pupa is directly related to the number of female mites that invade a brood cell. It is also

significant even if only one female mite is present (Duay *et al.*, 2003). Feeding activity of the mites during the brood stage causes appreciable losses (15-50 %) in haemolymph protein content and total haemolymph volume of the emerging bees (Engles and Schatton, 1986). The energy and nutritional demand of the parasitic mite *V. destructor* contribute to the malformation and weakening of bees (Gardew *et al.*, 2004). Many of the infested bees hatching in autumn do not survive the winter (Kovac and Crailsheim, 1988).

Infestation level in workers brood increases dramatically in late summer and often reaches an average of more than one mite per brood cell (De Jong *et al.*, 1984; Rosenkranz and Engels, 1985). *Varroa* infestation grows in relation to the amount of brood raising done by the bees. According to Fries *et al.* (1994) using population models it can be shown that with theoretical starting population of 10 mites the population size stays relatively low for the first two years. In the third year, the mite population escalates to 4000 and then in the fourth year population can grow to 10,000 mites. The model developed by Calis *et al.* (1999) predicts that a longer brood rearing period drastically increases the mite population size and that a relatively large number of drone brood cells leads to an increased population growth.

Varroa infestation can be fatal to a honey bee colony preparing to overwinter in temperate climates, since overwintering requires brood production to cease and workers to extend their longevity. By weakening individual worker bees, parasitic mites hinder their ability to live through the winter, thus decreasing the entire colony's chances of surviving until spring (Amdam *et al.*, 2004, Rice *et al.*, 2004). Amdam *et al.* (2004) suggest that parasitism on the developing pupae of workers weakens them and alters their physiological "programming" to the extent that they may not be capable of maintaining the longevity needed for overwintering. Workers that had been parasitized as pupae showed a rapid drop in vitellogenin following emergence. This has very negative implications for winter bees whose vitellogenin levels should remain high throughout the winter, significantly more so than typical summer workers (Fluri *et al.*, 1982). Vitellogenin is required for winter bees to have the extended lifespan necessary for successful overwintering (Amdam *et al.*, 2004), and it is also important in the

production of brood food that will be needed to feed the new work force once the queen resumes egg laying in the spring (Amdam *et al.*, 2003).

The mite *V. destructor* has also been reported to influence the flight duration and homing ability of infested *A. mellifera* foragers. Kralj and Fuchs (2006) found that the infested workers stay outside the colony significantly longer (1.7 times longer) than the uninfested ones. Moreover, it was also found that the loss of foragers per flight was 2.2 times higher in a highly infested colony compared to a less infested colony.

Parasitism by varroa mites has been found to have significant detrimental effect on drones, which can reduce the available population of drones for mating with local queens. Parasitized drones experience increased mortality as compared to their non-infested counterparts (Rinderer *et al.*, 1999, Collins and Pettis 2001). Collins and Pettis (2001) have shown that parasitized drones are more often deformed following pupation, making them immediately unfit because they cannot fly. Of those that were functional upon emergence, one third parasitized drones lived to sexual maturity compared to non-infested drones. And, although they did not go on fewer flights per drone, parasitized individuals died more frequently after reaching flight age. Besides having higher mortality, infested drones have lower sperm viability as well as decreased sperm counts, seminal gland weights, mucous gland weights, and overall body weights (Rinderer *et al.*, 1999, Zoltowska *et al.*, 2007). They also suffer from decreased sugar concentration, especially trehalose and glycogen, indicating increased sugar turnover resulting from parasitism, which could be detrimental (Zoltowska *et al.*, 2007).

Normally, all colonies that do not receive chemical treatment die within two to four years. Continuous influxes of mite from neighbouring colonies and apiaries can result in colony collapse in as little as single season. The feeding activity of the *Varroa* mite also provides a new route of transmission for some bee viruses, earlier found only in apparent form. The capability of *V. destructor* to transmit viruses and activate covert virus infections has been confirmed (Shen *et al.*, 2005a, 2005b) and it is well established that viruses vectored by *V. destructor* play an important role in varroa induced colony collapse as evident from field observations and supported by a modeling approach (Hung *et al.*, 1995, 1996; Martin 1998, 2001). It has been shown that Acute paralysis virus (Ball 1989; Batuev, 1979), Deformed wing virus and Kashmir bee virus can all

be successfully transmitted between honey bee colonies during mite feeding activity (Ball, 1989). Bees from dying *Varroa* infested colonies have been found to be infected with acute bee paralysis virus (Ball, 1983, 1985; Ritter *et al.*, 1984). According to Ball (1985) “Acute paralysis virus” was the primary cause of both adult bee and brood mortality in honey bee colonies severely infested with *Varroa*. Studies on Deformed wing virus (Martin, 2001) have shown that when it is transmitted by *Varroa* to pupae, pupal mortality increases and its subsequent adult longevity is reduced.

2.4 VARROA MONITORING

In a well-formed strategy for controlling any pest, the single most important element is that of monitoring pest populations. Sampling tools are intended to be used by the beekeeper in order to identify the time when mite populations reach their economic threshold, at which time treatment is necessary to reduce mite populations in order to avoid injury to the colony. It is suggested to monitor populations on at least a monthly basis normally and on a bi-weekly basis if mite populations seem to be increasing rapidly (Sammataro *et al.*, 2002). One common method for sampling mites within a colony is to place an adhesive sticky board on the bottom board of a hive to catch all mites falling to the hive bottom over a given period (usually 24, 48, or 72 hours). Sticky boards are thought by some to be more efficient and accurate than other invasive measures that monitor mites from adults, brood, or both (Sammataro *et al.*, 2000, Sammataro *et al.*, 2002, Branco *et al.*, 2006), but they are best used only under certain circumstances. For example, colonies should contain brood, and not be experiencing collapse from varroosis (Branco *et al.*, 2006). Delaplane and Hood (1999) calculated the season economic threshold for varroa mites in the southeastern US. They found that early-season colonies (with smaller adult populations of about 12,000 to 13,500) require treatment if sticky board counts are 1-10 in a 24 hours, but late season colonies (with larger populations of 24,808 - 33,699 bees) require treatment when sticky board counts exceed 59-187 mites in 24 hours. Similarly, Martin (1998) suggests economic thresholds of 250-500 in winter and 20-40 in the summer. Though generally 24-hour mite counts are highly correlated with total mite populations, there are some inconsistencies since colony population size is not the only factor for mite mortality (Ostiguy and Sammataro 2000, Branco *et al.*, 2006). For example, sticky board counts are known to correlate negatively with mite infestations in colonies that express high levels of grooming behavior; mite populations

are typically lower, but only because more mites are being actively removed from adult bees and fall to the hive bottom (Arechavaleta-Velasco and Guzman-Novoa 2001).

Ether rolls are another tool for monitoring varroa mite populations. About 100-200 bees are caught in a jar to which a few milliliter of ether is applied to cover the inner surface. The jar is then rolled to dislodge and count the mites. Similar to ether rolls, powdered sugar rolls, in which 100-200 adult bees are captured in a container, preferably with a solid bottom and finely powdered sugar is dusted over the bees are also used (Aliano and Ellis 2005a). This method, however, is non-lethal to the bees and has proven harmless to brood within the colony except in cases in which large amounts of powdered sugar are applied directly to the cells (Aliano and Ellis 2005b). The mites are removed from the bees when the small particles of powdered sugar interfere with their grip on the exoskeleton. Loose mites are then shaken through a mesh lid to be counted. Alcohol wash is another method for monitoring the *Varroa* population. It utilizes ethanol (usually 70%) added to a sample of adult bees, which is subsequently strained so that mites can be counted (Sammataro *et al.*, 2000). Each of these monitoring techniques provides an estimate of the mite-to-adult-bee ratio, because they allow the beekeeper to know exactly how many mites were present in a given number of bees sampled. According to Vander Dussen (2006) ether, sugar rolls and alcohol washes give good indication of phoretic mite. When brood is present number of phoretic mites, could be 20 to 30 per cent of total mites and total mite population in colony can be estimated using this criteria. However, under broodless conditions these methods give direct ratio of number of phoretic mite in a honey bee colony. Three thousand *Varroa* mites in a colony is considered as economic injury threshold with 2500 mites as maximum before treating (Vander Dussen, 2006).

Combined with measuring the percentage of adult bees infested with mites, examining brood samples can be a useful method of monitoring mite populations as well (Branco *et al.*, 2006). Uncapping pupal cells using a capping scratcher is a common technique used to achieve this measurement (Sammataro *et al.*, 2000).

2.5 CONTROL OF VARROA AND PROBLEMS ASSOCIATED WITH IT

Without treatment to control *V. destructor*, the vast majority of honey bee colonies will succumb to mite infestations within 1-3 years (Nordstrom *et al.*, 1999, Fries *et al.*, 2006). Whatever mite control method is used, it is very important to have *Varroa* populations under control by late summer or early fall. The infestation level at this time has direct implications for the winter survival of colonies, especially due to the

detrimental effects of parasitism on the worker population that will overwinter and be needed to care for brood in the spring (Martin 2001, Amdam *et al.*, 2004, De Grandi-Hoffman and Curry 2004, Amrine and Noel 2006, Fries *et al.*, 2006, Akyol *et al.*, 2007, Todd *et al.*, 2007).

2.5.1 Chemical acaricides

Among various chemicals, pyrethroid fluvalinate (Apistan®) applied as chemically impregnated strips placed between brood frames is used for controlling *Varroa destructor*. Apistan® is a contact poison that acts as an axonic nerve poison against varroa mites (Stanghellini and Raybold 2004). Pyrethroids show more rapid and higher toxicity to mite than organophosphates (Elzen *et al.*, 2000). The current formulation of this acaricide, however, is very toxic to honey bees as well (Frazier *et al.*, 2008). Fluvalinate has been shown to have sublethal effects on drones, such as decreased longevity, seminal gland weight, mucus gland weight, sperm count, and body weight. Thus, queen breeders are advised to treat for mites just prior to drone rearing to prevent increased drone mortality (Rinderer *et al.*, 1999). It is suggested that fluvalinate be used in rotation with an acaricide from a different chemical class to avoid mites resistance to this pesticide (Elzen *et al.*, 1998).

Coumaphos (Checkmite®) is an organophosphate acaricide. It is also applied in the form of chemically impregnated strips hung between brood frames. Treatments with this acaricide should be done in spring and fall as needed, but applications should be avoided during the nectar flow (Sammataro *et al.*, 2000).

2.5.1.1 Mite resistance to pesticides

Mite in many geographic locations have become resistant to fluvalinate (Elzen *et al.*, 1998, Rice *et al.*, 2004, Sammataro *et al.*, 2005, Frazier *et al.*, 2008). Resistance of mites to fluvalinate is an unstable trait, and as such Apistan® can be reintroduced only after waiting sufficiently long after previous use (Elzen *et al.*, 2000). Resistance may be due in part to the persistent residual presence of the chemical in wax combs; such exposure to sublethal amounts of acaricide can encourage resistance to it (Korta *et al.*, 2001). Spreafico *et al.* (2001) was the first to document *Varroa* resistance to coumaphos (Checkmite®) at label-recommended doses, and increasing resistance has been shown subsequently, even in colonies not treated with coumaphos (Elzen and Westervelt 2002, Rice *et al.*, 2004, Sammataro *et al.*, 2005). As is the case with fluvalinate, residues in wax combs encourage development of resistance to the chemical, though it has not been

found to leave behind chemical residues (Korta *et al.*, 2001), resistance to the formamidine, Amitraz[®] has been reported (Elzen *et al.*, 2000, Sammataro *et al.*, 2005).

2.5.1.2 Acaricidal residues

Coumaphos is lipophylic and accumulates in wax over time and has a half-life of 115 days in that medium (Thrasylvoulou and Pappas 1988, Kochansky *et al.*, 2001, Korta *et al.*, 2001, Martel *et al.*, 2007). Following application, coumaphos residues are four to fifteen times higher in wax than honey samples, and it is found throughout the colony in both brood and honey combs (Martel *et al.*, 2007). The pesticide has been shown to persist in honey for 30 days (Martel *et al.*, 2007), but Korta *et al.* (2001) have shown it to be stable in honey for over 9 months following application. Its levels in honey, though, decrease drastically after 3 months of storage, and after that have been shown to be safe for human consumption (Thrasylvoulou and Pappas 1988). It can persist in wax for over a year following treatment (Martel *et al.*, 2007). Coumaphos remains present in beeswax during industrial recycling processes, even after heating (Martel *et al.*, 2007). In a recent study, 100 per cent of wax samples analyzed for chemical residues contained coumaphos (Frazier *et al.*, 2008).

Like coumaphos, fluvalinate is also lipophylic and persists in wax throughout and following industrial recycling, even if it is boiled at higher temperatures or for longer period (Bogdanov *et al.*, 1998, Korta *et al.*, 2001, Martel *et al.*, 2007). In fact, in a recent study by Frazier *et al.* (2008), every sample of wax taken from 88 colonies in the US contained fluvalinate residues. Levels of up to 204 ppm have been found in brood wax and pollen (Frazier *et al.*, 2008). It is stable in honey for over 9 months after treatment (Korta *et al.*, 2001). Fluvalinate residues have been found in brood combs after only a single treatment, and residues were 5-10 times higher in the brood comb than in honey combs (Bogdanov *et al.*, 1998). Residues increase with increasing duration of treatment and persist at higher levels in new wax than in old (Bogdanov *et al.*, 1998).

2.5.2 Plant-derived and non-synthetic acaricides

Thymol (an essential oil component) is consistently been used in apiculture with good results against the mite (Imdorf *et al.*, 1999; Floris *et al.*, 2004). In fact, thymol has been shown to produce 90-100% mite mortality, without any relevant side effects to colony health (Imdorf *et al.*, 1999, Baggio *et al.*, 2004). However, Floris *et al.* (2004) reported significant reduction in brood area during treatment with thymol products, suggesting that the product should not be used during typical times of colony growth.

Calderone *et al.* (1997) found that thymol products used as fumigants were much less effective when applied in the presence of brood. This is another reason to wait for brood production to be low before applying this treatment. Api Life VAR[®] is a varroacide that has thymol as its active ingredient and also contains menthol, eucalyptol, and camphor (other essential oils) in smaller amounts. Api Life VAR[®] tablets are placed upon brood frames and its oils are passively evaporated into the colony to kill varroa mites (Imdorf *et al.*, 1999). Because it relies on passive evaporation, its effectiveness is variable depending on factors such as external temperature (20-25° C is optimum) and colony condition (strong colonies distribute the substance better than weak ones; Baggio *et al.*, 2004; Floris *et al.*, 2004). It is useful as an element of an IPM strategy, especially when applied in late fall, but probably is not effective as a stand-alone treatment (Imdorf *et al.*, 1999; Pettis and Shimanuki, 1999; Rice *et al.*, 2004; Stanghellini and Raybold, 2004). Api Life VAR[®] leaves very low residues behind in honey, even after long-term treatment, however, it should not be applied during the nectar flow to avoid affecting the taste of honey (Imdorf *et al.*, 1999). Apiguard[®] is another thymol-based product that is available in gel form and has proven effective against varroa mites. This formulation has been observed to release more thymol into the colony than Api Life VAR[®] during the first week of treatment. Apiguard has also shown variable mite control depending on temperature and other biological factors (Floris *et al.*, 2004).

Oxalic acid, a non-synthetic pesticide consumed by honey bees, is a protoplasmic poison active against *Varroa* that acidifies the bees' hemolymph (Stanghellini and Raybold 2004) and is known by many to have good mite control when the colony is broodless, but not when brood is present (Gregorc and Planinc, 2001; Stanghellini and Raybold; 2004). There have been no side effects concerning colony health from the use of oxalic acid, and it has been shown to leave behind no residues in beeswax and propolis because of its hydrophilic properties (Gregorc and Planinc 2001, Sammataro *et al.*, 2008).

Sucrose octanoate (Sucrocide[®]) is a non-synthetic acaricide deriving from the tobacco plant, shows good mite control, is safe to use, and can be used throughout the season (Stanghellini and Raybold 2004). However, Sammataro *et al.* (2008) found that Sucrocide[®] lacks ability to control varroa mites over a long period of time.

2.5.3 Biological control

Entomopathogenic fungi have proven to be prime candidates for biological control of *V. destructor* in honey bee colonies even though the environment within a colony is not one typically suitable for fungal growth, which generally requires high humidity for sporulation and moderate temperatures of about 15-27° C (Chandler *et al.*, 2001; Shaw *et al.*, 2002). However, there are many fungal strains and certain isolates have been shown to be capable of growth at the low humidity in the hive (up to 70% relative humidity), but often in the range of 40-50% RH) and high summer temperatures found within the colony that are often at 32-37° C (Chandler *et al.*, 2001; Shaw *et al.*, 2002).

The fungal pathogen, *Metarhizium anisopliae* has shown significant success in controlling mites when applied as both a dust and on strips coated with conidia (Shaw *et al.*, 2002; Kanga *et al.*, 2003; Kanga *et al.*, 2006). Strip application of *M. anisopliae* was most effective in reducing mite population (Kanga *et al.*, 2003). Numbers of mites sealed in brood cells of colonies treated with *M. anisopliae* were comparable to those of colonies treated with Apistan[®], and effects were seen 42 days after treatment (Kanga *et al.*, 2003, 2006).

Hirsutella thompsonii is a fungus that has been shown to be fatally infectious to varroa mites under laboratory conditions, and shows some promise as a biological control tool (Peng *et al.*, 2002, Shaw *et al.*, 2002). Also, it is capable of growth at the high temperatures found within the bee colony (Chandler *et al.*, 2001). Treatment with *Beauveria bassiana* conidial isolates collected from *V. destructor* have been shown to be fatal to the mite (Shaw *et al.*, 2002) and lead to significant knockdown, peaking between 5-8 days after treatment, in honey bee colonies when applied in a wax powder mix (Meikle *et al.*, 2007, 2008). However, such treatment is only effective to those mites exposed to the fungus at the time of application; those that emerge from sealed brood cells later will remain untreated (Meikle *et al.*, 2008).

2.5.4 Mechanical control

2.5.4.1 Screened bottom board

Screen bottom boards allow mites to fall out of the colony when they fall to the bottom of the hive, whereas with regular bottom boards, they may be able to ride back on another passing worker. These bottom boards have been shown to exhibit some level of

mite control, sometimes significantly (Pettis and Shimanuki 1999, Delaplane *et al.*, 2005, Coffey 2007). As an additional benefit, colonies with screen bottom boards have been shown to have significantly more sealed brood than those with normal bottom boards (Pettis and Shimanuki 1999). They can have variable results and should not be used as the only measure of mite control, but screen bottom boards may be a good addition to an integrated management system, especially considering the limited cost and effort required (Pettis and Shimanuki, 1999; Rice *et al.*, 2004; Coffey 2007).

2.5.4.2 Drone brood manipulation

One form of cultural control for *V. destructor* is drone-brood removal (Boot *et al.*, 1995a; Wilkinson and Smith, 2002b; Charriere *et al.*, 2003; Calderone, 2005). This technique takes advantage of the mite's natural preference for parasitizing drone brood. Mites selectively prefer drone brood because it increases their reproductive output as drones have a longer post-capping duration than workers. Drone brood enables mites to produce more offspring (Calderone and Kuenen, 2001). When practicing drone-brood removal, beekeepers provide entire frames of drone comb allowing queens to concentrate their drone-laying to isolated frames. Mite infested frames are later removed from the hive after the cells are capped. This allows the frames to act as a sink for the mite population, minimizing the loss of valuable worker brood. Most of the previous forms of this cultural practice involve the drone frames being destroyed or frozen, killing all mites present as well as drones (Huang 2001; Calderone, 2005). Drone-brood removal has achieved low mite levels through summer and early fall without the use of synthetic acaricides, and it is a form of control to which mites will not become resistant (Boot *et al.*, 1995a; Wilkinson and Smith, 2002b). The drawback to method of drone trapping currently practiced to control varroa mites is the negative impact on the drone population. Though the absence of drones is not harmful to individual colonies (Seeley, 2002), they are essential to colony reproduction. A healthy breeding population is vital to honey bee population health, and having low drone numbers or drones of poor quality could have negative effects on queen mating (Sammataro *et al.*, 2000; Coffey 2007, Kraus *et al.*, 2007). Hoopingartner (2001) also raised a potential objection to trapping with drone brood that it exerts constant selection against the mites that prefer drone brood which in long term is not in interest of *Varroa* reduction programme.

2.5.4.3 Sugar dusting

On a worldwide basis, there are numerous materials that have been used to control *Varroa* mite (Shimanuki *et al.*, 1992). In case of chemical control measures, extreme efforts are needed to prevent chemical contamination of honey, wax and other bee products (Nowotnick, 1988). In addition, the chemical control may have deleterious effect on unsealed brood (Choi, 1985) and may cause high bee mortality (Henderson, 1988). Mites often develop resistance against these chemicals within a few year of usage (Baxter *et al.*, 1998; Pettis *et al.*, 1998). Hence, there has been a shift towards reducing or minimizing the chemical use in bee colonies by focusing on Integrated Pest Management (IPM) strategies for varroa management. This includes monitoring varroa population using cultural, mechanical and biological prevention methods and delaying chemical control until *Varroa* population reaches the economic threshold (Delaplane *et al.*, 2005). Use of non toxic dust has been one of the chemical free method tried to control *Varroa* mite. A finely powdered glucose (Ramirez, 1987) or wheat flour has been used for dusting to control (Shah and Shah, 1988; Loglio and Pinessi, 1991, 1992) or to detect the *Varroa* mite. Loglio (1996) also used wheat flour to collect healthy *Varroa* mites.

It is hypothesized that varroa like other mites, has tarsal pads which allow it to adhere to the substrate. The dust on the bee adheres to the pads of *Varroa* mite and this prevents the mite from attaching to the bee surface. It loses its grip and falls on the floor of the hive where it dies by starvation (Ramirez, 1987; Ramirez, 1994). According to Fakhimzadeh (2000) sugar dust adheres to the mite ambulacra (foot pads) apparently causing the mite to loosen its grip on the bee. Dusting may in addition stimulate bee grooming behaviour (Macedo *et al.*, 2002). As an alternative to flour dusting with finely ground sugar has been investigated as a possible *Varroa* control method by some of the workers. (Fakhimzadeh, 2001a, 2001b; Macedo *et al.*, 2002; Aliano and Ellis, 2005a, 2005b). Fakhimzadeh (2000) dusted honey bee colonies with powdered sugar with the help of blower apparatus. The colonies were dusted at the rate dependent upon on colony size and maximum of 15 gram of sugar powder per colony was dusted with the help of blowing apparatus. The mites were collected by placing light

coloured strong paper under the colony which was protected from bees by a wooden wired frame (8 to 12 mesh per inch) as large as the hive floor. The frame was placed on the top of the paper, leaving approximately 0.6 cm space between the wired frame and paper. Three groups of colonies were dusted at interval of 3 (Group A), 7 (Group B) and 14 (Group C) days. From the results obtained it was found that mite fall after 24 hour was significantly higher in dusted colonies of all the groups than the untreated colonies. Moreover the mite fall was 47 to 56 fold greater after sugar dusting in the treated colonies. Fakhimzadeh (2001b) found that direct heavy dusting resulted in greater mite drop than light blow dusting and found no sugar particles were found in the bees' trachea. It was also found that dusting at frequent intervals did not appear to effect brood production, colony strength, queen survivorship or honey production. Macedo *et al.* (2002) used powdered sugar dust to detect and assess the *Varroa* infestation in honey bee colonies. They collected approximately 300 adult honey bees in a screened jar and dusted them with sugar powder. After one minute of dusting the jar was inverted to collect the mites. Using this method they achieved 92.9 ± 5.5 per cent mite fall after dusting 318 ± 7 adult bees and also reported that dusting caused no injury to bees or mites.

Aliano and Ellis (2005a) described a new technique to remove *Varroa* from adult honey bees using powdered sugar. The adult bee population of a colony was isolated in a detachable box (screened with mesh from two sides) prior to the application of sugar dust. The box was designed in such a manner that it was connected to the colony entrance. By applying a bee repellent, Bee GO[®], to a colony, the adult bees were forced into the box where they were dusted with 225 gram of powdered sugar. The boxes were then gently bounced over the white paper to recover mites. After five to ten minutes the bees were released near the colony entrance. To know the effectiveness of dusting, approximately 300 bees were collected in alcohol to remove the mites from bees so as to know the number of mites on bees before and after treatment of the colonies. The results showed that the honey bee populations treated in this manner dropped 76.7 ± 3.6 per cent of their mites. The average number of mites collected from each colony after dusting was 841 ± 94.6 . In the control colonies, the pre- treatment alcohol

samples had 0.06 ± 0.02 fewer mites per bee than samples taken two days post treatment. In contrast, in the treated colonies the pre treatment alcohol samples had 0.05 ± 0.02 more mites per bee than samples taken two days after treatment. Considering the alcohol samples taken two days post treatment the control colonies had 0.09 ± 0.05 more mites per bee than the treated colonies. Oliver (2007) described an easier method of dusting honey bees in the colony. He used moving screen over the frames and spread powdered sugar on the screen. Approximately 100 gram of sugar was dusted for one story colony. Bee brush was used to sift the sugar through screen. After this, the screen was lifted and the brush was used to sift the sugar fallen on top bars into the bee ways. According to him initial dusting once a week for several weeks knocks mite levels way back, dusting twice a month keeps the mite at low levels and dusting monthly (or even less frequently) keeps the mites at tolerable levels. According to model described by him monthly dusting keeps the mite population below moderate thresholds of 3000 mites and bimonthly dusting keeps them below 1000 mites.

Alliano and Ellis (2005b) in order to find the effect of sugar dusting on eggs and larvae, applied measured amount of powdered sugar directly to honey bee brood containing egg and larvae of known age group. Low and high doses of powdered sugar (0.3 and 0.6 gram per 151.5 \pm 1.0 cells, respectively) caused significant egg removal of 62.2 \pm 5.1 and 86.1 \pm 5.1 per cent, respectively, when compared to eggs that received no powdered sugar. However, the powdered sugar had no effect on five day old (days after oviposition) honey bee larvae but caused significant percentage (18 \pm 4.5%) of eight day larvae (days after oviposition) to be removed when applied at high dose. From the results they concluded that only large amounts of powdered sugar applied directly to brood cells harms immature honey bees.

Ellis *et al.* (2009) in order to find out the efficacy of powdered sugar as *Varroa* control tool, compared the mite population, adult bee population and brood area in untreated colonies with those colonies which were dusted every two weeks for 11 months with 120 gram powdered sugar per application. From the results obtained it was found that there was no significant difference in

population (10061.7 ± 629.4), brood area ($4521.9 \pm 342.8 \text{ cm}^2$), number of mites per colony (2112.2 ± 224.6), number of mites per sealed brood cell (0.112 ± 0.013) and number of mites per adult bee (0.080 ± 0.010) in the colonies treated with powdered sugar and those colonies which were not treated. Adult bee population, brood area (cm^2), number of mites per colony, per sealed brood cells and per adult bee were 10691.0 ± 554 , 4472.55 ± 365.85 , 2197.80 ± 207.75 , 0.106 ± 0.018 and 0.097 ± 0.010 , respectively. When the data were analyzed by sampling month, the treatment had significant effect on total mites per colony in May, mites per brood cell in September and February and mites per adult bee in February. In May the total number of mites per colony was significantly higher in control colonies and in September, the number of mites per brood cell was significantly higher in powdered sugar colonies. In February the number of mites per brood cell and mites per adult bee was significantly higher in control colonies. From the results obtained it was concluded that the dusting colonies with powdered sugar did not significantly affect mite population or the colony strength. Oliver (2008, 2009a, 2009b) also in order to find out the efficacy of this method, dusted his colonies with sugar dust and found that dusting caused only 34 per cent of total phoretic mites present on the adult bees to drop after one hour of the treatment. He also found that out of the total mites fallen, about 50 per cent fall within the first 10 minutes of sugar dusting and 80 per cent in the first hour. He compared 24 hour natural mite fall with mites collected after alcohol wash of 300 bees (method used for determining mite infection level). He found no correlation between the two, however, good correlation was observed between the 24 hour dust drop and alcohol wash.

There are some drawbacks of sugar dusting. First its application depends upon fair weather. It may also draw ants and dusting during a nectar dearth may initiate robbing (Oliver, 2007). Moreover Ellis *et al.* (2009) found that dusting sugar does not cause significant varroa control and they attributed that this may be due to the reproduction rates of varroa mites under differing population pressure. The increase in varroa population despite sugar dusting may be due to the reason that mite may be able to compensate for population loss due to dusting by increasing its reproductive rates. It was thought to be a possible reason by

Ellis *et al.* (2009) because in a study Eguaras *et al.* (1994) found that the reproductive rate of varroa increases at lower mite densities.

2.6 SELECTION OF VARROA TOLERANT/ RESISTANT BEES

Acaracide resistant strains, contamination of hive products and limitation of chemical control have led scientists to explore the possibility of selecting *Varroa* resistant strains. Some studies have indicated the natural defense mechanism in *A. mellifera* (Peng *et al.*, 1987b; Buchler *et al.*, 1992; Ruttner and Hanel, 1992) which can be incorporated in selection programme to produce *Varroa* resistant strains. According to Buchler (1994) several characteristics which are involved in *Varroa* tolerance of honey bees are hygienic behaviour, grooming behaviour, non reproduction of female mites, post capping duration and larval attractiveness. According to Liebig (1997), six factors that have been used for selection of varroa tolerant colonies are percentage of damaged mites in natural mite fall, cleaning out by workers of dead brood, the ratio of mites on adult bees and in brood, development time of sealed brood, percentage of sterile female mites and mite fall after autumn treatment.

2.6.1 Hygienic behaviour

The first detailed observations on hygienic behavior of honey bee, *A. mellifera*, were recorded in 1930's during efforts to determine resistance to American foul brood (AFB). Park (1936, 1937) presented comb sections containing the remains of larvae killed by AFB. Out of 31 colonies, 7 colonies did not show any symptoms and these were those colonies that removed the entire inoculation comb and then rebuilt it with new wax. These colonies were considered resistant and later on used to determine whether the resistance was heritable. For this purpose they raised queens from one of the resistant colonies and allowed them to mate with drones from other resistant colonies in an isolated mating yard. From the result it was found that the bees raised were also resistant to the foulbrood and thus they concluded that the resistance was heritable. Later on hygienic behaviour was studied by many workers and was considered primary natural defense against AFB (Woodrow and Holst, 1942; Rothenbuhler, 1964) and chalk brood (Gillam *et al.*, 1983). Hygienic bees have the ability to detect,

uncap and remove diseased brood from the rest before the causative organism reaches the sporulating stage.

Rothenbuhler (1964) postulated that hygienic behavior is controlled by two independent assorting recessive genes. Rapid hygienic behavior occurs at relatively low frequency in most honey bee populations so far studied (Spivak and Gilliam, 1993).

Due to inherent problems in inoculating colonies with AFB spores, different types of methods were developed to screen colonies for hygienic behavior. Newton *et al.* (1975) were the first to use a freeze-killed brood assay to survey colonies in USA for hygienic behavior. In their study, an entire frame of sealed brood was frozen for 24-72 hours at -17°C and then placed in the test colonies. The time required by the bee colonies to remove the frozen brood was recorded. Taber (1982) and Taber and Gilliam (1987) modified the assay by presenting the bees with frozen brood in 5X6 cm comb section containing approximately 100 larvae and pupae per side and then recording the time required for colonies to detect, uncap and remove the brood. The brood was frozen at -20°C for 24 hours. The colonies that removed all of the freeze killed brood within 48 hours were considered hygienic.

There are several difficulties inherent in the freeze-killed brood. First the amount of freeze killed brood removed within 48 hours by a particular colony may vary between assays (Oldroyd, 1996; Rodrigues *et al.*, 1996) and secondly environmental conditions and the age of frozen brood can influence the test results. (Message and Goncalves, 1980; Momot and Rothenbuhler, 1971; Thompson, 1964). Another assay for hygienic behavior was developed by Newton and Ostasiewski (1986). The assay involved inserting a fine insect pin through larvae or pupae covered by wax capping and then recording the time required for colonies to remove the pierced brood. The assays is not as labour intensive as the freeze killed brood and does not cause as much damage to comb. They found a significant co-relation between the freeze killed and the pierced brood assays ($r = 0.956$). Similarly, Spivak and Downey (1998) also reported a significant co-relation between the two assays. If the pierced brood assay is used

to screen colonies it is recommended that the colonies should be inspected within 24 hours of the treatment.

Testing colonies of honey bees for hygienic behavior may also serve to predict their reaction to *Varroa* infestation (Spivak *et al.*, 1994). The removal of mite-infested worker brood or hygienic behaviour is one of the defense mechanism of *A. cerana* towards *Varroa* (Peng *et al.*, 1987a; Peng *et al.*, 1987b; Rath and Drescher, 1990). The defense mechanism of the Asian honey bees against the mite is also present in European honey bees up to some extent. *A. mellifera* also removes mite infested pupae from capped brood cells but to a limited extent as compared to *A. cerana* (Bar and Rosenkranz, 1992; Boecking, 1992; Boecking and Drescher, 1990). Mite removal behavior could limit the growth of *Varroa* population in colonies in three ways. First immature mites which have begun to develop in brood cells are killed, decreasing the average number of off springs per mother mite, secondly removal of female mites extends the phoretic period of those mites which survive the removal process and thirdly, the mite removal increases the mortality of mother mites within the brood (Fries *et al.*, 1994). Hygienic behavior of worker honey bees is determined largely by two behavioral components, the uncapping and removal of dead brood. Few colonies (10% or less) in nature demonstrate hygienic behavior (Oldroyd, 1996; Spivak and Gilliam, 1991; Spivak and Gilliam, 1993). These colonies can be readily selected for the behavior using field assays (Spivak and Gilliam, 1998) or by direct inoculation with mites (Boecking and Drescher, 1990; Spivak, 1996).

Boecking *et al.* (1992) investigated the removal behavior of *A. mellifera*. After 10 days the mean removal rate of brood artificially infested with *Varroa* was 19.9 per cent (0.0-32.0%) and in those infested with *Tropilaelaps* it was 49.9 per cent (28.0-61.5%) and to brood cells infested with both mites was 66.7 per cent (65.5-75%). However, in case of naturally infested cells high percentage (mean 58.6% \pm SD 22.3) of the estimated amount of mite-infested brood cells were removed. Boecking and Drescher (1992) experimentally introduced *Varroa* mites into *A. mellifera carnica* colonies in Germany and found that the mean rate of removal by the Carniolan bees on day 10 after infestation was 29.3 per cent

when one mite per cell was introduced but 55.1 per cent when two mites per cell were introduced. Positive co-relation ($r = 0.74$) was found between the removal rates of mite infested brood and the removal of freeze killed brood. They suggested that freeze killed brood assay could facilitate the selection of colonies that would remove brood infested with *Varroa*.

The removal behavior of *A. mellifera intermissa* against *Varroa* was investigated in Tunisia to know the behavioral strategies in *Varroa* tolerant colonies of honey bees. In 15 test colonies, mean removal rate towards brood cells infested with one mite per cell was 38 (15 - 75%) and 37.5 per cent (5 - 75%) in each of the two trials. Mean removal rate of freeze killed brood was 45.9 (14.4 - 96.6%) and 57.6 per cent (22.2 - 98.6%), respectively, in first and second trial (Boecking and Ritter, 1993). On the basis of freeze killed brood assay, Spivak (1996) selected and bred hygienic and non-hygienic colonies. The colonies that removed the freeze killed brood within 48 hours in two consecutive trials were considered hygienic, colonies that took longer than one week to remove the dead brood in both trials were considered non-hygienic. The results revealed that the hygienic colonies (bred from hygienic colonies previously selected) removed significantly more pupae ($69.2 \pm 16.41\%$) infested with mite than the non-hygienic colonies ($10.0 \pm 10.0\%$).

Thakur *et al.* (1997) using infra red photography found that in total 30 and 26.6 per cent artificially infested cells were uncapped and removed in two experiments. The co-relation between uncapping and removal and grooming behavior was found to be $r=0.37$, $P=0.001$; $r=0.03$, $P=0.5146$ and $r=0.01$, $P=0.8179$, respectively. Large significant differences between colonies with respect to uncapping, removing and grooming were found in three colonies in two experiments. Significantly positive co-relation between uncapping and removal of *Varroa* infested cells indicated that both these traits are controlled partly genetically. Thakur *et al.* (1999) showed genetic improvement with regard to uncapping and removal of artificially *Varroa* infested brood since a higher percentage of bees from F1 were found to be involved in uncapping and removal, compared to the control. Spivak and Reuter (1998) compared the colonies with

naturally mated queen from hygienic line of Italian honey bees to colonies from a commercial line of Italian bees not selected for hygienic behavior. The results revealed that naturally mated hygienic colonies removed significantly more (82.9 ± 10.49) freeze killed brood than the commercial lines (58.9 ± 21.5) and hygienic colonies also had significantly less mite ($0.6 \pm 0.8/100$ bees) than the commercial colonies ($1.0 \pm 1.0/100$ bees). Honey bee colonies bred for hygienic behavior were later on tested by Spivak and Reuter (2001) to determine if these were able to resist the parasitic mite *Varroa* better than unselected colonies of Starline stock. The results indicated that the hygienic colonies with queen mated naturally to unselected drones had significantly fewer mites on adult bees and within worker brood cells than the Starline colonies.

Guerra *et al.* (2000) showed that Africanized honey bees are more efficient at removing worker brood artificially infested with the parasitic mite *Varroa* than Italian bees and Italian/Africanized hybrids. The Africanized honey bees removed significantly more (51%) artificially infested brood than Italian/Africanized hybrids (25%). Africanized colonies (59%) also removed a significantly greater proportion of infested brood than Italian colonies (31%). The capacity of Africanized honey bees to detect and react to brood infested with mite was thought to be possible factor determining this tolerance. Similar mechanism was thought to be possible factor determining *Varroa* tolerance in Africanized honey bees in other studies also (De Jong, 1997; Correa-Marques and De Jong, 1998). In order to test the co-relation between “freeze killed” brood assay and “pin killed” brood assay, a comparison of removal rate of seven pin killed cells (at 3 different location in a comb) with twenty five frozen capped brood cells was made in 28 colonies. The co-relation co-efficient for uncapping was $r = 0.534$ ($P < 0.003$) and for brood removal $r = 0.572$ ($P < 0.0001$). The two methods gave very similar results and thus any of the methods can be used to screen colonies for hygienic behavior (Szabo, 1998).

In order to select colonies for *Varroa* tolerance Szabo (1999) selected colonies showing lowest number of fallen mites per 24 hours, highest bee population development and the best hygienic behavior. Szabo and Szabo (2000)

later on, modified the pin kill method from 7 cell kill section to one 25 cell kill block. One point was counted for each cell uncapped and a separate point counted for each cell emptied. The most hygienic colonies uncapped all cells and removed the cell contents within 24 hours (receiving a perfect score of 50). The method was used by them in six years of their breeding programme to evaluate colonies for better hygienic behavior (Szabo, 1998, 1999; Szabo and Szabo, 2000, 2001, 2002, 2003). The results revealed that hygienic behavior of bees increased from 57 per cent in 1999 to 90 per cent in 2002 and the number of naturally fallen *Varroa* per colony per day was also reduced from 60.7 in 1999 to 2.0 in 2002, by constant selection for 6 years.

2.6.2 Grooming behaviour

Feral and domesticated honey bee colonies have evolved elaborate defense mechanism to protect both themselves and their food from pathogen and parasite invasion. The defense mechanism of an individual bee serves to minimize the threat for the whole colony (Boecking and Spivak, 1999). Constitutional defense mechanism such as chitinous cuticle which serves as a barrier between internal and external environment, and the intestinal microflora of the bee gut, can protect each individual bee against infectious diseases (Dustmann, 1993; Glinski and Jarosz, 1995). Cellular defense mechanisms (haemocytes) and humoral reactions (enzyme and antimicrobial factors) can contribute to resistance towards infections (Casteels *et al.*, 1985, Jacobs *et al.*, 1990; Mitro, 1994). The proventricular valve enables the bees to filter ingested spores which serves as a mechanism of physiological resistance to disease (Dustmann, 1993). Likewise, the behavioral defense (like grooming and hygienic behaviour) provides resistance to honey bees (*A. mellifera*) against ectoparasitic mite *V. destructor* (Anderson and Trueman, 2000).

Grooming behaviour was first described by Peng *et al.* (1987a) in *A. cerana* as a resistance mechanism to *Varroa*. In grooming, adult bees detect and remove phoretic mites from themselves (autogrooming) or from nest mates (allogrooming). Peng *et al.* (1987a) suggested that the grooming behaviour of the Asian honey bee is one reason for the low infestation level of *A. cerana* colonies

as the bees catch and kill *Varroa* with their mandibles. In an experiment *Varroa* mite from *A. mellifera* were collected and inoculated with adult *A. cerana* bee in observation hives. The Asian bees removed 99.6 per cent of the mites from their bodies within 15 minutes and about 73.8 per cent of those mites that were dropped had injuries. The findings of Peng *et al.* (1987a) concerning the extensive grooming ability of *A. cerana* provoked conclusion concerning the potential for grooming of *A. mellifera*. Buchler *et al.* (1992) investigated the grooming success of *A. cerana*, using one colony in an observation hive. They observed that within 5 minutes, 75 per cent of the inoculated mites were either removed by auto and allogrooming of the bees. The grooming behaviour towards mites exists to a lower degree in *A. mellifera* compared to *A. cerana* (Peng *et al.*, 1987b; Wallner, 1990; Rath, 1991; Moretto *et al.*, 1991) but despite of lower degree of grooming behaviour, *A. mellifera* colonies showing higher level of grooming behaviour can be selected and can be incorporated in breeding programme to produce *Varroa* tolerant bees. In the process of grooming, the legs of the mite may be cut off or the cuticle of the idiosoma may be damaged by the bees' mandibles, causing the damaged mite to fall to the bottom of the colony (Ruttner and Hanel, 1992; Stanimirovic *et al.*, 2003).

Using infra red photographs Thakur *et al.* (1997) studied the defense mechanism in *A. mellifera carnica* and found that bees detect mites moving on brood frames and developing larvae. They catch the mite tightly in their mandibles with the help of their forelegs. The bees chew and bite the mites vigorously before dropping and the infested bees perform the self cleaning with the help of their fore legs and hind legs, removing mites from their body. Grooming behaviour of honey bees can be measured by collecting and examining the mites fallen on the board with microscope to detect with missing legs, dents on their body etc. and then determining the percentage of mite damaged by bees (Sammataro, 1996). The colony with more damaged mites is considered more tolerant. Ruttner and Hanel (1992) examined natural mortality of mites in five *A. mellifera* colonies in Austria and found that on an average 26 per cent of the mites collected showed injuries to the legs but rarely to the cuticle of the idiosoma. To investigate the grooming behaviour of *A. mellifera intermissa*

against *Varroa*, Boecking and Ritter (1993) inserted (25x30cm) protected plastic sheets on the bottom board (45x40cm) of 15 honey bee colonies from 0900 to 1800 h. The mites falling on the sheets were collected and classified as living or dead. All mites were carefully examined for injuries under dissecting microscope at 40X magnification. On an average 56.6 per cent of mites were found alive and 43.4 per cent were found dead. The percentage of mites that were injured ranged between 0 to 39.9 in different colonies, however, on an average 19.3 per cent of mites were damaged. The damage to the mites included separated segments to total loss of all legs. Similarly, grooming behaviour was detected by Flores *et al.* (1998) in *A. mellifera iberica* sub species of honey bees. It was observed that 0.05 per cent of living mites and 50.85 per cent of dead mites were injured. The damage was present in legs and body.

Lodesani *et al.* (1996) investigated, kinds of damage found in *Varroa* located in worker brood, on adult honey bees and on the bottom board and found that 10.2 per cent of the mites entering the cells to reproduce had dorsal shield damage. The same damage was observed in 5.2 per cent of the offspring, apparently when inside the brood cell. On the mites collected from adult bees, only dorsal shield damage was detected (7.2% damaged mites) and on an average 24.2 per cent of mites on the bottom board were damaged. For all damaged mites those with the leg damage were found in proportionally greater number on the bottom boards.

Wallner, (1997) suggested that resistant colonies could be selected by determining the percentage of mites found on the bottom board that have damaged marks indicating that bees had attacked the mites. He called this percentage of damaged mites as “Killer Factor” but Liebig (1997) questioned the usefulness of selection based on the percentage of damaged mites. One serious potential flaw is that the mite bodies can dry and break spontaneously, without interference by the bees or become injured or killed inside honey bee brood cells (Lobb and Martin, 1997). Even wax moth (*Galleria mellonella*) larvae have been found to damage *Varroa* (Szabo and Walker, 1995). In a study conducted by Bienefeld *et al.* (1999) damaged mites increased when wax moth larvae were

present in the colony and the mites were left on floorboard for more than two days. Possibly a classification of the various types of damage into categories would provide more objective parameters for selection, as some may be more indicative of active defense (against mite) by the bees. Particular kind of damage could be co-related with resistant capabilities of the colonies.

Several researchers have reported and quantified damaged mites found in honey bee colonies, however, the types of damage found were generally not categorized. Ruttner and Hanel (1992) found that 30-50 per cent of fallen mites had damaged legs, especially the first pair of legs. Rosenkranz *et al.* (1997) reported that 25 per cent of damaged mites lost part or all of one leg, 75 per cent had more than one leg damaged and dorsal shield injuries were rare. Lodesani *et al.* (1996) found three types of damage, depression in the dorsal shield, mutilated legs and multiple damages. Correa-Marques *et al.* (2000) classified and quantified the fallen mites collected in 24 hours interval according to the type of damage found. The damaged mites were reported into six categories (1) damaged legs (2) depression or hollow in the dorsal shield (3) carcass or empty dorsal shield (mite that lacks legs and all or nearly all of the ventral shield) (4) damaged shield + damaged legs (5) hollow in the dorsal shield+ damaged legs and (6) damaged shield. Among the 1201 adult female mites collected 36.9 per cent were found damaged. The most frequent category of damage was damaged legs (58%) followed by the depression in the dorsal shield (15.6%). Out of the total mites fallen on the bottom board 79.3 per cent were also found dead and 67.8 per cent of them were mature, dark coloured mites. There was no co-relation between the percentage of mites found damaged and the brood or adult bee infestation levels. However, a significantly greater proportion of damaged young (lightly pigmented) adult female mites among those collected in the hive debris was found in the colonies with lower brood infestation rates. There was also relatively high negative co-relation between the percentage of brood cell infested and the proportion of mites with damaged legs.

Correa-Marques *et al.* (2002) looked for possible cause of *Varroa* tolerance in the Italian honey bees kept in an isolated island in Brazil. Colonies

were kept there without treatment since 1984. The colonies were considered *Varroa* tolerant and six honey bee colonies were prepared from the queen from Brazilian island population. The colonies were compared from the Carniolan colonies, both tested at the same time in Germany. It was found that 35.8 and 42.3 per cent of mites were damaged in Italian and Carniolan bees, respectively, which was significantly at par. The most frequent damage was damaged legs (47.4%) in Italian bees which was similar to that found in Carniolan colonies (46%). However, in study of Africanized bees (considered *Varroa* tolerant) about 72 per cent of mites had damaged legs (Correa-Marques *et al.*, 2000). Thus on the basis of results obtained it was concluded that percentage of damaged mites does not appear to be good indicator of *Varroa* tolerance levels, since similar percentages of damage (36-42%) was found as compared to Africanized bees (37%) kept in Brazil. However, difference in the rates of specific type of damage to the mites, such as damaged legs may be indicative of tolerance capabilities by the bees. Similar categorization and quantification of kind of damage was done by Stanimirovic *et al.* (2005) who observed 36.05 to 39.61 per cent damaged mites in potent honey bee colonies and the most frequent type of damage was damaged leg that was found to 53.38 per cent in potent colonies with one year old queen and 52.02 per cent in colonies with two-year old queen.

2.6.3 Post capping period

In general development stages of worker honey bee include egg stage of 3 days, uncapped brood of 5-6 days and capped brood stage of about 12 days. However, developmental stages show variation in different honey bee colonies. Harbo *et al.* (1981) showed that eggs of Africanized bees hatched four hours sooner than eggs of European bees. Similarly variations have also been found in the capped brood stage which is also termed as 'Post capping period' (PCP). Moritz (1985) found that the mean duration of PCP of cape bee (*A. mellifera capensis*) was 2 day shorter than the European sub-species (*A. mellifera carnica*). Similarly, Schousboe (1986) observed difference of 1.15 days in the duration of capped stage of worker brood in *A. mellifera ligustica* colonies. Later on Le Conte and Cornuet (1989) confirmed significant difference in duration of capped stage for the European races. As reproduction of *Varroa* occurs inside the capped

brood, PCP is of special interest (Harbo, 1992). A female mite enters a cell shortly before it is capped and cannot leave until the cell is uncapped. A mite requires 24 hours in a capped cell to produce one mature female and additional females are produced at 30 hours intervals (Rehm and Ritter, 1989). During one reproduction cycle the mite can lay up to 6 eggs in intervals of 30 hours on worker brood (Ifantidis, 1983). Males and immature female mites die when the cell is uncapped by adult bee that emerges from the cell. Thus a shorter capped period for the bee would provide less time for *Varroa* to reproduce and may provide the bee colony with some resistance to mite (Harbo, 1992). Moritz and Hanel (1984) observed more mites with adult off spring in *A. mellifera carnica* compared to *A. mellifera capensis* which has a capped stage approximately 2 days shorter. Limitation in the population increases of *Varroa* in *A. mellifera capensis* colonies was confirmed by the investigation of Moritz and Mautz (1990)

Buchler and Drescher (1990) described a time saving method for determining the duration of the capped stage for large number of colonies. To determine the period of the sealed brood, one comb with many larvae shortly before cell capping was placed in the upper brood chamber. At an interval of 12 hours during capping and 8 hours during the emerging period of young bees, the selected comb was photographed. By comparing subsequent photographs, the time of capping and emerging was determined. The results revealed up to 9 hour differences between strains and up to 19 hour within individual colonies. The average period was 7 hour shorter in late spring than in late summer. The correlation between the capped period and the susceptibility of colonies to mite was calculated as $r = 0.48$ and heritability was estimated to be $h^2 = 0.232$. It was indicated that with one hour reduction in the capped period of honey bees, there is a corresponding 8.7 per cent decline in *Varroa* population. By testing small honey bee colonies in a flight room Moritz (1985) estimated the heritability of the length of the capped stage ($h^2=0.8$). However, Buchler and Drescher (1990) estimated the heritability of PCP for European honey bees under field condition to be $h^2=0.23$.

In another study workers from 26 different colonies averaged 114.5 ± 4.3 hours for the uncapped larval period and 285 ± 5.1 hour for the capped period. Heritability \pm SEM was 0.41 ± 0.15 for uncapped and 0.61 ± 0.19 for the capped period. It was predicted that selective breeding from 10 per cent of the population should reduce the mean capped period of workers by 5 hours in a single generation (Harbo, 1992). Harbo (1993) tested previously selected honey bee stocks for short and long PCP. Twenty colonies were established from bees that had been collected into a large cage. Initial population consisted of 8578 ± 111 bees and 148 varroa mites in each colony. Each queen for the experiment was inseminated with semen from two drones from a different colony than the queen but of the same stock type. The results revealed that after 60 days, bee population was 7326 ± 1521 and mite population of 942 ± 387 per colony. When evaluating mite population on adult bees only, duration of capped brood had a significant effect in the group selected for short duration of capped brood but not within the group selected for long duration of capped brood. There was a significant relationship between duration of capped brood and mite population and there were only 41 fewer mites for each hour that the capped period was shortened.

In order to find the influence of shorter post-capping period on the rate of *Varroa* reproduction, Siuda and Wilde (1995) bred honey bee queens that produced workers emerging in less than 276 hours. The control group contained workers produced by queens from the same breeding lines and emerging in less than 288 hours. The difference between the *Varroa* mites found after the incubation of the brood on the bees with short post capping period (SPCP) was (64.9 mites) significantly different from bees with long post capping period (LPCP; 72.2 mites). The calculated growth of *Varroa* mite population per generation was lower in the SPCP group (1.75) than in LPCP group (1.98), which was highly significant.

Harbo and Hoopingarner (1997) evaluated honey bee colonies for hygienic behaviour, grooming behavior, the duration of post capping period and the frequency of non-reproducing mites in brood cells. Out of 4 characters only non reproduction of mites was highly related and duration of post capping was

marginally related to change in the mite population. The duration of post-capping in different colonies ranged between 267 to 286 hours.

2.7 BREEDING SUCCESS FOR VARROA RESISTANCE.

Some studies have indicated the natural defense mechanism in *A. mellifera* (Buchler *et al.*, 1992; Peng *et al.*, 1987a; Ruttner and Hanel, 1992) which can be incorporated in selection programme to produce *Varroa* resistant strains. According to Buchler (1994) several characteristics which are involved in *Varroa* tolerance of honey bees are hygienic behaviour, grooming behaviour, non-reproduction of females mites, post capping duration and larval attractiveness. These characteristics can be utilized for selecting honey bee colonies resistant to *Varroa*. Kulinčević *et al.* (1992) conducted five year experimental selection of honey bees for resistance and susceptibility to *Varroa*. Two lines of honey bees were selectively bred through four generations. The evaluation of the parental colonies was based on the presence of reproducing mites during the active bee keeping seasons. The resulting lines of bees obtained were comparatively resistant and susceptible. Siuda and Wilde (1995) used post capping period (PCP) in various races and hybrids of honey bees for selecting bees for *Varroa* tolerance. By selecting queens with a short PCP, the average PCP in worker was reduced from 284.2 hours in second generation in *A. mellifera carnica* to 279.4 in the fifth generation. Spivak (1996) selected and bred hygienic and non-hygienic colonies on the basis of freeze killed brood assay. The results obtained revealed that the hygienic colonies removed significantly more pupae infested with one mite per cell than the non hygienic colonies.

The honey bee colonies, which were thought to have potential for *Varroa* resistance, were assembled by Harbo and Hoopingarner (1997). The colonies were subjectively chosen for grooming behaviour, hygienic behaviour, a short post capping period or for maintaining low mite populations. Queens and drones were propagated randomly from this group to produce 43 instrumentally inseminated queens, each mated to only one drone. At the end of the experiment three of the colonies had fewer mites at the end of test than at beginning. From the different character tested in experimental colonies, non-reproduction of mites

was highly related to change in mite population. The duration of the post capping was marginally related, however, grooming behaviour and hygienic behaviour were apparently unrelated. The studies revealed that non-reproduction of mites may be a valuable characteristic for selecting bees resistant to *Varroa*. Wallner (1997) based his selection on the Varroa killer factor (VKF) of a colony; this is the percentage of damaged mite due to bee grooming. The colonies with high VKF values were selected and bred. In a six year selection programme the average VKF increased from 27 to 77 per cent and total mite fall doubled.

The first study to evaluate hygienic stock in large field colonies with naturally mated queens was made by Spivak and Reuter (1998). Colonies with naturally mated queen from a hygienic line of *A. mellifera ligustica* were compared with colonies not selected for hygienic behaviour (commercial colonies). The hygienic colonies were selected by using freeze killed brood assay. The results obtained revealed that hygienic colonies had significantly fewer mites ($0.6 \pm 0.86/100$ adult bees) than the commercial colonies (1.0 ± 1.0) not selected for hygienic behaviour. Moreover the hygienic colonies produced significantly more honey (40 ± 16.45 kg) compared to commercial colonies (30 ± 14.49 kg). The hygienic colonies also removed significantly more freeze killed brood and had significantly less chalk brood than the commercial colonies. The results indicated that it is possible to select hygienic behaviour without compromising honey production.

Szabo (1998) selected honey bee breeder queens based on the lowest number of fallen mites within 24 hours, the best hygienic behaviour and the highest bee population. Same criteria were used for selecting the best queens in subsequent generation for rearing the new generation of queens. By selecting and breeding colonies for six years the number of naturally fallen *Varroa* per colony per day was reduced from a mean of 60.7 in 1999 to 2.0 in 2002. The percentage of fallen alive *Varroa* increased from 13 to upto 40 per cent suggesting that the colonies activity expelled *Varroa*. The hygienic behaviour of bees also increased from a mean of 57 per cent in 1999 to 90 percent in 2002. (Szabo 1999; Szabo and Szabo, 2000, 2001, 2002)

Abdullah and Spivak (2006) compared the mechanism, of resistance to *V. destructor* displayed by bees bred for suppression of mite reproduction (SMR) and hygienic behaviour (HYG). The results indicated that the SMR colonies also express hygienic behaviour as in their experiment the SMR colonies removed significantly more mite infested pupae ($84.5\% \pm 7.4$) than the HYG colonies ($61.7\% \pm 7.3$). Ibrahim *et al.* (2007) selectively bred colonies for both hygienic behaviour and suppression of mite reproduction (HYG/SMR) and compared them with colonies solely bred for hygienic behaviour (HYG) and unselected control colonies. The results revealed that in September the HYG/SMR colonies had significantly fewer mites on adult bees and in worker brood compared to the control colonies. The colonies were kept in apiaries at Minnesota (MN) and North Dakota (ND). The results revealed that in the month of September during two years, the HYG/SMR colonies had significantly fewer mites on adult bees and in worker brood compared to the control colonies. The HYG/SMR and HYG colonies had also significantly fewer mites in worker brood compared to the control colonies. The hygienic colonies had intermediate levels of mite infestation. The proportion of colonies within each lines that expressed hygienic behaviour (i.e. removed > 95% of the freeze killed brood within 24 hours) was higher in HYG/SMR colonies (64 and 94% in 2003 and 2004, respectively) and HYG colonies (47 and 75%) compared to the control colonies (12 and 23%). The results of hygienic behaviour indicated that the genes and neural mechanism for the SMR trait may be the same as for hygienic behaviour. The results also revealed that there was no significant difference in honey production, indicating that selection for SMR/HYG trait does not affect honey production of colony.

The heritability for tolerance to varroa is 0.29, and this must be taken into account in selective breeding programmes (Boigenzahn, 1999). According to McCann and Otis (2003) a closed population breeding programme, initiated with a diverse population of partially resistance colonies, provides the best approach to selecting males for characters that increases resistance of colonies to mite.

Chapter-3

MATERIALS AND METHODS

The present investigations on “Screening of *Apis mellifera* L. colonies for *Varroa* tolerance and evaluation of colony performance of selected stock” were undertaken in different apiaries. Detailed observations were made in an apiary at Nauni (Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan) situated at 32° 52’ north latitude, 77°11’ east longitude and 1270 m above mean sea level. The experiments were carried out during 2007 – 2009. The methodology adopted for these experiments is described in this chapter.

3.1 CRITERIA USED FOR SCREENING OF COLONIES AT NAUNI, SOLAN:

The different criteria used for screening of bee colonies, located in the University are described below:

3.1.1 Colony parameters

The observations on various colony parameters were made at 21 day interval during different periods of colony development. The data were recorded for three to four brood cycles during build up (February to March), honey flow (April to mid June), dearth (July to September) and winter period (October to December). These observations were started on 12 bee colonies headed by one year old queens (queens mated during last breeding season) and later 8 more colonies were added during honey flow period. These colonies were not given any chemical treatment for the control of *Varroa* mites. The susceptible colonies naturally perished due to severe attack of mites. Therefore, later observations revealed data of surviving colonies only.

3.1.1.1 Population

Number of frames covered by bees was recorded to determine the strength of a colony. To know the approximate number of bees in a colony, the number of frames covered by bees in each colony was multiplied by a factor of 1574, as one Langstroth frame of honey bees, contains about 1574 bees (Sharma, 2005).

3.1.1.2 Brood area and pollen stores

Brood area and pollen stores in the experimental colonies were measured in cm² with the help of a scale. The data were recorded for all the periods described above.

3.1.1.3 Honey stores:

The honey stores of the colonies were estimated visually on the assumption that one Langstroth frame sealed with honey weighed 2 kg.

3.1.2 Varroa mite infestation of honey bee colonies

3.1.2.1 Natural mite fall

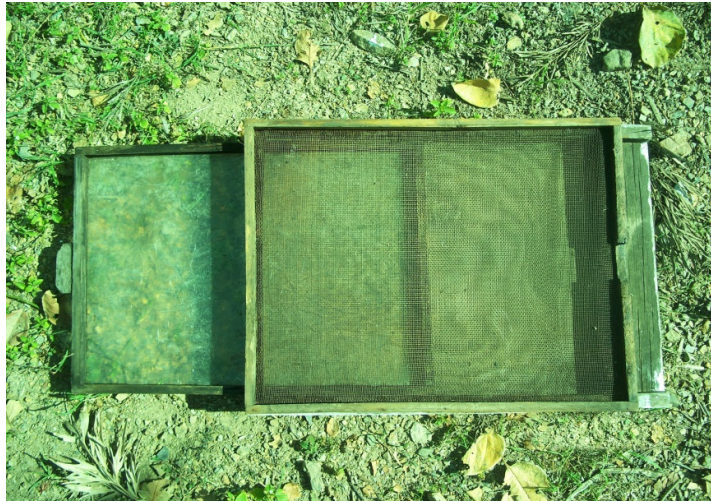
For recording 24 hour natural mite fall, bottom boards of the experimental colonies were modified. For this purpose on the back side of the bottom board, bar was removed and a movable tray of the dimension of bottom board was inserted (Photograph 1). This tray had translucent fibre sheet boarded by wooden frame (having dimension of 48.5 (length) x 36.0 (breadth) x 1.8 cm (height)). This movable tray was covered with a wire mesh screen (having mesh size of 3 mm). Movable tray opened from the back of the hive. This also avoided disturbing normal working of the colony during observation period. For observing natural mite fall, a white filter paper sheet was placed, on the movable tray beneath the screened mesh. After 24 hours the number of mites falling on the movable tray were counted. Natural mite fall was recorded at 21 days interval during colony build up, honey flow, dearth and winter period.

3.1.2.2 Determination of phoretic mite population by sugar dusting

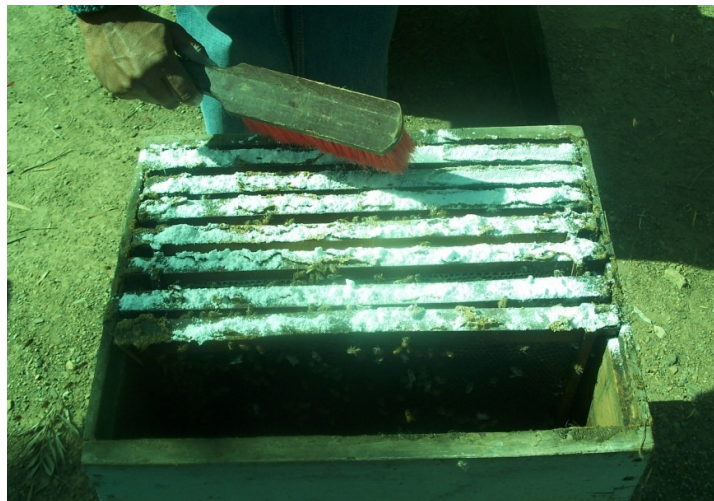
For determining phoretic mite population in the experimental colonies, finely powdered sugar was dusted over the frames of honey bee colonies @ 15 gram/frame. The sugar was kept over the top bar of every frame and this sugar was gently dusted with the help of bee brush (Photograph 2). Mites falling after dusting got collected in the movable tray of screened bottom board and were counted after 24 hours (Photograph 3). Sugar dusting was done in all the colonies one day after taking 24 hour natural mite fall count. The data were recorded during build up period, honey flow, dearth and winter periods, by repeating three times during each period.

3.1.2.3 Mites per 100 bees

Random sample of about 100 bees from the brood nest was drawn from each colony in a half kg wide mouthed glass bottle. Finely powdered sugar (5 g) was dusted



Photograph 1. Screened bottom board with movable tray



Photograph 2. Dusting colony with finely powdered sugar



Photograph 3. Sugar dust fallen on the bottom board along with mites

over these bees to motivate them for grooming and bees were slightly agitated. After 4-5 minutes when bees had groomed themselves, the jar was inverted on a paper and dislodged mites falling on the paper were counted. The data were recorded during build up period, honey flow, dearth and winter periods, three times during each period.

3.1.2.4 Mites per 50 brood cells

Infestation of honey bee brood with *Varroa* was determined at pupal stage by random inspection of 50 sealed brood cells per hive. The sealed brood cells were uncapped using forceps and mites in each cell were counted. The data for brood infestation were recorded for all the colony developmental periods as described for adult bees.

3.1.2.5 Degree of infestation of brood

Degree of infestation of honey bee brood with *Varroa* was determined at pupal stage by random inspection of 50 cells of brood per hive as described earlier. From the number of mites observed in brood cells, the degree of infestation was worked out by the formula given by Ritter (1980).

$$B \text{ brood} = \frac{M \text{ brood}}{n \text{ brood}} \times 100$$

B brood = infestation of brood (%)

M brood = Number of mites in the brood cell examined

n brood = Number of brood cells examined

3.1.2.6 Degree of infestation of adult honey bees

Degree of infestation of adult bees was worked out by formula given by Ritter (1980) from the data collected from 100 adult bees by sugar dusting.

$$B \text{ bees} = \frac{M \text{ bees}}{n \text{ bees}} \times 100$$

B bees = Infestation of adult bees (%)

M bees = Number of mites on bees examined

n bees = Number of bees examined

3.1.2.7 Overall infestation of bee colony:

The overall infestation of bee colonies was calculated by following formula:

$$B_{colony}(\%) = \left[M_{bees} \times \frac{N_{bees}}{n_{bees}} + M_{brood} \times \frac{N_{brood}}{n_{brood}} \right] \times \left[\frac{100}{N_{bees} + N_{brood}} \right]$$

B colony = Overall infestation of bees in the colony (%)

N bees = Total number of bees in a colony (bee population)

N brood = Total number of brood cells in a colony (calculated from, brood area x 3.7; since each cm² covers on an average 3.7 brood cells; Harbo and Harris, 2004).

3.1.2.8 Total number of mites in a colony

The total number of mites in a colony of bees was determined by estimating the number of mites on adult bees and the number of adult mites within the capped brood cells, from the data collected on number of mites per 100 bees (M bees), mites per 50 brood cells (M brood), bee population and sealed brood area of the colony.

3.1.3 Hygienic behaviour

Hygienic behaviour was determined by pin killing 25 capped pupae. The pupae in the sealed stage were killed by piercing the caps with sharp needle. The pierced area was observed after 24 hours for removal of pin killed brood. The per cent removal of killed brood was worked out by following formula.

$$\text{Per cent removal} = \frac{\text{Number of pin killed pupae removed}}{25} \times 100$$

The data on hygienic behaviour were taken at 21 days interval for three brood cycles each during build up, honey flow and dearth period.

3.1.4 Grooming behaviour

The mites naturally falling within 24 hours on the screened bottom board were collected and were observed under microscope. The damage to different body parts was recorded. In each colony the per cent damaged mites was worked out by following the formula

$$\text{Per cent damaged mites} = \frac{\text{Number of damaged mites}}{\text{Total number of mites}} \times 100$$

Extent of damage on the different parts was worked out separately for each type of damage based on following parameters as described by Correa – Marques *et al.* (2000).

- i) Damaged legs
- ii) Damaged shield
- iii) Damaged shield + damaged legs
- iv) Carcass or empty dorsal shield
- v) Hollow in dorsal shield
- vi) Hollow in dorsal shield + damaged legs

$$\text{Type of damage(\%)} = \frac{\text{Number of mites with particular type of damage}}{\text{Total number of mites observed}} \times 100$$

Data on the damaged mites were taken five times at 10 days interval during June – August.

3.1.5 Growth rate of mites

To determine mite fecundity in the experimental colonies, initial observations on total mite count per colony (described under 3.1.2.8) was recorded in April (pre count) and later after 60 days the mite count was again taken in June (post count). From this mite count, per cent growth rate per day was calculated using following formula

$$\text{Per cent growth rate} = \frac{\text{Post count of mites} - \text{Pre count of mites}}{\text{Pre count of mites} \times 60} \times 100$$

3.1.6 Bee activity

Observations on the bee activity of each experimental colony were recorded during March-April, June and September, 2007. These observations were recorded by counting the number of bees leaving the hive (out going) and those landing at the entrance (incoming) per minute during 1000 to 1100 hours from each colony. On each colony the activity was recorded three times a day and repeated on alternate day to record 3 observations on each colony. The data on incoming and outgoing bees were recorded to know the effect of mite infestation on foraging activity of the colony.

3.2 CRITERIA USED FOR SELECTION OF COLONIES FROM OTHER APIARIES

Seventeen colonies from the migratory unit of the University were used for screening of *Varroa* tolerance. The following data were recorded during the month of May and September, (2007) and February (2008).

- Population of bee colonies
- Infestation of adult bees (mites per 100 bees)
- Infestation of brood (mites per 50 brood cells)

Similar observations were recorded on nine colonies in an apiary at RHFRS, Bhota which is situated at 650 m above mean sea level. Data were recorded at Bhota during the month of September and December, 2007 and then in March, 2008.

One apiary of a private beekeeper was also used for selection of *Varroa* tolerant colonies at Karnal (Haryana), situated at 245 m above mean sea level. Similar observations were recorded in this apiary during March, April and June, 2008.

3.3 RAISING OF FIRST GENERATION QUEENS FROM THE SELECTED STOCK

Out of the three colonies screened for varroa tolerance, the best performing colony (C3) was selected and was used to raise next generation queens for further studies. During the month of April and May, 2008, the Doolittle method of grafting was used for raising new queens (Photograph 4 to 9). Seven healthy queens were raised and were allowed to mate in the apiary at, Nauni, Solan. Successfully mated queens by the month of May were used for further studies. For comparison three queens raised from general stock, also open mated in the apiary, were used. Each mated queen was introduced in colonies having bee strength of 4 frames.

3.4 PERFORMANCE OF FIRST GENERATION QUEENS:

Observations on varroa tolerance of four colonies headed by first generation queens (designated as D colonies) were recorded in terms of all the parameters used for selection of the stock (described under 3.1.1 to 3.1.3) and compared with the mother colonies (designated as M colonies) initially selected for raising these queens as well as with queens of general stock (G colonies) during June 2008 to October, 2008. In addition to these parameters observations were also recorded on the post capping duration of



Photograph 4. Taking out 24 hr old larvae using grafting needle



Photograph 5. Grafting of larvae inside artificial queen cups



Photograph 6. Young workers raising queen cells



Photograph 7. Fully sealed queen cells



Photograph 8. Queen cells enclosed by queen cages a day before emergence of queens



Photograph 9. Newly emerged queen (←)

Photographs (4 – 9) Mass queen rearing

these experimental colonies following method of Sammataro (1996). Three colonies from each group were used for this study.

To know the length of post capping duration, the empty worker brood frame was given to each colony for egg laying by the queen. The brood frame was observed twice a day for egg laying. When the eggs were laid in the frames, the side of frame containing the eggs was marked with a marker. After eight days of egg laying the frame was inspected for first sealing of brood at 24 hours interval. The group of brood cells which sealed first was marked by making a template of sealed brood cells on a transparent sheet. The time of first sealing was noted and after 265 hours of the first sealing the marked area was observed for the opening of the sealed cells starting at 0730 h and continued at an interval of 1 hour till most of the cells (80 % or so) opened. The template made on the transparent sheet was used to observe the marked area. The difference between the sealing and opening of the brood cells was calculated to know the post capping duration of the colony. Three such areas were marked in each colony and the post capping duration (in hours) was recorded in July and again repeated during August and September.

These test colonies were migrated to Kadasan area of Ambala in the month of October, later to Hisar in the month of December and after that to Saha area of Ambala in the month of February, 2009 and were kept there up to the month of March. During this period in addition to other colony parameters, data on honey harvested were also recorded.

3.5 RAISING OF SECOND GENERATION QUEENS FROM THE BEST FIRST GENERATION STOCK

On the basis of mite tolerance (minimum 24 h mite fall, low mite infestation, hygienic behaviour and colony performance) colony D₂ was selected for raising next generation queens at Nauni, during March-April, 2009, using Doolittle method of mass queen rearing. The newly emerged queens were transferred to mating nuclei (having dimension of 23.0 (length) x 18.0 (width) x 12.5 cm (height)) which were prepared two days before the date of emergence of queen. The mating nuclei (Photograph 10) were prepared to contain four worker brood frames and one frame modified for using as feeder in which sugar candy (prepared by mixing powdered sugar in honey in the ratio of 8:1; w/w) was used for feeding bees (Photograph 11).

For raising combs for mating nuclei, two frames of mating nuclei with foundation sheet were tied to a empty frame of Langstroth hive and were given to strong colonies (Photograph 12). For preparation of mating nuclei, frames containing young workers were shaken over the mating nuclei. Care was taken so that the frames had no drones. At least three full size frames were shaken to ensure covering 4 small sized frames of mating nuclei and compensate for any shifting of bees to the parent colonies. The newly emerged queens were introduced in the mating nuclei using queen cage. In total, 10 second generation healthy queens were used for further studies.

3.6 SELECTION OF DRONE MOTHER COLONY

To infuse new genetic material, best performing (mite tolerant, higher population) colony from Karnal was used as drone mother colony.

3.7 MATING OF QUEENS

3.7.1 Mating in isolated mating yard

The isolated mating yard was selected at Khundidhar near Solan. For selecting the area it was ensured that there were no other colonies of *A. mellifera* up to 10 km. Observations were recorded during 0900, 1200 and 1600 h on the bee flora of the area for knowing the possible presence of *A. mellifera* foragers. Five queens were transferred to isolated mating yard in the mating nuclei, along with one strong drone mother colony. After mating the queens were brought back to the main apiary at Nauni. Out of five only three queens mated successfully and were used for further studies.

3.7.2 Mating of queen under open condition

For mating queens under open condition, the mating nuclei containing five unmated queens were kept in the apiary at Nauni which had about 50 honey bee colonies. Out of five only two queens mated and were used for further studies.

3.7.2.1 Performance of second generation queens:

For evaluation of queens mated at isolated mating yard and those mated under open conditions, all the queens were transferred to the colonies having bee strength of 2 frames in the month of April, 2009. Observations on varroa tolerance and colony performance of 3 colonies headed by queens mated under isolation (designated as I colonies) were recorded and compared with colonies headed by the queens mated in open condition (designated as O colonies). The data were recorded during April to



Photograph 10. Mating nucleus



Photograph 11. Sugar candy feeding in mating nucleus



Photograph 12. Mating nuclei frames given to full size colonies for raising

August, 2009 in terms of all the parameters used for selection of the stock (described under 3.1.1 to 3.1.3)

3.8 EFFICACY OF SUGAR DUSTING

To know the efficiency of sugar dusting in controlling *Varroa* mites, four sugar dustings were made during one brood cycle (June-July) in twenty colonies. Total number of mites on adult and brood in a colony was worked out before start of sugar dusting as well as in the end of brood cycle.

To know the percentage of mites falling after sugar dusting at different time intervals, mite counts from the screened bottom board were taken after one, two, sixteen and twenty four hours of sugar dusting in the experimental colonies. Data on this aspect were recorded three times during the month of July-August.

3.9 STATISTICAL ANALYSIS

The data recorded for different parameters were subjected to completely randomized design (CRD) and the co-efficient of co-relation was worked out between different parameters like mite infestation level, bee activity and colony performance parameters (Gomez and Gomez, 1986).

Chapter-4

EXPERIMENTAL RESULTS

The results of present study are presented in this chapter:

4.1 SELECTION OF COLONIES AT NAUNI, SOLAN

Data on different aspects used for selection of *Varroa* tolerant colonies in the apiary of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan during the build up (February to March), honey flow (April to mid June), dearth (July to September) and winter period (October to December) are presented in Tables 1 to 10.

Natural variations occurring in the experimental colonies headed by one year queen, having different level of mite infestation have been recorded and discussed below. No manipulation in terms of different parameters was done but all the colonies were given uniform management practices whenever required.

4.1.1 Performance during build up period

4.1.1.1 Population

The data on average population of honey bee colonies (Table 1) showed that the colonies C3 and C4 had higher average population of 13641.33 bees each, during the build up period which was significantly more than found in C2, C7 and C11. These colonies had average bee population of 5246.67, 8132.33 and 6820.67 bees, respectively. Similarly C1, C6, C8, C10 and C12 having average honey bee population of 11280.33, 12592.00, 12067.33, 11542.67 and 10493.33 bees, respectively, had significantly higher bee population than C2, C7 and C11. However, the difference was non- significant among colonies C1, C3-C6, C8-C10 and C12. The difference was also non-significant among colonies C5, C7, C9 and C11.

Table 1. Performance of the honey bee colonies used for screening for *Varroa* tolerance during build up period (February to March, 2007)

Colony number	Bee population (number of bees)	Brood area (cm ²)	Pollen area (cm ²)	Honey stores (g)	24 hour natural mite fall (number of mites)	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
C1	11280.33 (4.04)*	4642.33 (3.67)	354.00 (2.43)	850.00 (2.68)	44.67	215.33 (2.33)	8.67	11.33
C2	5246.67 (3.72)	1220.00 (3.08)	54.67 (1.59)	466.67 (2.62)	16.33	174.67 (2.24)	5.33	6.33
C3	13641.33 (4.13)	2997.00 (3.39)	136.00 (2.11)	3100.00 (3.36)	3.33	36.67 (1.37)	0.67	2.33
C4	13641.33 (4.13)	7248.33 (3.86)	120.33 (2.08)	1183.33 (3.06)	23.33	159.33 (2.20)	3.33	3.33
C5	9968.67 (3.96)	4662.33 (3.63)	204.33 (2.31)	616.67 (2.78)	19.00	208.33 (2.30)	3.33	7.33
C6	12592.00 (4.09)	5097.67 (3.68)	154.00 (1.99)	966.67 (2.98)	13.33	101.67 (1.97)	2.33	3.67
C7	8132.33 (3.91)	2090.67 (3.32)	386.67 (2.55)	816.67 (2.82)	14.67	83.00 (1.92)	3.67	5.00
C8	12067.33 (4.07)	4104.67 (3.49)	309.67 (2.48)	1391.67 (2.88)	16.33	109.67 (2.02)	2.67	5.67
C9	9444.00 (3.97)	3414.67 (3.46)	278.00 (2.29)	1000.00 (2.75)	15.67	106.00 (2.01)	5.00	4.67
C10	11542.67 (4.05)	4781.33 (3.63)	78.00 (1.86)	423.33 (2.46)	31.67	126.33 (2.08)	6.67	5.33
C11	6820.67 (3.82)	1800.33 (3.06)	173.33 (2.21)	300.00 (2.46)	6.33	75.00 (1.84)	1.33	0.33
C12	10493.33 (4.02)	1888.67 (3.06)	222.67 (2.08)	3583.33 (3.48)	10.33	105.67 (1.97)	2.67	6.67
CD_{0.05}	0.18	0.54	0.62	0.71	17.57	0.38	1.64	3.64

*Figures in parentheses are log transformed values

4.1.1.2 Brood area

The amount of brood reared by the top five colonies during the build up period was 4642.33, 7248.33, 4662.33, 5097.67 and 4781.33 cm² in C1, C4, C5, C6 and C10, respectively. The brood rearing in these colonies was significantly higher than C2, C11 and C12 which had average brood area of 1220.00, 1800.33, and 1888.67 cm², respectively. However, there was no significant difference in C1, C3 to C10 colonies. Similarly differences were non-significant in C2, C3, C7-9, C11 and C12. Brood area in C3, C7, C8 and C9 was 2997.00, 2090.67, 4104.67 and 3414.67 cm², respectively.

4.1.1.3 Pollen stores

Pollen stores of honey bee colonies during build up period (Table 1) averaged to 354.00, 136.00, 120.33, 204.33, 154.00, 386.67, 309.67, 278.00, 173.33 and 222.67 cm² in C1, C3, C4, C5, C6, C7, C8, C9, C11 and C12, respectively. The differences were, however, non-significant. The pollen stores in C7 were significantly higher than C2 and C10 which had pollen stores of only 54.67 and 78.00 cm², respectively. There were also no significant differences among the colonies C1, C3-6, C8 to C12 and also among the colonies C2-4, C6, C10-11 and C12.

4.1.1.4 Honey stores

During the build up period (Table 1) significantly higher honey stores of 3100.00 and 3583.33 grams were found in C3 and C12 in comparison to colonies C2, C10 and C11 which had honey stores of 466.67, 423.33 and 300.00 grams, respectively. However, there were no significant differences among C1, C2, and C4-11 which had honey stores in the range of 300.00 to 1391.67 grams, respectively.

4.1.1.5 24-hour natural mite fall

The number of mites falling in 24 hours on the bottom board i.e. 24 hour mite count was significantly less (3.33 mites) in colony number C3 as compared to 44.67, 23.33 and 31.67 in colony number C1, C4 and C10, respectively.

However, the difference were non-significant between C2, C3, C5-9, C11 and C12 having mite fall varying between 3.33 to 19.00 mites per colony.

4.1.1.6 *Varroa* infestation level of bee colonies as determined by sugar dusting

The data on mite falling within 24 hours of sugar dusting revealed that colony C3 had least number of mites (36.67) which was significantly lower than all other colonies. C3 colony was followed by C11 in mite infestation having a count of 75.00 mites which was significantly less than in C1, C2 and C5. Differences in mite count were non-significant between colony number C4 and C6 to C12 having mite count per colony in the range of 75.00 to 159.33. Similarly the mite count varied non-significantly between the colony number C1, C2, C4-6, C8-10 and C12 which was in the range of 101.67 to 215.33.

4.1.1.7 Number of mites per 100 adult bees.

The data on number of mites per 100 bees (Table 1) collected by sugar dusting showed that C3 and C11 colonies had significantly less number of mites on adult bees (respective values being 0.67 and 1.33 mites/100 bees) as compared to C1, C2, C4-5, C7, C9 and C10 (mites/100 bees being in the range of 3.33 to 8.67). Colony C1 had maximum number of 8.67 mites per 100 bees which was significantly higher than all other colonies.

4.1.1.8 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells during the build up period revealed that colony number C11 had significantly less number of mites per 50 brood cells (0.33 mites) than C1-2, C5, C7-10 and C12 (having mites in the range of 4.67 to 11.33 mites/50 brood cells). However, the mite infestation of the brood in colony C11 was at par with C3, C4 and C6; varying non-significantly between 0.33 to 3.67 mites per 50 brood cells.

4.1.1.9 Per cent brood, adult and colony infestation levels

On the basis of per cent brood, adult and colony infestation there is an indication that colonies C3 and C11 had significantly low mite infestation

showing some tolerance as compared to most of the other colonies viz. C1, C2, C5, C7 to C10 and C12 (Table 2) being susceptible to mite attack. The mite infestation in C3 and C11 varied non-significantly from each other, the respective values for per cent brood, adult and colony infestation level were in the range of 0.67 to 4.67, 0.67 to 1.33 and 0.97 to 1.54 per cent as compared to susceptible group described above, which had the mite infestation level in the range of 9.33 to 22.67, 2.67 to 8.67 and 5.93 to 17.01 per cent, respectively.

Table 2. Per cent brood, adult and colony infestation level in the experimental colonies during the build up period (February to March, 2007)

Colony number	Per cent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
C1	22.67(4.73)*	8.67(2.93)	17.01(4.11)
C2	12.67(3.54)	5.33(2.31)	08.64(2.94)
C3	04.67(1.63)	0.67(0.67)	01.54(1.18)
C4	06.67(2.58)	3.33(1.82)	05.54(2.35)
C5	14.67(3.73)	3.33(1.79)	10.54(3.16)
C6	07.33(2.70)	2.33(1.52)	05.30(2.30)
C7	10.00(3.15)	3.67(1.91)	06.68(2.58)
C8	11.33(3.36)	2.67(1.63)	07.03(2.62)
C9	09.33(3.03)	5.00(2.23)	07.46(2.70)
C10	10.67(3.20)	6.67(2.57)	09.15(2.98)
C11	00.67(0.47)	1.33(1.14)	00.97(0.96)
C12	13.33(3.61)	2.67(1.63)	05.93(2.40)
CD _{0.05}	1.29	0.48	0.79

*Figures in parentheses are square root transformed values

4.1.2 Performance during honey flow period

4.1.2.1 Population

Data on average population of honey bee colonies during honey flow period revealed that the colonies C1, C3, C5, C9 and C11 had average population of 15740.00, 12592.00, 17314.00, 20462.00 and 13379.00 bees, respectively, which was significantly higher than C2, C12, C15 and C17-20 having average

Table 3. Performance of the honey bee colonies used for screening for *Varroa* tolerance during honey flow period (April to June, 2007)

Colony number	Bee population (number of bees)	Brood area (cm ²)	Pollen area (cm ²)	Honey stores (g)	24 hour natural mite fall (number of mites)	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
C1	15740.00 (4.20)*	4367.00 (3.49)	315.33 (1.88)	1033.33 (3.00)	36.33	253.00 (2.39)	7.67	20.33
C2	6820.67 (3.83)	1298.67 (3.06)	19.67 (1.15)	650.00 (2.80)	13.67	169.00 (2.14)	9.33	20.00
C3	12592.00 (4.08)	3614.00 (3.34)	132.67 (1.94)	1466.67 (3.08)	03.00	63.33 (1.74)	2.00	1.00
C4	11542.67 (4.02)	1805.67 (3.20)	344.67 (2.24)	1866.67 (3.27)	31.33	107.00 (2.01)	3.33	18.67
C5	17314.00 (4.24)	2937.67 (3.42)	101.00 (1.74)	1766.67 (3.24)	98.33	404.33 (2.59)	10.00	18.00
C6	10231.00 (4.00)	253.33 (2.38)	143.33 (2.07)	1133.33 (3.03)	15.67	174.33 (2.23)	12.67	13.00
C7	11805.00 (4.07)	2395.00 (3.22)	431.33 (2.11)	2716.67 (3.43)	32.67	273.33 (2.43)	11.33	10.67
C8	10231.00 (4.00)	500.00 (2.58)	84.00 (1.68)	1233.33 (2.93)	25.33	330.67 (2.01)	8.33	19.67
C9	20462.00 (4.31)	5226.33 (3.60)	759.00 (2.25)	1633.33 (3.18)	58.00	585.33 (2.65)	11.67	10.00
C10	11018.00 (4.02)	706.67 (2.60)	225.00 (1.88)	2266.67 (3.20)	98.67	485.67 (2.65)	12.00	14.66
C11	13379.00 (4.12)	4484.33 (3.56)	271.33 (2.14)	1016.67 (2.97)	4.00	64.00 (1.68)	2.00	2.00
C12	5246.67 (3.72)	1581.33 (3.17)	22.00 (1.23)	516.67 (2.69)	28.33	241.33 (2.34)	12.00	19.00
C13	8919.33 (3.94)	3280.33 (3.47)	216.00 (1.97)	633.33 (2.69)	03.00	20.00 (1.24)	1.33	2.67
C14	11018.00 (4.04)	4088.00 (3.57)	50.67 (1.44)	1066.67 (2.99)	05.33	37.33 (1.56)	2.00	4.67
C15	5246.67 (3.72)	1808.00 (3.20)	24.00 (1.27)	583.33 (2.75)	22.33	177.33 (2.24)	14.00	18.00
C16	9444.00 (3.97)	1936.67 (3.26)	36.00 (1.54)	766.67 (2.87)	34.00	212.00 (2.30)	13.33	18.00
C17	6033.67 (3.78)	1473.33 (3.09)	23.33 (1.31)	433.33 (2.62)	23.33	180.00 (2.21)	14.33	18.00
C18	6296.00 (3.80)	1538.00 (3.17)	34.00 (1.42)	533.33 (2.72)	21.33	186.67 (2.26)	14.33	18.67
C19	6820.67 (3.83)	1196.67 (3.05)	37.33 (1.52)	733.33 (2.86)	17.33	172.00 (2.19)	12.00	18.00
C20	4459.67 (3.65)	1344.67 (3.06)	36.00 (1.48)	650.00 (2.81)	30.67	261.67 (2.39)	12.67	20.33
CD_{0.05}	0.16	0.58	NS	0.40	23.76	0.51	9.40	15.16

*Figures in parentheses are log transformed values

population in the range of 4459.67 to 6820.67 bees. However, there were no significant differences among colonies C1, C5 and C9 and also among the colonies C1, C3, C5 and C11.

4.1.2.2 Brood area

The amount of brood reared by top four colonies during the honey flow period was 4367.00, 5226.33, 4484.33 and 4088.00 cm² in C1, C9, C11 and C14, respectively. The amount of brood reared in these colonies was significantly higher than colonies C6, C8 and C10 which had average brood area of 253.33, 500.00 and 706.67 cm², respectively. However, there were no significant differences among colonies C1-5, C7, C9 and C11-20 which had average brood area ranging between 1196.67 to 5226.33 cm². The differences were also non-significant among colonies C6, C8 and C10.

4.1.2.3 Pollen stores

The pollen stores of honey bee colonies during the honey flow period varied non-significantly between 19.67 to 759.00 cm² in all the colonies. However, the colonies C4, C7, C9 and C11 were the top four colonies having pollen stores of 344.67, 431.33, 759.00 and 271.33 cm², respectively.

4.1.2.4 Honey stores

During the honey flow period significantly higher honey stores of 1866.67, 1766.67 and 2716.67 gram were found in C4, C5 and C7, respectively, in comparison to C2, C12, C13, C15, C17, C18, C19 and C20 which had average honey stores of 650.00, 516.67, 633.33, 583.33, 433.33, 533.33, 733.33 and 650.00 grams, respectively. However, the honey stores were at par in C1, C3-11, C14 and C16 (varying non-significantly between 766.67 to 2716.67 g).

4.1.2.5 24-hour natural mite fall

The data on number of mites falling within 24 hours during the honey flow period revealed that colonies C3, C11, C13, and C14 had significantly less number of mites (respective values being 3.00, 4.00, 3.00 and 5.33) falling

compared to C1, C4-5, C7, C9-10, C16 and C20 (24 hour mite fall being in the range of 30.67 to 98.67). The difference in mite count was non-significant between colony number C2-3, C6, C11, C13-15 and C17-19 having mite count per colony in the range of 3.00 to 23.33. The colony C5 and C10 had maximum number of mites fallen (respective values being 98.33 and 98.67) within 24 hours, which was significantly higher than rest of the colonies.

4.1.2.6 Sugar dusting

The number of mites falling within 24 hour on the bottom board after sugar dusting was significantly less in colonies C3, C11, C13 and C14 (respective mite fall being 63.33, 64.00, 20.00 and 37.33) compared to colonies C1, C5, C7, C9-10, C12, C16, C18 and C20 which had mite fall in the range of 186.67 to 585.33. However, the differences were non-significant among the colonies C3, C11, C13 and C14.

4.1.2.7 Mites per 100 bees

The data on number of mites per 100 bees during the honey flow period revealed that colonies C3, C11, C13 and C14 had significantly less number of mites on adult bees (respective values being 2.00, 2.00, 1.33 and 2.00 mites/100 bees) as compared to C6, C9-10, C12 and C15-20 (mites/100 bees being in the range of 11.67 to 14.33). However, the differences were non-significant among colonies C1-5, C8, C11 and C13-14 (mites/100 bees being in the range of 1.33 to 10.00)

4.1.2.8 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells during the honey flow period revealed that the colonies C3, C11 and C13 had 1.00, 2.00 and 2.67 mites per 50 brood cells which was significantly less than in colonies C1-2, C4-5, C8, C12 and C15-20 which had mites per 50 brood cells varying between 18.00 to 20.33. However, mite per 50 brood cells varied non-significantly between the colonies C3, C6-7, C9-11, and C13-14 (mites per 50 brood cells were in the range of 1.00 to 14.66).

4.1.2.9 Per cent brood, adult and colony infestation

The data on per cent brood, adult and colony infestation (Table 4) indicated that colonies C3, C11, C13 and C14 had significantly low mite infestation than most of the colonies viz. C2, C5, C12 and C15-20, which were susceptible to mite attack. The mite infestation in C3, C11, C13 and C14 varied non-significantly from each other, respective values for per cent brood, adult and colony infestation level were in the range of 2.00 to 9.33, 1.33 to 2.00 and 2.00 to 5.77. However, the susceptible colonies (C2, C5, C12 and C15-20) had respective brood, adult and colony infestation varying between 36.00 to 40.67, 9.33 to 14.33 and 18.91 to 25.23 per cent.

Table 4. Per cent brood, adult and colony infestation level in the experimental colonies during honey flow period (April to June)

Colony number	Per cent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
C1	40.67(39.21)*	07.67(2.62)**	17.90(24.70)*
C2	40.00(38.68)	09.33(2.96)	21.61(26.95)
C3	02.00(08.13)	02.00(1.14)	02.00(08.13)
C4	37.33(37.25)	03.33(1.82)	16.06(23.03)
C5	36.00(36.66)	10.00(3.05)	18.91(25.60)
C6	26.00(29.83)	12.67(3.45)	13.84(21.20)
C7	21.33(26.72)	11.33(3.33)	14.27(21.90)
C8	39.33(38.27)	08.33(2.71)	11.53(19.27)
C9	20.00(24.43)	11.67(3.29)	13.35(20.45)
C10	29.33(32.11)	12.00(3.41)	14.05(21.79)
C11	04.00(11.53)	02.00(1.38)	03.15(10.21)
C12	38.00(37.62)	12.00(3.41)	23.42(28.68)
C13	05.33(12.17)	01.33(1.14)	03.01(09.52)
C14	09.33(17.52)	02.00(1.38)	05.77(13.79)
C15	36.00(36.58)	14.00(3.68)	24.66(29.62)
C16	36.00(36.63)	13.33(3.56)	22.35(28.02)
C17	36.00(36.63)	14.33(3.71)	22.98(28.52)
C18	37.33(37.39)	14.33(3.69)	24.28(29.27)
C19	36.00(36.71)	12.00(3.41)	20.79(27.04)
C20	40.67(39.47)	12.67(3.52)	25.23(30.10)
CD_{0.05}	18.43	0.79	8.59

*Figures in parentheses are arc sin transformed values

**Figures in parentheses are square root transformed values

4.1.3 Performance during dearth period

4.1.3.1 Population

The data on the average honey bee population during the dearth period (Table 5) revealed that colonies C3, C11 and C14 had respective average population of 14166.00, 9444.00 and 9247.25 bees, which was significantly higher than the colonies C2, C8, C9, C12, C16 and C20 which had average bee population of 2361.25, 3345.00, 5115.75, 2951.50, 5115.75 and 984.00 bees, respectively. However, the differences were non-significant among the colonies C1, C3-7, C10-11, C13-15 and C17-19 which had bee population in the range of 2361.00 to 14166.00. The differences were also non-significant among the colonies C1-2, C4, C6, C8-9, C12 and C16-20 in which the population ranged between 984.00 to 2951.25 bees.

4.1.3.2 Brood area

The data on average brood reared by honey bee colonies during the dearth period indicated that the colony C3, C11 and C14 reared significantly higher amount of brood (respective amount of brood reared being 3066.50, 2142.00 and 1900.25 cm²) than colonies C2, C8, C9, C12, C16 and C20 (respective amount of brood reared being 362.75, 404.50, 994.00, 461.50, 851.75 and 100.25 cm² respectively). However, the differences were non-significant among the colonies C1, C3-5, C7, C10-11, C13-15 and C17-19 (average brood area ranged between 189.25 to 3066.50 cm²).

4.1.3.3 Pollen stores

Pollen stores of honey bee colonies during the dearth period (Table 5) averaged to 18.50, 96.00, 56.25, 29.25, 47.00, 21.25, 69.00, 57.75, 26.50, 40.50 and 29.50 cm² in C1, C3, C5, C7, C10, C11, C13, C14, C15, C17, C18 and C19, respectively. The differences were, however, non-significant among these colonies. The pollen stores in C3, C11, C14 and C15 were significantly higher than colonies C2, C8, C9, C12, C16 and C20. In addition to above colonies the colony C3 also had significantly higher pollen stores (96.00 cm²) than colonies C4 and C6 which had pollen stores of 10.88 and 15.63 cm², respectively.

Table 5. Performance of the honey bee colonies used for screening for *Varroa* tolerance during dearth period (July to September, 2007)

Colony number	Bee population (number of bees)	Brood area (cm ²)	Pollen area (cm ²)	Honey stores (g)	24 hour natural mite fall (number of mites)	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
C1	3344.75 (3.44)*	511.25 (2.36)	18.5 (1.17)	625 (2.76)	19.70	170.00 (2.18)	9.00	52.00
C2	2361.25 (1.68)	362.75 (1.26)	3.75 (0.25)	112.75 (1.02)	7.50	86.00 (1.93)	6.00	82.00
C3	14166.00 (4.15)	3066.50 (3.44)	96.00 (1.86)	2250.00 (3.34)	9.33	93.67 (1.82)	1.67	7.00
C4	4722.13 (2.67)	479.37 (2.01)	10.88 (0.79)	225.12 (1.72)	13.00	103.30 (2.00)	5.33	65.70
C5	7870 (3.84)	1184.75 (2.85)	56.25 (1.59)	850.00 (2.90)	20.30	98.00 (1.97)	7.00	54.30
C6	2557.88 (2.47)	200.87 (1.72)	15.63 (0.89)	500.12 (1.98)	14.70	178.30 (2.07)	14.70	38.70
C7	5115.50 (3.63)	1092.25 (2.83)	18.75 (1.25)	1337.50 (3.06)	12.00	102.30 (1.91)	8.33	41.30
C8	3345.00 (1.76)	404.50 (1.29)	16.50 (0.53)	637.75 (1.39)	17.00	389.50 (2.53)	15.50	78.00
C9	5115.75 (1.84)	994.00 (1.41)	15.75 (0.59)	407.75 (1.30)	39.50	398.00 (2.39)	15.00	45.50
C10	5312.25 (3.70)	590.75 (2.57)	29.25 (1.33)	450.00 (2.55)	17.00	350.00 (2.47)	19.70	50.30
C11	9444.00 (3.96)	2142.00 (3.29)	47.00 (1.62)	1337.50 (3.12)	5.33	37.33 (1.56)	1.67	11.00
C12	2951.50 (1.73)	461.50 (1.33)	15.25 (0.56)	505.25 (1.35)	19.00	188.00 (2.26)	18.00	67.50
C13	5705.75 (3.71)	1336.00 (2.93)	21.25 (1.29)	462.50 (2.65)	6.33	71.67 (1.81)	1.67	17.00
C14	9247.25 (3.97)	1900.25 (3.25)	69.00 (1.75)	492.50 (2.65)	5.33	67.67 (1.77)	2.67	10.70
C15	5312.25 (3.72)	462.00 (2.64)	57.75 (1.70)	1425.00 (3.12)	13.70	155.70 (2.18)	13.70	54.00
C16	5115.75 (1.85)	851.75 (1.46)	26.75 (0.70)	525.25 (1.36)	25.00	202.50 (2.31)	17.00	34.50
C17	2361.00 (3.32)	189.25 (2.14)	26.50 (1.33)	250.00 (2.29)	20.00	132.70 (2.08)	10.00	27.30
C18	3541.50 (3.45)	409.75 (2.38)	40.50 (1.49)	525.00 (2.68)	20.00	128.30 (2.08)	11.30	25.30
C19	2951.25 (3.30)	278.00 (2.28)	29.50 (1.20)	700.00 (2.83)	15.00	171.30 (2.22)	12.30	39.70
C20	984 (1.45)	100.25 (0.90)	3.75 (0.27)	162.75 (1.09)	28.00	142.50 (2.06)	19.50	32.00
CD_{0.05}	2.03	1.66	0.90	1.56	CD1=15.98 CD2=19.57 CD3=17.86	0.48 0.59 0.54	5.99 7.34 6.70	26.48 32.43 29.60

*Figures in parentheses are log transformed values

CD 1 = for comparison among colonies C1, C3-7, C10-11, C13-15 and C17-19 (Group A).

CD 2 = for comparison among colonies C2, C8, C9, C12, C16 and C20 (Group B).

CD 3 = for comparison of Group A colonies with Group B colonies.

4.1.3.4 Honey stores

During the dearth period significantly higher honey stores of 2250.00, 1337.50, 1337.50 and 1425.00 grams were found in colonies C3, C7, C11 and C15, respectively, in comparison to colonies C2, C8, C9, C12, C16 and C20 which had honey stores of 112.75, 637.75, 407.75, 505.25, 525.25 and 162.75 grams, respectively. However, the differences were non-significant among colonies C1, C3-7, C10-11, C13-15 and C17-19 (honey stores ranged between 225.12 to 2250.00 grams) and also among C2, C4, C6, C8-10, C12, C16-17 and C20 (average honey stores ranged between 112.75 to 637.75 grams)

4.1.3.5 24-hour natural mite fall

The number of mites falling in 24 hours on the bottom board was significantly less in colonies C11, C13 and C14 (respective mites fallen being 5.33, 6.33 and 5.33) as compared to colonies C1, C5, C8-10 and C15-20 (mite count being in the range of 20.00 to 39.50). However, there were no significant differences among the colonies C2-4, C6-7 and C11-14 (mite count varied between 5.33 to 19.00)

4.1.3.6 *Varroa* infestation level of bee colonies as determined by sugar dusting

The data on mite falling within 24 hours of sugar dusting revealed that mite count after of sugar dusting was significantly less (37.33) in colony C11 as compared to colonies C1, C6, C8-10, C12 and C15-20, in which average mite fall varied between 142.50 to 398.00. However, the differences were non-significant among C2-5, C7, C11 and C13-14, having mite fall between 37.33 to 103.30 mites per colony.

4.1.3.7 Number of mites per 100 adult bees

The data on number of mites per 100 bees (Table 5) showed that the colonies C3, C11 and C13 had significantly less mites (1.67 mites each) on adult bees as compared to C1, C6-10, C12 and C15-20 (mites per 100 bees being in the range of 8.33 to 19.70). However, the mite infestation of adult bees in colonies

C3, C11 and C13 was at par with the colonies C2, C4, C5 and C14 (varying non-significantly between 1.67 to 7.00)

4.1.3.8 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells during the dearth period showed that C3, C11 and C14 colonies had significantly less number of mites per 50 brood cells (respective values being 7.00, 11.00 and 10.70 mites/50 brood cells) compared to C1-2, C4-10, C12, C15 and C19 (having mites in the range of 38.70 to 82.00 mites/50 brood cells). However, the brood infestation of colonies C3, C11 and C14 was at par with the colonies C13, C16-18 and C20 (varying non-significantly between 7.00 and 34.50 mites per 50 brood cells).

4.1.3.9 Per cent brood, adult and colony infestation level

The data on per cent brood, adult and colony infestation (Table 6) revealed that colonies C3, C11 and C14 had significantly low mite infestation

Table 6. Per cent brood, adult and colony infestation level in the experimental colonies during dearth period (July to September, 2007)

Colony number	Per cent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
C1	104.00	09.00(2.93)*	54.10(51.27)**
C2	164.00	06.00(2.45)	62.23(52.84)
C3	14.00	01.67(1.24)	06.73(14.23)
C4	131.30	05.33(2.28)	42.47(40.59)
C5	108.70	07.00(2.62)	46.71(43.33)
C6	77.33	14.70(3.78)	27.69(31.29)
C7	82.67	08.33(2.85)	39.52(38.68)
C8	156.00	15.50(3.92)	72.64(64.91)
C9	91.00	15.00(3.87)	41.16(39.80)
C10	100.70	19.70(4.41)	45.86(42.59)
C11	22.00	01.67(1.24)	11.26(19.43)
C12	135.00	18.00(4.24)	60.87(51.28)
C13	34.00	01.67(1.28)	14.95(22.56)
C14	21.33	02.67(1.63)	10.55(18.91)
C15	108.00	13.70(3.57)	33.25(35.13)
C16	69.00	17.00(4.12)	35.91(36.80)
C17	54.67	10.00(3.14)	20.93(27.02)
C18	50.67	11.30(3.28)	21.86(27.84)
C19	79.33	12.30(3.46)	28.79(32.32)
C20	64.00	19.50(4.42)	30.09(33.21)
CD_{0.05}	CD 1 = 52.96 CD 2 = 64.86 CD 3 = 59.21	0.96 1.17 1.07	21.67 26.53 24.22

*Figures in parentheses are square root transformed values.

**Figures in parentheses are arc sin transformed values.

CD 1 = for comparison among colonies C1, C3-7, C10-11, C13-15 and C17-19 (Group A).

CD 2 = for comparison among colonies C2, C8, C9, C12, C16 and C20 (Group B).

CD 3 = for comparison of Group A colonies with Group B colonies.

(values for brood, adult and colony infestation level were in range of 14.00 to 22.00, 1.67 to 2.67 and 6.73 to 11.26 per cent, respectively) than the highly susceptible colonies C1, C5, C8, C10 and C12 (values for brood, adult and colony infestation level were in range of 100.70 to 164.00, 7.00 to 19.70 and 45.86 to 72.64 per cent, respectively). However, the difference were non-significant among colonies C3, C11 and C14.

4.1.4 Performance during winter period

4.1.4.1 Population

The data on average population of the surviving honey bee colonies during the winter period (Table 7) revealed that colony C11 maintained the maximum population of 4459.67 bees which was significantly higher than the colony C13 which had average adult bee population of 2098.67 bees. However, there was no significant difference among the colonies C3, C11 and C14 and also among the colonies C3, C13 and C14. The colonies C3 and C14 had average population of 2885.67 and 3935.00 adult bees.

4.1.4.2 Brood area

The data on brood rearing presented in Table 7 revealed that on an average colony C3, C11, C13 and C14 reared 277.33, 296.00, 106.67 and 345.33 cm² of brood, respectively. However, the difference were non-significant.

4.1.4.3 Pollen Stores

The data on pollen stores of honey bee colonies during the winter period revealed that the colony C11 had significantly higher pollen stores of 39.33 cm² than the colonies C3, C13 and C14 which had average pollen stores of 10.00, 18.00 and 21.33 cm², respectively. The colony number C14 also had significantly higher pollen stores than the colony C3. However, there were no significant differences among the colonies C13 and C14 and also among the colonies C3 and C13.

Table 7: Data on surviving honey bee colonies (mite tolerant) during the winter period (October to December, 2007)

Colony number	Bee population (number of bees)	Brood area (cm ²)	Pollen area (cm ²)	Honey stores (g)	24 hour natural mite fall (number of mites)	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
C3	2885.67 (3.41)*	277.33 (2.03)	10.00	850.00 (2.93)	5.67	38.00	7.67	12.33
C11	4459.67 (3.64)	296.00 (2.06)	39.33	783.33 (2.87)	1.00	9.00	2.66	3.00
C13	2098.67 (3.26)	106.67 (1.65)	18.00	866.67 (2.93)	3.00	23.67	9.00	5.33
C14	3935.00 (3.58)	345.33 (1.92)	21.33	676.66 (2.80)	2.33	12.00	3.66	2.33
CD 0.05	0.35	NS	11.16	NS	NS	22.18	NS	NS

*Figures in parentheses are log transformed values

4.1.4.4 Honey stores

The colonies C3, C11, C13 and C14, on an average had honey stores of 850.00, 783.33, 866.67 and 676.66 grams, respectively, during the winters. However, there were no significant differences among all these colonies.

4.1.4.5 24-hour natural mite fall

The data on 24 hour natural mite fall are presented in Table 7 which revealed that the mites falling on the bottom board within 24 hours were 5.67, 1.00, 3.00 and 2.33 in colonies C3, C11, C13 and C14, respectively. However, the difference was non-significant among all these colonies.

4.1.4.6 Varroa infestation level of bee colonies as determined by sugar dusting

The data on number of mites falling after sugar dusting (Table 7) revealed that the colonies C11 and C14 had significantly less mite fall after sugar dusting and than colony C3. However, the difference was non-significant among the colonies C11, C13 and C14 and also between the colonies C3 and C13. The colonies C3, C11, C13 and C14 had average mite fall of 38.00, 9.00, 23.67 and 12.00 mites, respectively.

4.1.4.7 Number of mites per 100 bees

The data on number of mites per 100 bees during the winter period (Table 7) showed that the colony C3, C11, C13 and C14 had 7.67, 2.66, 9.00 and 3.66 mites per 100 bees, respectively. The differences were non-significant among these colonies.

4.1.4.8 Number of mites per 50 brood cells

The number of mites examined in 50 brood cells during the winter period varied non-significantly between 2.33 to 12.33 mites per 50 cells in colonies C3, C11, C13 and C14.

4.1.4.9 Per cent brood, adult and colony infestation level

The data on per cent brood, adult and colony infestation revealed that (Table 8) there were no significant differences among all the surviving colonies (C3, C11, C13 and C14) with respect to mite infestation. The per cent brood, adult and colony infestation in these colonies ranged between 4.67 to 24.67, 2.66 to 9.00 and 3.93 to 12.81.

Table 8: Per cent brood, adult and colony infestation level in surviving honey bee colonies (mite tolerant) during the winter period (October to December, 2007)

Colony number	Per cent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
C3	24.67(3.94)	7.67(2.52)	12.81(3.09)
C11	6.00(1.80)	2.66(1.56)	4.14(1.80)
C13	10.67(2.62)	9.00(2.82)	9.80(2.62)
C14	4.67(1.76)	3.66(1.82)	3.93(1.76)
CD 0.05	NS	NS	NS

*Figures in parentheses are square root transformed values.

4.1.5 Hygienic behaviour

For estimating hygienic behaviour, pin killed brood assay was used (Photograph 13 and 14) during the build up, honey flow and dearth period. The data on the per cent pin killed brood cleaned by honey bees after 24 hours are depicted in Table 9. The data on percentage of pin killed brood removed by honey bees after 24 hours of piercing revealed that bees of C3 and C11 cleaned significantly more pin killed brood (93.33 to 96.00%) during the build up period than other colonies (48.00 to 84.00 %). However, the differences were non-significant between C3 and C11. Colonies C1, C7 and C12 removed significantly less amount of killed brood (varying between 48.00 to 61.33%) as compared to C5, C8 and C10 (varying between 78.67 to 84.00 %).

Table 9. Hygienic behaviour of the experimental colonies during different periods as determined by per cent brood cleaning of pin-killed brood after 24 hours

Colony Number	Cleaned cells (Per cent)		
	Build up (February-March)	Honey flow (April- mid June)	Dearth (July-September)
C1	61.33(51.53)*	65.33(54.06)	48.00(43.83)
C2	72.00(58.07)	69.33(56.56)	58.00(49.59)
C3	93.33(78.26)	97.33(82.28)	93.33(75.17)
C4	70.67(57.26)	80.00(63.48)	66.67(54.87)
C5	78.67(62.49)	74.67(59.82)	69.33(56.57)
C6	62.67(52.42)	65.33(53.96)	65.33(54.06)
C7	54.67(47.67)	72.00(58.07)	68.00(55.62)
C8	84.00(66.50)	70.67(57.26)	64.00(53.14)
C9	73.33(58.99)	66.67(54.83)	68.00(55.57)
C10	78.67(62.49)	77.33(61.62)	69.33(56.47)
C11	96.00(80.65)	97.33(82.28)	97.33(82.28)
C12	48.00(43.83)	68.00(55.56)	58.00(49.59)
C13	-	94.67(76.80)	90.67(72.26)
C14	-	90.67(72.26)	85.33(67.50)
C15	-	54.67(47.66)	42.67(40.76)
C16	-	66.67(54.75)	64.00(53.11)
C17	-	89.33(70.98)	65.33(54.06)
C18	-	65.33(53.96)	62.67(52.35)
C19	-	68.00(55.55)	54.67(47.73)
C20	-	68.00(55.55)	70.00(56.78)
C.D._(0.05)	8.44	6.56	CD 1 = 7.66 CD 2 = 9.38 CD 3 = 8.57

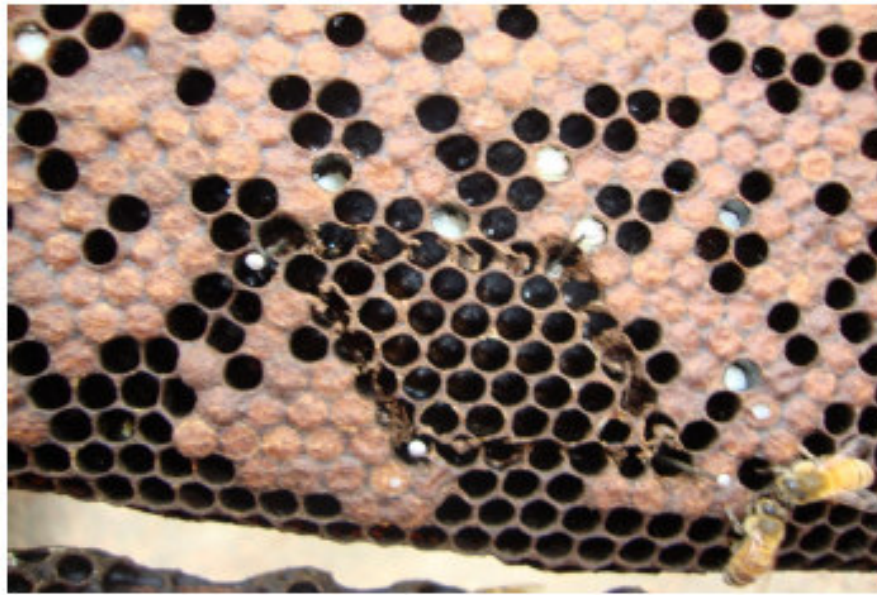
* Figures in parentheses are arc sin transformed values

CD 1 = for comparison among colonies C1, C3-7, C10-11, C13-15 and C17-19 (Group A).

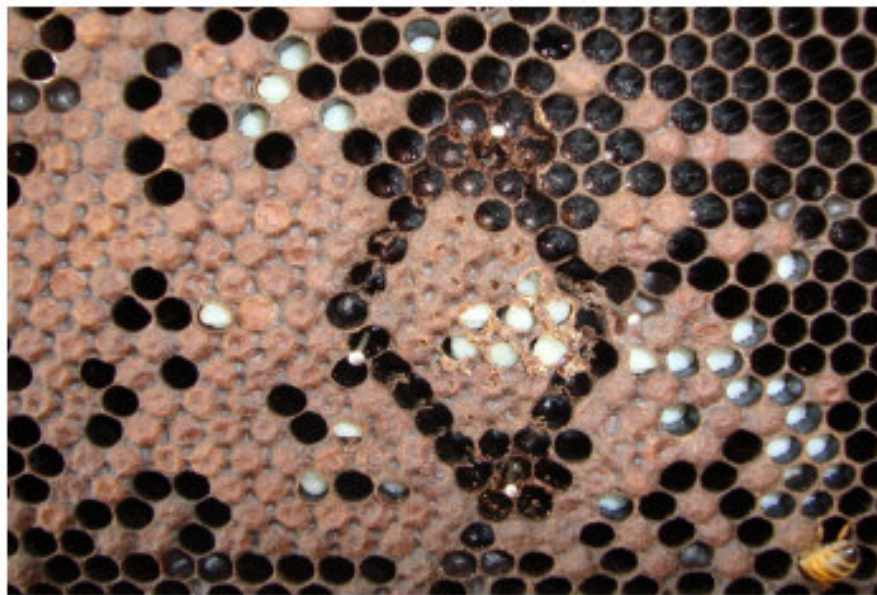
CD 2 = for comparison among colonies C2, C8, C9, C12, C16 and C20 (Group B).

CD 3 = for comparison of Group A colonies with Group B colonies

During the honey flow period the colonies C3 and C11 cleaned significantly more pin killed brood (97.33% each) than rest of the colonies (54.67 to 90.67 %); except the colony C13 (94.67 %). The colonies C13, C14 and C17 also removed significantly more pin killed brood (respective cleaned cells being 94.67, 90.67 and 89.33 %) than C1-2, C4-10, C12, C15-16 and C18-20 (per cent cleaned cells varied between 54.67 to 80.00). However, differences were non-significant among the colonies C3, C11 and C13 and also among the colonies C11, C13 and C14.



Photograph 13. Pin killed brood cells cleaned by hygienic honey bee colony



Photograph 9. Pin killed brood cells cleaned by unhygienic honey bee colony

The data on percentage of pin killed brood removed by bees during the dearth period revealed that the colonies C3, C11, C13 and C14 cleaned significantly more pin killed brood (respective cleaned cells being 93.33, 97.33, 90.67 and 85.33 %) than rest of the colonies(42.67 to 70.00 %). The colony C11 also removed significantly more killed brood than C13 and C14. However, the differences were non-significant between C3 and C11 and also between C3 and C13.

4.1.6 Grooming Behaviour

Grooming behaviour of the experimental colonies was determined from the damage caused to body parts of mites. Data recorded on damaged mites (Table 10) revealed that C11 and C14 colonies had significantly more damaged mites (69.83 and 65.21%) as compared to C1-2, C4-10, C12, C15-17 and C19-20 which had damaged mites in the range of 24.79 to 39.84 per cent. Except for C14

Table 10. Damaged mites (per cent) from the 24-h natural mite fall in the experimental colonies during July- August, 2007

Colony Number	Per cent damaged mites
C1	33.35(35.26)*
C2	33.21(35.17)
C3	60.97(51.36)
C4	39.84(39.14)
C5	27.54(31.63)
C6	32.64(34.81)
C7	36.64(36.99)
C8	37.00(37.37)
C9	30.44(33.24)
C10	34.33(35.75)
C11	69.83(57.12)
C12	31.20(33.99)
C13	58.33(49.86)
C14	65.21(53.96)
C15	24.79(29.69)
C16	29.30(32.65)
C17	28.42(32.18)
C18	49.19(44.54)
C19	26.33(30.81)
C20	35.13(36.25)
C.D_(0.05)	05.56

*Figures in parentheses are arc sin transformed values

(differences non-significant) the colony number C11 had maximum damaged mites in comparison to all other colonies. However, damaged mites in C14 were at par with those of C3 and C13 (varying non-significantly between 58.33 to 65.21%).

4.1.7 Type of damage to mites

Type of damage done by honey bees to the mites (Photograph 15 to 20) is presented in Table 11. The observations revealed that in general, irrespective of colony number, maximum damage (in 24.71 % of mites) was done to legs of the mites, followed by damage to shield (5.44 %). Damage to other parts varied non significantly between 1.48 to 3.42 per cent. However, among the colonies there was great variation in the type of damage done to different body parts. Damage to legs varied significantly from as low as 11.07 per cent (C19) to as high as 52.67 per cent (C11). Similarly for damaged shield the values varied between 2.38 (C15) to 11.68 (C7); damaged shield + damaged legs between 0.00 (C2, C8) to 6.97 (C4); empty dorsal shield between 0.00 (C1, C8, C10, C11 and C17) to 5.00 (C13); hollow or depression in dorsal shield between 0.00 (C2, C11, C12 and C15) to 7.58 (C8) and hollow in dorsal shield + damaged legs varied between 0.00 (C2,C4, C10, C13, C15 and C19) to 4.44 (C8).

4.1.8 Growth rate of mites

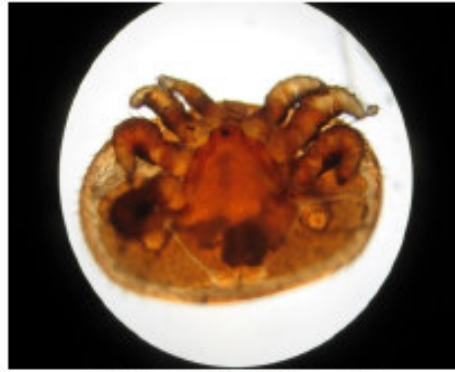
Per cent growth rate per day of the varroa mite as calculated from pre and post count for a period of 60 days is depicted in Table 12. Lower growth rate of -0.16 and -0.01 was found in C10 and C3, respectively. The growth rate of the mites varied between 0.09 to 0.16 in C5, C11 and C17. On the other hand the maximum growth rate of 7.19 per cent was found in colony number C6 followed by 4.68 in C2, 3.57 in C13 and 1.75 per cent in colony number C12. The mean growth rate of mites in all colonies was 1.35 ± 0.41 (mean \pm SD).

4.2 BEE ACTIVITY

The data on outgoing and incoming bees during the months of March, June and September are presented in Table 13 to 15.



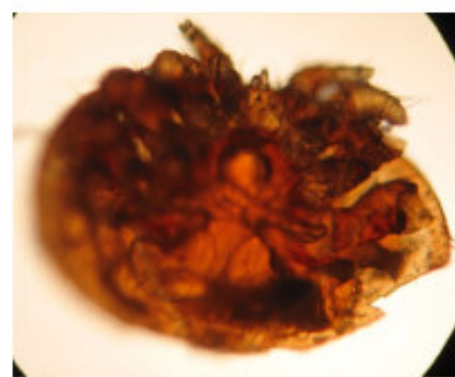
Photograph 15. Undamaged mite



Photograph 16. Damaged leg



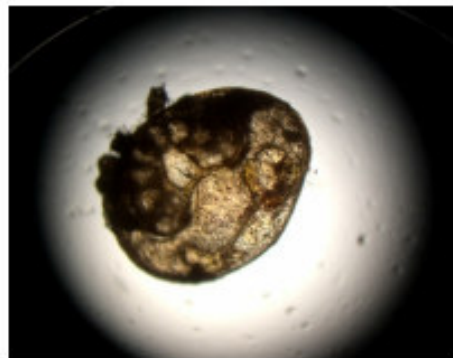
Photograph 17. Damaged shield



Photograph 18. Damaged leg + damaged shield



Photograph 19. Carcass or empty dorsal shield



Photograph 20. Depression in dorsal shield

Photographs (15-20) Different types of damage in mites

Table 11. Different types of damage to body parts of *Varroa* in the experimental colonies during July-August, 2007

Type of damage	Per cent mites with type of damage																				
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	Mean
Damaged legs	16.95 (24.18)	22.66 (27.80)	47.52 (43.60)	20.49 (26.76)	15.45 (23.01)	16.98 (24.06)	16.10 (23.44)	21.83 (27.64)	13.92 (21.75)	19.26 (25.85)	52.67 (46.59)	21.87 (27.85)	43.89 (41.46)	46.64 (42.99)	18.50 (25.41)	17.18 (24.04)	35.95 (36.76)	18.07 (25.05)	11.07 (17.34)	17.15 (24.44)	24.71 (29.00)
Damaged shield	06.11 (12.63)	06.11 (11.14)	04.49 (07.79)	05.33 (10.37)	05.33 (10.37)	04.36 (09.34)	11.68 (19.61)	03.14 (06.51)	05.39 (13.36)	09.37 (17.42)	10.50 (14.77)	03.68 (08.41)	03.33 (04.82)	02.86 (04.44)	02.38 (05.62)	03.44 (08.12)	05.84 (11.89)	03.30 (08.03)	03.96 (08.80)	08.17 (15.98)	05.44 (10.47)
Damaged shield +Damaged legs	02.18 (05.39)	00.00 (00.00)	01.82 (03.51)	06.97 (11.73)	02.67 (05.94)	04.86 (08.13)	03.00 (06.35)	00.00 (00.00)	01.89 (04.99)	02.85 (05.93)	03.33 (04.82)	03.21 (06.58)	03.89 (07.25)	05.36 (08.58)	02.86 (06.20)	01.15 (03.90)	00.54 (01.89)	01.34 (04.22)	01.00 (02.58)	01.70 (04.68)	02.53 (05.13)
Carcass or empty dorsal shield	00.00 (00.00)	04.44 (07.79)	02.00 (03.69)	02.86 (04.44)	00.69 (02.14)	01.11 (02.73)	01.33 (02.99)	00.00 (00.00)	00.98 (03.59)	00.00 (00.00)	00.00 (00.00)	00.91 (02.46)	05.00 (08.18)	05.00 (06.00)	01.05 (02.65)	00.53 (01.87)	00.00 (00.00)	00.63 (02.04)	00.91 (02.46)	02.22 (06.57)	01.48 (02.98)
Hollow or depression in dorsal shield	04.47 (09.37)	00.00 (00.00)	03.15 (06.50)	04.19 (07.43)	02.71 (06.04)	03.33 (04.82)	01.43 (03.10)	07.58 (14.30)	05.78 (13.88)	02.86 (04.44)	00.00 (00.00)	00.00 (00.00)	02.22 (03.89)	02.86 (04.44)	00.00 (00.00)	03.67 (07.04)	05.10 (09.60)	04.46 (09.22)	09.39 (13.47)	05.28 (10.14)	03.42 (06.39)
Hollow in dorsal shield +damaged legs	03.65 (07.00)	00.00 (00.00)	02.00 (03.69)	00.00 (00.00)	00.69 (02.14)	02.00 (03.69)	03.10 (06.46)	04.44 (05.63)	02.48 (06.96)	00.00 (00.00)	03.33 (04.82)	01.54 (03.22)	00.00 (00.00)	02.50 (04.14)	00.00 (00.00)	03.33 (04.82)	01.77 (04.78)	00.63 (02.04)	00.00 (00.00)	00.59 (01.98)	01.60 (03.07)
	05.56 (09.76)	05.54 (07.79)	10.16 (11.46)	06.64 (10.12)	04.59 (08.27)	05.44 (08.79)	06.11 (10.33)	06.17 (09.01)	05.07 (10.75)	05.72 (08.94)	11.64 (11.83)	05.20 (08.09)	09.72 (10.93)	10.87 (11.77)	04.13 (06.65)	04.88 (08.30)	08.20 (10.82)	04.74 (08.43)	04.39 (07.44)	05.85 (10.63)	

* Figures in parentheses are arc sin transformed values

CD_{0.05}

Colony = 03.77

Damaged = 02.06

Colony x Damaged = 09.25

Table 12. Growth rate of *Varroa* in different experimental colonies during April – June, 2007

Colony	Per cent growth rate per day*
C1	0.31
C2	4.68
C3	-0.01
C4	0.74
C5	0.13
C6	7.19
C7	0.24
C8	1.70
C9	1.19
C10	-0.16
C11	0.09
C12	1.75
C13	3.57
C14	0.58
C15	0.91
C16	1.23
C17	0.16
C18	0.97
C19	1.13
C20	0.51
Mean ± SD	1.35 ± 0.41

*Calculated from pre and post count of mites for a period of 60 days

4.2.1 Bee activity during March-April.

The data on the number of outgoing and incoming bees are presented in Table 13. The number of outgoing bees was significantly more in C3, C10 and C12 being 30.11, 30.89 and 30.89 bees per minute, respectively, than all other test colonies. The minimum number of outgoing bees was in C4 and C7 (respective values being 16.44 and 16.22 bees/ minute, differences non-significant). In rest of the colonies (C1-2, C5-6, C8-9 and C11) the values ranged between 23.67 to 27.89 bees per minute. The data on incoming bees revealed that on an average colony C3 had 27.33 bees coming into the hive, which was significantly higher than in all the other colonies. On the other hand C4 had significantly minimum number of 14.33 bees entering the hives followed by C7

Table 13. Number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of March-April 2007

Number of bees leaving or entering/minute									
Colony number	Date	Outgoing bees				Incoming bees			
		30 th March	1 st April	3 rd April	Mean	30 th March	1 st April	3 rd April	Mean
C1		26.00	26.67	26.00	26.22	24.00	25.67	24.33	24.67
C2		23.33	25.00	24.33	24.22	21.67	26.67	16.67	21.67
C3		31.33	28.67	30.33	30.11	25.33	28.67	28.00	27.33
C4		16.67	17.33	15.33	16.44	14.33	14.33	14.33	14.33
C5		26.33	27.33	25.00	26.22	23.67	26.00	22.00	23.89
C6		25.33	24.00	21.67	23.67	24.33	26.33	24.00	24.89
C7		16.67	16.00	16.00	16.22	19.33	17.67	18.00	18.33
C8		27.00	28.33	28.33	27.89	18.33	17.33	26.33	20.67
C9		27.33	28.33	25.33	27.00	27.00	21.33	21.33	23.22
C10		31.00	33.00	28.67	30.89	26.67	27.00	21.33	25.00
C11		27.33	27.00	24.67	26.33	30.33	20.00	22.00	24.11
C12		31.33	31.00	30.33	30.89	25.33	25.67	24.67	25.22
Mean		25.81	26.06	24.67		23.36	23.06	21.92	

CD_{0.05}

Colony	2.01	2.06
Date	1.00	1.03
Colony X Date	3.47	3.57

Coefficient of correlation

- i. Outgoing bees and colony infestation level ($r=-0.2305$; non significant at 10 df)
- ii. Incoming bees and colony infestation ($r=-0.2006$; non significant at 10 df)

and C8; respective values being 18.33 and 20.67. The number of incoming bees varied non-significantly between 23.22 to 25.22 bees in colonies C1, C5-6 and C9-12.

The analysis of data on outgoing and incoming bees revealed that there was no significant co-relation between the bee activity of the colony and level of infestation/overall colony infestation (Table 13), r being 0.23 and 0.2006, respectively, for outgoing and incoming bees.

4.2.2 Bee activity during June

The data on number of outgoing and incoming bees presented in Table 14 revealed that number of outgoing bees per minute was significantly more in colonies C3, C11, C13 and C14 being 11.00, 12.33, 12.56 and 8.44 bees, respectively, than all the other colonies (number of outgoing bees varied between 0.89 to 6.22). The colony C11 and C13 also had significantly more number of outgoing bees than colonies C3 and C14, however, the difference were non-significant between C11 and C13. On the other hand C4 had significantly minimum number of 0.89 bees going out of the colony. The data on incoming bees revealed that on an average the colonies C3, C11, C13 and C14 had 14.33, 13.78, 12.67 and 9.89 bees coming into the hives, which was significantly higher than rest of the colonies (number of incoming bees varied between 2.11 to 8.44). The colony C3 also had significantly more number of incoming bees than C13 and C14. However, the differences were non-significant between C3 and C11 and also between C11 and C13.

The analysis of data on outgoing and incoming bees revealed that there was significant negative co-relation between the bee activity of the colony and level of infestation, r being -0.8090 and -0.8868, respectively, for outgoing and incoming bees.

4.2.3 Bee activity during September

The data on number of outgoing bee per minute during the month of September (Table 15) revealed that the colonies C11 and C14 had 14.78 and

Table 14. Number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of June, 2007

Date Colony number	Number of bees leaving or entering/minute							
	Outgoing bees				Incoming bees			
	26 th June	28 th June	30 th June	Mean	26 th June	28 th June	30 th June	Mean
C1	5.00	6.33	4.67	5.33	6.67	6.00	7.33	6.67
C2	4.33	3.00	2.67	3.33	2.67	5.67	5.33	4.56
C3	12.00	10.33	10.67	11.00	16.00	13.33	13.67	14.33
C4	00.67	1.00	1.00	00.89	1.33	2.00	3.00	2.11
C5	4.67	3.33	3.00	3.67	6.00	6.00	6.33	6.11
C6	5.33	4.33	3.33	4.33	7.00	8.33	8.67	8.00
C7	6.00	6.33	5.00	5.78	6.67	7.67	8.00	7.44
C8	4.33	4.33	3.67	4.11	7.67	8.00	8.00	7.89
C9	6.00	6.33	6.33	6.22	7.67	7.00	8.00	7.56
C10	7.33	5.67	5.67	6.22	7.00	8.33	10.00	8.44
C11	12.00	12.67	12.33	12.33	13.67	14.00	13.67	13.78
C12	4.67	6.33	5.33	5.44	6.67	6.67	7.00	6.78
C13	13.67	12.33	11.67	12.56	13.00	12.33	12.67	12.67
C14	8.00	8.67	8.67	8.44	10.00	9.67	10.00	9.89
C15	5.67	5.67	4.33	5.22	3.67	5.67	4.33	4.56
C16	6.33	6.67	5.67	6.22	3.33	5.33	3.67	4.11
C17	4.67	5.67	4.67	5.00	4.00	4.33	3.00	3.78
C18	6.00	5.33	5.00	5.44	1.33	2.00	4.00	2.44
C19	7.33	5.67	5.33	6.11	2.00	3.00	3.00	2.67
C20	6.33	5.67	5.00	5.67	6.00	4.00	3.67	4.56
mean	6.52	6.28	5.70		6.62	6.97	7.17	

CD_{0.05}

Colony	1.13	1.35
Date	0.44	0.52
Colony X Date	1.96	2.34

Coefficient of correlation

- i. Outgoing bees and colony infestation level ($r=-0.8090$; significant at 18 df)
- ii. Incoming bees and colony infestation ($r=-0.8868$; significant at 18 df)

Table 15. Number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of September, 2007

Number of bees leaving or entering/minute								
Date Colony number	Outgoing bees				Incoming bees			
	1 st Sept	3 rd Sept	5 th Sept	Mean	1 st Sept.	3 rd Sept	5 th Sept	Mean
C1	8.33	7.00	8.00	7.78	9.33	7.33	7.33	8.00
C3	12.00	11.00	8.33	10.44	13.67	13.33	9.33	12.11
C4	4.00	3.67	4.67	4.11	5.33	5.00	6.00	5.44
C5	4.67	3.33	5.33	4.44	5.00	4.33	6.33	5.22
C6	6.00	6.33	6.00	6.11	7.00	7.33	6.67	7.00
C7	7.00	6.00	4.67	5.89	7.00	7.00	6.33	6.78
C10	3.67	3.33	4.33	3.78	3.33	4.33	5.00	4.22
C11	13.33	15.33	15.67	14.78	14.67	16.67	17.33	16.22
C13	10.67	11.67	9.67	10.67	12.00	13.00	10.33	11.78
C14	11.33	13.00	11.33	11.89	12.00	14.67	15.00	13.89
C15	4.00	2.67	2.33	3.00	4.00	4.33	2.33	3.56
C17	2.33	3.00	2.33	2.56	2.00	3.33	3.00	2.78
C18	3.00	3.33	2.67	3.00	3.33	4.33	2.67	3.44
C19	1.67	1.33	1.33	1.44	3.33	3.00	2.67	3.00
Mean	6.57	6.50	6.19		7.29	7.71	7.17	

CD_{0.05}

Colony	=	1.16	1.38
Date	=	0.54	0.64
Colony X Date	=	2.01	2.39

Coefficient of correlation

- i. Outgoing bees and colony infestation level ($r=-0.3475$; non significant at 12 df)
- ii. Incoming bees and colony infestation ($r=-0.2847$; non significant at 12 df)

11.89 bees per minute leaving the hive, which was significantly higher than rest of the test colonies (number of outgoing bees varied between 1.44 to 10.67). Number of outgoing bees was also higher in C3 and C13 (number of outgoing bees being 10.44 and 10.67, respectively) compared to C1, C5-7, C10, C15 and C17-19 (number of outgoing bees varied between 1.44 to 7.78). The data on incoming bees showed that the colonies C3, C11, C13 and C14 had 12.11, 16.22, 11.78 and 13.89 bees, respectively, coming into the hive. These colonies had significantly higher number of incoming bees than rest of the colonies in which incoming bees per minute ranged between 2.78 to 8.00. The colony C11 also had more incoming bees than C14, C3 and C13 and C14 also had more incoming bees than C3 and C13.

Data on outgoing and incoming bees revealed that there was no significant co-relation between the bee activity of the colony and level of infestation, r being 0.3475 and 0.2847, respectively, for outgoing and incoming bees.

4.3 SELECTION OF COLONIES FROM OTHER APIARIES

4.3.1 Migratory bee colonies of Nauni, Solan

The data on average bee population, number of mites per 100 bees and number of mites per 50 brood cells of migratory unit of honey bee colonies of Nauni, Solan during May, 2007 to February, 2008 are presented in Table 16.

4.3.1.1 Population

The data on average bee population of migratory honey bee colonies revealed that out of seventeen colonies, 11 colonies had almost same average bee population varying non-significantly between 7607.67 to 11542.67 bees per colony (in colony M1, M3, M5-7, M9, M10-11, M13-14 and M17). Two colonies viz. M12 and M16 had significantly low bee population varying between 4459.67 to 5771.33 bees per colony as compared to M3, M10 and M14 which had bee population in the range of 8919.33 to 11542.67 bees per colony.

4.3.1.2 Number of mites per 100 adult bees

The data on average number of mites per 100 bees in colonies of migratory unit revealed that the count of mites per 100 bees in M10 and M11 was significantly less (1.33 to 1.67 mites/100 bees) as compared to ten other experimental colonies which had mite count in the range of 4 to 6 mites/100 bees (in colonies M1-4, M6, M8-9, M12 and M15-16). The difference of mite count in M10 and M11 was, however, non-significant when compared with five other colonies having mite count of 2 to 3.33 mites per 100 bees (colony M5, M7, M13-14 and M17).

Table16. Bee population and level of mite infestation of adult bees and brood in bee colonies from the migratory unit screened for *Varroa* tolerance

Colony number	Bee population (number of bees)	Number of mites per 100 bees	Number of mites per 50 brood cells
M1	7870.00(3.89)	5.00	17.67
M2	6558.33(3.81)	6.00	13.00
M3	11542.67(4.05)	4.00	11.67
M4	6820.67(3.83)	4.00	13.33
M5	8132.33(3.91)	3.33	10.67
M6	7607.67(3.87)	4.33	14.00
M7	8919.33(3.94)	2.67	6.33
M8	6296.00(3.79)	4.00	10.00
M9	7607.67(3.87)	5.00	16.67
M10	9444.00(3.96)	1.67	2.33
M11	8394.67(3.92)	1.33	3.67
M12	4459.67(3.62)	5.00	11.33
M13	8657.00(3.93)	2.67	5.00
M14	8919.33(3.95)	2.00	2.67
M15	7083.00(3.84)	4.33	10.33
M16	5771.33(3.75)	5.67	19.00
M17	8394.67(3.90)	3.00	6.00
CD _{0.05}	0.19	2.32	8.67

*Figures in parentheses are log transformed values

4.3.1.3 Number of mites per 50 brood cells

The number of mites per 50 brood cells was between 2.33 to 2.67 mites in M10 and M14 which was significantly less as compared to seven other colonies having mite count in the range of 11.67 to 19.00 mites per 50 brood cells (colony number M1-4, M6, M9 and M16). The differences were, however, non-significant in these two colonies (M10 and M14) compared with seven other experimental colonies, which had count of 3.67 to 10.67 mites/ colony (colony number M5, M7-8, M11, M13, M15 and M17).

4.3.2 Bee colonies from bhota

The data on average bee population, number of mites per 100 bees and number of mites per 50 brood cells of apiary at RHFRS, Bhota from September 2007 to March, 2008 are presented in Table 17

Table 17. Bee population and level of mite infestation of adult bees and brood in bee colonies of the Bhota apiary screened for *Varroa* tolerance

Colony number	Bee population (number of bees)	Number of mites per 100 bees	Number of mites per 50 brood cells
B1	8132.33(3.85)*	04.00	03.67
B2	5509.00(3.70)	05.00	08.00
B3	2885.67(3.46)	04.33	04.67
B4	4197.33(3.61)	05.00	04.33
B5	6296.00(3.76)	11.00	26.67
B6	3148.00(3.46)	05.67	12.00
B7	2623.00(3.42)	03.67	04.00
B8	4722.00(3.67)	04.33	04.00
B9	8657.00(3.87)	02.33	00.67
	CD _{0.05} = 0.19	NS	23.01

*Figures in parentheses are log transformed values

4.3.2.1 Population

The data on population of bee colonies (Table 17) showed that the colony number B9 had significantly higher population of 8657.00 bees than the colonies B3, B4, B6, B7 and B8 which on an average had 2885.67, 4197.33, 3148.00, 2623.00 and 4722.00 bees, respectively, per colony. However, the colony B9 had significantly same population compared to B1, B2 and B5 (population varied between 5509.00 to 8657.00 bees/colony).

4.3.2.2 Number of mites per 100 bees

The data on number of mites per 100 bees (Table 17) revealed that there were 4.00, 5.00, 4.33, 5.00, 11.00, 5.67, 3.67, 4.33 and 2.33 mites per 100 bees in colonies B1, B2, B3, B4, B5, B6, B7, B8 and B9, respectively. However, these colonies had mite infestation at par with each other.

4.3.2.3 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells revealed that colony B9 had significantly less (0.67) mites than B5 which on average had 26.67 mites per 50 brood cells. However, the mite infestation varied non-significantly between 0.67 to 12.00 mites per 50 brood cells in colonies B1-4 and B6-9.

4.3.3 Bee colonies from Karnal

The data of different parameters (population, number of mites per 100 bees and per 50 brood cells) used for screening of varroa tolerant colonies at Karnal from March, 2008 to June, 2008 are presented in Table 18.

4.3.3.1 Population

Colonies K10, K18, K39 and K40 on an average had population of 16264.67, 18888.00, 18363.33 and 18363.33 bees, respectively (differences non-significant). These colonies had significantly higher population than colonies K3, K7-8, K17, K19-24, K27, K29, K32-35, K37 and K44 in which the population ranged between 3672.67 to 9444.00 bees. The colony K18 in addition to above colonies also had significantly higher population of bees than colonies K9, K14, K31, K35, K38, K41, K45 and K46 in which average population ranged from 9444.00 to 10496.00 bees.

4.3.3.2 Number of Mites per 100 bees

The data on number of mites per 100 bees of colonies at Karnal revealed that (Table 18) there were no mites on the adult bees in the colonies K11, K18 and K35. These colonies (K11, K18 and K35) had significantly less mite infestation than colonies K1-6, K8, K16-17, K19-21, K23, K25, K28-34, K36, K38 and K41 to K46 (mite infestation varied between 1.67 to 4.33 mites/100 bees).

Table 18. Bee population and level of mite infestation of adult bees and brood in bee colonies of the Karnal apiary screened for *Varroa* tolerance

Colony number	Bee population (number of bees)	Number of mites per 100 bees	Number of mites per 50 brood cells
K1	14690.67 (4.14)*	2.00	3.00
K2	13116.67 (4.12)	2.00	1.67
K3	8919.33 (3.95)	2.67	1.00
K4	13116.67 (4.12)	4.33	0.33
K5	12067.33 (4.08)	2.00	1.33
K6	11542.67 (4.06)	2.00	0.67
K7	8919.33 (3.95)	0.67	0.33
K8	3672.67 (3.50)	2.33	1.67
K9	9968.67 (3.99)	1.33	1.33
K10	16264.67 (4.16)	0.67	1.00
K11	13641.33 (4.13)	0.00	0.33
K12	12067.33 (4.07)	0.67	0.33
K13	14166.00 (4.15)	1.33	1.67
K14	9968.67 (4.00)	1.00	1.00
K15	14690.67 (4.17)	1.00	1.00
K16	13641.33 (4.13)	2.00	1.67
K17	7870.00 (3.86)	2.00	0.33
K18	18888.00 (4.27)	0.00	.000
K19	4722.00 (3.66)	2.00	0.67
K20	6820.67(3.76)	2.00	2.33
K21	7345.33(3.86)	2.67	2.00
K22	8919.33(3.89)	0.33	0.67
K23	7345.33(3.86)	2.00	2.00
K24	9444.00(3.95)	0.33	0.33
K25	14166.00(4.15)	3.00	2.67
K26	14166.00(4.15)	0.33	0.00
K27	6296.00(3.80)	0.67	1.33
K28	11542.67(4.06)	2.00	2.00
K29	6820.67(3.83)	2.67	3.00
K30	13116.67(4.12)	2.67	1.33
K31	9968.67(4.00)	6.00	3.33
K32	5246.67(3.72)	2.67	3.00
K33	8394.67(3.92)	2.33	1.33
K34	8394.67(3.92)	2.00	2.00
K35	10493.33(4.01)	0.00	0.00
K36	13641.33(4.13)	2.33	2.67
K37	3672.67(3.56)	0.67	0.33
K38	10493.33(4.02)	2.67	1.33
K39	18363.33(4.24)	0.33	0.33
K40	18363.33(4.25)	1.00	0.67
K41	10493.33(4.02)	1.67	1.67
K42	13116.67(4.12)	2.33	1.00
K43	13116.67(4.12)	2.00	0.67
K44	8394.67(3.92)	1.67	1.67
K45	9444.00(3.97)	3.00	1.00
K46	10496(4.02)	3.00	2.67
CD _{0.05}	0.19	1.43	1.38

*Figures in parentheses are log transformed values

4.3.3.3 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells revealed that colonies K18, K26 and K35 had no mites (0.00 mites each) per 50 brood cells. The colonies K1- 2, K13, K16, K20-21, K23, K25, K28-29, K31-32, K34, K36, K38, K41, K44 and K46 had mite infestation varying between 1.33 to 3.00/50 brood cells).

4.4 RAISING AND EVALUATION OF FIRST GENERATION QUIENS

Daughter queens were raised during the month of April and May, 2008 from the colony C3 out of the four surviving colonies, since this colony showed better mite tolerance (minimum 24 hour mite fall, low mite infestation, higher hygienic and grooming behaviour) along with better colony performance (higher population, brood, pollen and honey stores) throughout the year. These queens were openly mated with drones from the colonies in the apiary at Nauni, Solan.

The data on different parameters relating to performance of first generation daughter queens raised from selected mother colonies have been compared with those of mother colonies and queens raised from general unselected stock (Table 19 to 32). The data were recorded on different parameters under Nauni condition (from June to October, 2008) and under migratory conditions (December 2008 to March 2009).

4.4.1 Population

In general, irrespective of number of the colonies, the population of colonies (Table 19) headed by daughter queens was significantly higher (10652.61 bees) than the general stock (7232.9 bees) but less than the mother colonies (13004.24 bees).

Average population of bees in colonies headed by daughter queens was significantly higher in case of C3D1, C3D2 and C3D4 (10680.71 to 13603.86 bees/colony) as compared to colonies headed by queens from general stock viz. colonies G1 to G3 (6633.29 to 7757.00 bees/colony). However, one daughter colony C3D3 had bee population at par with G1 to G3 (population varying non-

significantly between 6633.29 to 7757.00 bees/colony). One mother colony MC3 had significantly highest bee population of 16639.43 bees than all other colonies. Mother colonies MC11 and MC14 had population at par with the colonies headed by daughter queens C3D1 and C3D4 (population varying non-significantly between 10680.71 to 11580.14 bees)

Table19. Bee population in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	Bee population (number of bees)	Group mean
A (Mother colonies)	MC3	16639.43*(4.22)	13004.24(4.10)*
	MC11	10793.14(4.02)	
	MC14	11580.14(4.06)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	10905.57(4.03)	10652.61(4.01)
	C3D2 ♀(MC3) X ♂(OM)	13603.86(4.12)	
	C3D3 ♀(MC3) X ♂(OM)	7420.29(3.87)	
	C3D4 ♀(MC3) X ♂(OM)	10680.71(4.02)	
C (General stock)	G 1	7757.57(3.89)	7232.90 (3.86)
	G 2	6633.29(3.82)	
	G 3	7307.86(3.86)	
	CD _{0.05}	0.07	A,CXB = 0.04 AXC = 0.04

*Figures in parentheses are log transformed value

OM = open mated

4.4.2 Brood area

The data obtained on brood reared by experimental colonies from June to October, 2008 presented in Table 20, revealed that irrespective of number of the colonies, the colonies headed by daughter queens reared significantly more brood (2752.25 cm²) than the general stock (1471.14 cm²) but reared significantly less brood than the mother colonies (3541.95 cm²).

Irrespective of the group, the colonies C3D1, C3D2 and C3D4 headed by daughter queens reared significantly more brood (2386.00 to 4106.40 cm²) than the colonies G1 to G3 (1362.90 to 1607.10 cm²) headed by queens from general stock. However, daughter colony C3D3 had average brood area at par with

general stock colonies G1 and G3 (average brood area varying between 1362.90 to 1945.10 cm²). The brood area of daughter colony C3D2 (4106.40 cm²) was significantly at par with the mother colony MC3 (4430.60 cm²). The daughter Queens C3D1 and C3D4 had brood area significantly at par with the mother colonies MC11 and MC14 (average brood area varying between 2386.00 to 3221.00 cm²).

Table 20. Brood area(cm²) in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	Brood area (cm ²)	Group mean
A (Mother colonies)	MC3	4430.60(3.63)*	3541.95
	MC11	3221.00(3.49)	(3.52)
	MC14	2974.30(3.46)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	2386.00 (3.37)	2752.25
	C3D2 ♀(MC3) X ♂(OM)	4106.40(3.60)	(3.41)
	C3D3 ♀(MC3) X ♂(OM)	1945.10(3.27)	
	C3D4 ♀(MC3) X ♂(OM)	2571.40(3.41)	
C (General stock)	G 1	1607.10(3.20)	1471.14
	G 2	1362.90(3.13)	(3.16)
	G 3	1443.40(3.15)	
	CD _{0.05}	0.12	A,CXB = 0.06 AXC = 0.07

*Figures in parentheses are log transformed value

4.4.3 Pollen stores

Pollen stores, irrespective of the number of colonies averaged to 171.14 and 206.38 cm² (differences non-significant) in colonies headed by daughter queens and mother colonies, respectively (Table 21). These colonies had significantly higher pollen stores than the colonies headed by queens from general stock which had pollen stores of 95.86 cm².

In general, average pollen stores of bee colonies headed by daughter queens from selected stock was significantly higher in case of C3D2 (273.57 cm²) as compared to colonies headed by queens from general stock viz. G1 to G3 (85.57 to 104.57 cm²). However, the colony C3D2 had pollen stores at par with

the mother colonies MC3 to MC14 (average pollen stores varying between 165.00 to 273.57 cm²). The daughter queen C3D1, C3D3 and C3D4 also had pollen store at par with the general stock colonies G1 to G3 (average pollen stores varying between 85.57 to 148.86 cm²).

Table 21. Pollen area (cm²) in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	Pollen area (cm ²)	Group mean
A (Mother colonies)	MC3	265.57(2.38)*	206.38 (2.26)
	MC11	188.57(2.20)	
	MC14	165.00(2.19)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	148.86(2.14)	171.14 (2.17)
	C3D2 ♀(MC3) X ♂(OM)	273.57(2.42)	
	C3D3 ♀(MC3) X ♂(OM)	118.43(2.00)	
	C3D4 ♀(MC3) X ♂(OM)	143.71(2.11)	
C (General stock)	G 1	104.57(1.99)	95.86 (1.96)
	G 2	85.57(1.91)	
	G 3	97.43(1.98)	
	CD _{0.05}	0.23	A,CXB =0.12 AXC =0.13

*Figures in parentheses are log transformed values

4.4.4 Honey stores

The data on average honey stores in the experimental colonies (Table 22) from June to October, 2008 revealed that significantly higher honey stores of 1275.36 and 1502.38 grams(differences non-significant) were present in colonies headed by daughter queens and mother colonies compared to colonies headed by queens from general stock which had honey stores of 864.29 grams.

Individually, significantly higher honey stores of 1258.57, 1457.14 and 1314.29 grams were present, respectively, in colonies C3D1, C3D2 and C3D4 headed by daughter queens as compared to the colonies G2 and G3 headed by queens from general stock, which had honey stores of 842.86 and 828.57 grams, respectively. However, daughter colony C3D3 had honey stores at par with colonies G1 to G3 (honey stores varied between 828.57 to 1071.43 grams,

respectively). One mother colony MC3 also had significantly higher honey stores of 2064.29 grams than all other colonies. Mother colonies MC11 and MC14 had honey stores at par with the daughter colonies C3D1 to C3D4 (honey stores varied between 1071.43 to 1457.14 grams).

Table 22. Honey stores (grams) in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	Honey stores (grams)	Group mean
A (Mother colonies)	MC3	2064.29(3.30)	1502.38 (3.14)
	MC11	1228.57(3.05)	
	MC14	1214.29(3.08)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	1258.57(3.08)	1275.36 (3.08)
	C3D2 ♀(MC3) X ♂(OM)	1457.14(3.13)	
	C3D3 ♀(MC3) X ♂(OM)	1071.43(3.01)	
	C3D4 ♀(MC3) X ♂(OM)	1314.29(3.10)	
C (General stock)	G 1	921.43(2.96)	864.29 (2.92)
	G 2	842.86(2.91)	
	G 3	828.57(2.89)	
	CD _{0.05}	0.15	A,CXB = 0.08 AXC = 0.09

*Figures in parentheses are log transformed values

4.4.5 24-hour natural mite fall

The data (Table 23) on number of mites falling within 24 hours on bottom board indicated that the mite fall was significantly less (1.07) in colonies headed by daughter queens as compared to colonies headed by queens from general stock (3.90). However, the mother colonies had significantly less mite fall (0.24) than colonies headed by daughter queens and general stock.

Individually, the 24 hour mite fall was significantly less in colonies C3D1 to C3D4 (varying between 0.43 to 1.43) as compared to colonies headed by queens from general stock viz G1 to G3 (varying between 3.43 to 4.29). However, the colonies C3D2 and C3D3 had mite fall at par with the mother colonies MC3 to MC14 (mite fall varying non-significantly between 0.00 to 0.57)

Table 23. 24 hours natural mite fall in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	24 hour natural mite fall (number of mites)	Group mean
A (Mother colonies)	MC3	0.00	0.24
	MC11	0.14	
	MC14	0.57	
B (Daughter Queens)	C3D1 ♀(MC3) x ♂(OM)	1.43	1.07
	C3D2 ♀(MC3) x ♂(OM)	0.43	
	C3D3 ♀(MC3) x ♂(OM)	0.57	
	C3D4 ♀(MC3) x ♂(OM)	1.86	
C (General stock)	G 1	4.29	3.90
	G 2	4.00	
	G 3	3.43	
	CD _{0.05}	0.93	A,CXB =0.50 AXC =0.54

4.4.6 Number of mites per 100 bees

The data (Table 24) on number of mites per 100 bees revealed that in general, irrespective of number of the colonies, the colonies headed by daughter queens had significantly less (0.75 mites/100 bees) number of mites on adult bees than colonies headed by queens from general stock (2.29 mites/100 bees) but had significantly higher mite infestation compared to the mother colonies (0.24 mites/100 bees).

Individually, the average number of mites per 100 bees in colonies headed by daughter queens was significantly less in case of C3D1 to C3D4 (varying between 0.42 to 1.14 mites/100 bees) as compared to colonies G1 to G3 headed by queens from general stock (varying between 2.14 to 2.43 mites/100 bees). However, the colonies C3D2 and C3D3 had adult bee infestation at par with the mother colonies MC3 and MC11 (mites per 100 bees varying non-significantly between 0.00 to 0.43).

Table 24. Mite infestation of adult bees and brood in colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008

Group	Colony number	Number of mites per 100 bees	Group mean	Number of mites per 50 brood cells	Group mean
A (Mother colonies)	MC3	0.00	0.24	0.00	0.05
	MC11	0.14		0.00	
	MC14	0.57		0.14	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	1.14	0.75	0.00	0.25
	C3D2 ♀(MC3) X ♂(OM)	0.42		0.14	
	C3D3 ♀(MC3) X ♂(OM)	0.43		0.14	
	C3D4 ♀(MC3) X ♂(OM)	1.00		0.71	
C (General stock)	G 1	2.29	2.29	1.43	1.95
	G 2	2.43		2.14	
	G 3	2.14		2.29	
	CD _{0.05}	0.53	A,CXB = 0.29 AXC = 0.31	0.59	A,CXB = 0.32 AXC = 0.34

4.4.7 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells (Table 24) revealed that colonies headed by daughter queens had significantly less (0.25 mites/50 brood cells) number of mites per 50 brood cells than the colonies headed by queens from general stock (1.95 mites/50 brood cells) but had mite infestation of brood significantly at par with the mother colonies (0.05 mites/50 brood cells).

The average number of mites per 50 brood cells in colonies headed by daughter queen was significantly less in case of C3D1 to C3D4 (varying between 0.00 to 0.71 mites/50 brood cells) as compared to colonies headed by queens from general stock viz. G1 to G3 (varying between 1.43 to 2.29 mites/50 brood cells). However, the colonies C3D1, C3D2 and C3D3 had mite per 50 brood cells significantly at par with MC3 to MC14 (mite per 50 brood cells varying non-significantly between 0.00 to 0.71).

4.4.8 Hygienic behaviour

For estimating hygienic behaviour, pin killed brood assay was used. The data on the per cent pin killed brood cleaned by honey bees after 24 hours are depicted in Table 25. The data on percentage of pin killed brood removed by

honey bees after 24 hours of piercing revealed that bees of colonies headed by daughter queens cleaned significantly more pin killed brood (92.57%) than colonies headed by queens from general stock (69.33 %). However, the differences were non-significant between colonies headed by daughter queens compared to mother colonies (92.00%).

Table 25. Hygienic behaviour of the experimental colonies headed by daughter queens from the selected stock from Nauni, Solan in comparison to mother colonies and colonies from the general stock during June to October 2008 as determined by per cent brood cleaning of pin-killed brood

Group	Colony no	Colonies mean (% cleaned cells)	Group mean
A (Mother colonies)	MC3	96.00(80.33)*	92.00 (74.65)
	MC11	90.85(72.59)	
	MC14	89.14(71.03)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	93.71(76.89)	92.57 (76.22)
	C3D2 ♀(MC3) X ♂(OM)	97.14(83.62)	
	C3D3 ♀(MC3) X ♂(OM)	89.14(71.15)	
	C3D4 ♀(MC3) X ♂(OM)	90.29(73.22)	
C (General stock)	G 1	74.86(60.05)	69.33 (56.56)
	G 2	67.43(55.36)	
	G 3	65.71(54.27)	
	CD _{0.05}	6.20	A,CXB = 3.35 AXC = 3.58

*Figures in parentheses are arc sin transformed values

All the colonies headed by daughter queens, C3D1 to C3D4 removed significantly higher amount of pin killed brood (varying between 89.14 to 97.14 %) as compared to colonies G1 to G3 headed by queens from general stock (varying between 65.71 to 74.86 %). However, the differences were non-significant between colonies C3D1, C3D3 and C3D4 as compared to mother colonies MC11 and MC14 (per cent cleaned brood varied between 89.14 to 93.71 per cent). One daughter colony C3D2 had significantly higher percentage (97.14%) of cleaned brood cells than rest of the colonies except for the colony MC3 (96.00%).

4.4.9 Post capping duration

The data on the post capping period of brood in mother colonies, daughter colonies and general stock are presented in Table 26. These observations revealed that irrespective of months of observations the colony MC11 and C3D2 had significantly short post capping duration of 268 and 269.78 h, respectively, as compared to C3D3, MC3, MC14 and G1 to G3 having post capping period in the range of 272.89 to 277.78 h. However, the post capping duration of C3D2 (269.78 h) was at par with C3D1 (270.78 h). It was interesting to note that irrespective of type of colony; the post capping period during September (275.7) was significantly higher from that observed during July (272.07 h) or August (272.11 h).

Table 26. Post capping duration (in hours) of brood in honey bee colonies selected for *Varroa* tolerance during different months (2008)

Colony \ Month	Post capping duration (hours)			
	July	August	September	Mean
MC3	271.67	272.33	277.00	273.67
MC11	265.33	268.33	270.33	268.00
MC13	274.67	274.00	278.00	275.56
C3D1	270.33	270.00	272.00	270.78
C3D2	267.00	268.67	273.67	269.78
C3D3	271.67	271.33	275.67	272.89
G1	272.67	274.33	278.67	275.22
G2	276.00	274.00	278.00	276.00
G3	279.33	276.00	278.00	277.78
CD_{0.05}	272.07	272.11	275.70	

CD_{0.05}

Colony = 1.80

Month = 1.04

Colony X Month = 3.12

4.5 FURTHER EVALUATION OF COLONIES HEADED BY FIRST GENERATION QUEENS UNDER MIGRATORY CONDITIONS

For further evaluation the experimental colonies were migrated to Kadasan area of Ambala in the month of October, later to Hisar in the month of December and after that to Saha area of Ambala in the month of February, 2009 and were kept there up to the month of March.

4.5.1 Population

The data on population of experimental colonies under migratory conditions revealed that, irrespective of colonies, the population of colonies (Table 27) headed by daughter queens was significantly higher (12526.42 bees) than the general stock (7520.22 bees) but was at par with the mother colonies (11717.56 bees).

Table 27. Bee population in colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, during December, 2008 to March 2009 under migratory conditions

Group	Colony number	Bee population (number of bees)	Group mean
A (Mother colonies)	MC3	12854.33(4.10)*	11717.56 (4.06)
	MC11	11280.33(4.05)	
	MC14	11018.00(4.04)	
B (Daughter Queens)	C3D1 ♀(MC3) x ♂(OM)	11805.00(4.07)	12526.42 (4.09)
	C3D2 ♀(MC3) x ♂(OM)	15215.33(4.18)	
	C3D3 ♀(MC3) x ♂(OM)	11542.00(4.06)	
	C3D4 ♀(MC3) x ♂(OM)	11542.67(4.06)	
C (General stock)	G 1	8919.33(3.95)	7520.22 (3.87)
	G 2	7345.33(3.86)	
	G 3	6296.00(3.79)	
	CD _{0.05}	0.11	A,CXB = 0.06 AXC = 0.06

*Figures in parentheses are log transformed value

Average population of bees in colonies headed by daughter queens was significantly higher in case of C3D1 and C3D2 (11805.00 and 15215.33 bees/colony) as compared to colonies headed by queens from general stock viz. colonies G1 to G3 (6296.00 to 8919.33 bees/colony). However, two daughter colonies C3D3 and C3D4 had bee population at par with G1 (population varying non-significantly between 8919.33 to 11542.00 bees/colony). Daughter queens

C3D1, C3D3 and C3D4 had population at par with the mother colonies MC3 to MC14 (population varying non-significantly between 11018.00 to 12854.33 bees/colony)

4.5.2 Brood area

The data obtained on brood reared by experimental colonies from December 2008 to march 2009 presented in Table 28, revealed that irrespective of colonies, the colonies headed by daughter queens reared significantly more brood (2789.33 cm²) than the general stock (1451.22 cm²) but reared significantly equal brood compared to the mother colonies (2210.78 cm²).

Table 28. Average Brood area (cm²) in colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, during December, 2008 to March 2009 under migratory conditions

Group	Colony number	Brood area (cm ²)	Group mean
A (Mother colonies)	MC3		2210.78
	MC11	2307.33(3.35)*	(3.33)
	MC14	2246.33(3.35) 2078.67(3.30)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	2634.33(3.39)	2789.33
	C3D2 ♀(MC3) X ♂(OM)	3742.67(3.57)	(3.43)
	C3D3 ♀(MC3) X ♂(OM)	2298.67(3.35)	
	C3D4 ♀(MC3) X ♂(OM)	2481.67(3.39)	
C (General stock)	G 1	1750.67(3.24)	1451.22
	G 2	1327.67(3.10)	(3.13)
	G 3	1275.33(3.06)	
	CD _{0.05}	0.25	A,CXB = 0.13 AXC = 0.14

*Figures in parentheses are log transformed value

However, irrespective of the group, the colonies C3D1, C3D2 and C3D4 reared significantly more brood (respective amount of brood reared being 2634.33, 3742.67 and 2481.67 cm²) than the colonies G2 and G3 (respective amount of brood reared being 1327.67 and 1275.33 cm²) headed by queens from general stock. However, daughter colony C3D3 had average brood area at par with general stock colonies G1 and G2 (average brood area varying between 1327.67 to 2298.67 cm²). The daughter colonies C3D1 to C3D4 had brood area significantly at par compared to the mother colonies MC3 and MC11 (average brood area varying between 2246.33 to 3742.67 cm²). One daughter colony

C3D2 reared significantly more brood than all the colonies headed by queens from general stock (G1, G2 and G3) and also one mother colony MC14.

4.5.3 Pollen stores

Pollen stores, irrespective of the different colonies averaged to 373.00 and 285.00 cm² (differences non-significant) in colonies headed by daughter queens and mother colonies, respectively. These colonies had significantly higher pollen stores than the colonies from general stock which had pollen stores of 171.67 cm² (Table 29).

Table 29. Pollen stores (cm²) in colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, during December, 2008 to March 2009 under migratory conditions

Group	Colony number	Pollen area (cm ²)	Group mean
A (Mother colonies)	MC3	289.67(2.46)*	285.00 (2.43)
	MC11	240.33(2.38)	
	MC14	325.00(2.44)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	281.00(2.44)	373.00 (2.52)
	C3D2 ♀(MC3) X ♂(OM)	669.67(2.76)	
	C3D3 ♀(MC3) X ♂(OM)	259.67(2.41)	
	C3D4 ♀(MC3) X ♂(OM)	281.67(2.44)	
C (General stock)	G 1	204.33(2.31)	171.67 (2.22)
	G 2	156.00(2.17)	
	G 3	154.67(2.18)	
	CD _{0.05}	0.25	A,CXB= 0.14 AXC = 0.15

*Figures in parentheses are log transformed values

Average pollen store of bee colonies headed by daughter queens was significantly higher in case of C3D1, C3D2 and C3D4 (281.00 to 669.67cm²) as compared to colonies headed by queens from general stock viz. G1 to G3 (154.67 to 204.33 cm²). However the colony C3D3 had pollen stores at par with the general stock colonies G1 to G3 (average pollen stores varying between 154.67 to 259.67 cm²). Daughter colony C3D2 had significantly higher pollen stores than all the other colonies. The daughter colonies C3D1, C3D3 and C3D4 also had pollen store at par compared to the mother colonies MC3 to MC14 (average pollen stores varying between 240.33 to 325.00 cm²).

4.5.4 Honey stores

The data on average honey stores in the experimental colonies (Table 30) revealed that significantly higher honey stores of 1529.17 and 1466.67 grams (differences non-significant) were present in colonies headed by daughter queens and mother colonies than the colonies headed by queens from general stock having stores of 988.89 grams.

Table 30. Honey stores (grams) in colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, during December, 2008 to March 2009 under migratory conditions

Group	Colony number	Honey Stores (grams)	Group mean
A (Mother colonies)	MC3	1616.67(3.20)*	1466.67 (3.15)
	MC11	1466.67(3.15)	
	MC14	1316.67(3.09)	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	1683.33(3.20)	1529.17 (3.16)
	C3D2 ♀(MC3) X ♂(OM)	1866.67(3.26)	
	C3D3 ♀(MC3) X ♂(OM)	1233.33(3.07)	
	C3D4 ♀(MC3) X ♂(OM)	1333.33(3.11)	
C (General stock)	G 1	1200.00(3.06)	988.89 (2.93)
	G 2	866.67(2.80)	
	G 3	900.00(2.94)	
	CD _{0.05}	0.35	A,CXB = 0.19 AXC = 0.20

*Figures in parentheses are log transformed values

Individually, significantly higher honey stores of 1683.33 and 1866.67 grams were present, respectively, in colonies C3D1 and C3D2 as compared to the colony G2 which had honey store of 866.67 grams. However, daughter colonies C3D3 and C3D4 had honey stores at par with colonies G1 to G3 (honey stores varied between 866.67 to 1333.33 grams). Mother colonies MC3 to MC14 also had honey stores at par with the daughter colonies C3D1 to C3D4 (honey stores varied between 1233.33 to 1866.67 grams).

4.5.5 Amount of honey harvested

Surplus honey was harvested from the experimental colonies during the month of January to March, 2008. Each time during harvesting some amount of honey was left in colonies so as to ensure the availability of food (honey) for bees. The data on total amount of honey harvested from different colonies are presented in Figure 1. Maximum amount of honey (13800 grams) was harvested from colony C3D2 followed by colonies MC3, C3D1, C3D4, MC14, C3D3, G1, MC11, G2 and G3 from which 12000, 8500, 8300, 8000, 7700, 5000, 4400, 2000 and 1800 grams of honey was harvested, respectively.

4.5.6 Number of mites per 100 bees

The data on number of mites per 100 bees (Table 31) revealed that in general, irrespective of colonies, the colonies headed by daughter queens from selected stock had significantly less (0.92 mites/100 bees) number of mites on adult bees compared to colonies headed by queens from general stock (4.00 mites/100 bees). But the mite infestation of adult bees was at par compared to the mother colonies (0.44 mites/100 bees).

Taking individual colonies into consideration, the average number of mites per 100 bees in colonies headed by daughter queens was significantly less in case of C3D1 to C3D4 (varying between 0.67 to 1.33 mites/100 bees) as compared to colonies G1 to G3 headed by queens from general stock (varying between 3.67 to 4.33 mites/100 bees). However, the colonies C3D1 to C3D4 had adult bee infestation at par with the mother colonies MC3 to MC14 (mites per 100 bees varying non-significantly between 0.00 to 1.33).

4.5.7 Number of mites per 50 brood cells

The data on number of mites per 50 brood cells (Table 31) revealed that colonies headed by daughter queens had significantly less (0.33 mites/50 brood cells) number of mites per 50 brood cells than the colonies headed by queens from general stock (2.44 mites/50 brood cells). However, these colonies had mite infestation of brood significantly at par with the mother colonies (0.11 mites/50 brood cells).

Table 31. Mite infestation of adult bees and brood in colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, during December, 2008 to March 2009 under migratory conditions

Group	Colony number	Colonies mean (mites/100 bees)	Group mean	Colonies mean (mites/50 brood cell)	Group mean
A (Mother colonies)	MC3	0.00	0.44	0.00	0.11
	MC11	0.33		0.00	
	MC14	1.00		0.33	
B (Daughter Queens)	C3D1 ♀(MC3) X ♂(OM)	0.67	0.92	0.33	0.33
	C3D2 ♀(MC3) X ♂(OM)	0.67		0.00	
	C3D3 ♀(MC3) X ♂(OM)	1.00		0.33	
	C3D4 ♀(MC3) X ♂(OM)	1.33		0.67	
C (General stock)	G 1	3.67	4.00	2.33	2.44
	G 2	4.33		3.00	
	G 3	4.00		2.00	
	CD _{0.05}	1.62	A,CXB=0.87 AXC= 0.93	0.88	A,CXB=0.48 AXC= 0.51

Taking individual colonies into consideration, the average number of mites per 50 brood cells in colonies headed by daughter queens was significantly less in case of C3D1 to C3D4 (varying non-significantly between 0.00 to 0.67 mites/50 brood cells) as compared to colonies headed by queens from general stock viz. G1 to G3 (varying non between 2.00 to 3.00 mites/50 brood cells). However, the colonies C3D1 to C3D4 had mite per 50 brood cells significantly at par with MC3 to MC14 (mite per 50 brood cells varying non-significantly between 0.00 to 0.67).

4.6 RAISING AND EVALUATION OF SECOND GENERATION QUEENS

Based on all the parameters together (minimum 24 hour mite fall, low mite infestation, higher hygienic behaviour and colony performance) the colony C3D2 was selected and was used for raising second generation queens during the month of March to April, 2008. Colony K18 selected from Karnal apiary on the basis of low mite infestation and higher bee population was used as drone mother colony for mating of these queens under isolation. Out of the raised queens three of the queens successfully mated under isolation with drones of colony K18 and two of the queens openly mated with drones from the colonies of apiary at Nauri.

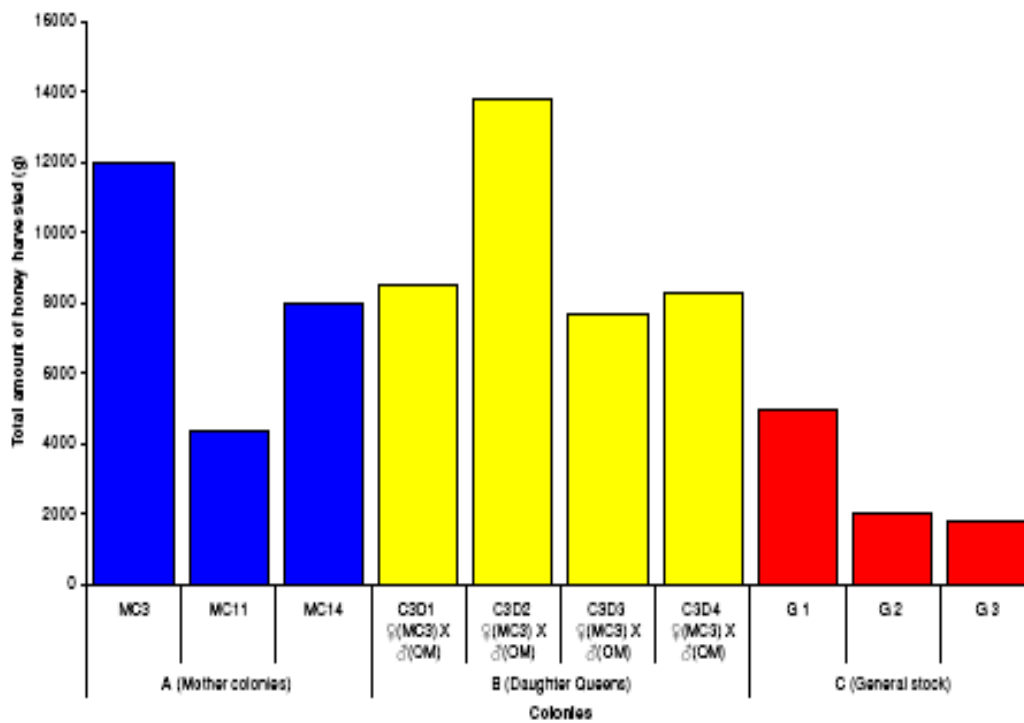


Fig. 1 Total amount of honey harvested from colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, from January, 2009 to March 2009 under migratory conditions

The data on different parameters relating to performance of second generation daughter queens raised from selected colony and mated under isolation (colonies C3D2-I-1, C3D2-I-2 and C3D2-I-3) and open mating conditions (colonies C3D2-O-1 and C3D2-O-2) are compared in Table 32 to 39. The data were recorded on different parameters under Nauni condition from May to August, 2009.

4.6.1 Population

The data on average population of second generation daughter colonies mated under isolated conditions and under open conditions are presented in Table 32. The results obtained revealed that on an average the colonies headed by queens mated under isolation had significantly higher population (5771.33 bees/colony) of bees than the colonies headed by queens mated under open conditions (4249.80 bees/colony). However, when individual colonies were compared, all the colonies had population at par with each other (population varying non-significantly between 4249.80 to 6296.00 bees).

Table 32. Population of colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	Bee population (number of bees)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	5194.20 (3.70)*	5771.33 (3.74)
	C3D2-I-2 ♀(C3D2) X ♂(K18)	5823.80(3.74)	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	6296.00(3.77)	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	4249.80(3.62)	4249.80 (3.62)
	C3D2-O-2 ♀(C3D2) X ♂(OM)	4249.80(3.62)	
	CD _{0.05}	NS	0.11

*Figures in parentheses are log transformed value

4.6.2 Brood area

The data on amount of brood reared (Table 33) by the experimental colonies revealed that the colonies headed by queens mated under isolation reared significantly more brood (1802.53cm²) than the colonies headed by queens mated under open conditions (1295.10 cm²).

Table 33. Brood area (cm²) of colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	Brood area(cm ²)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	1660.80(3.22)*	1802.53 (3.25)
	C3D2-I-2 ♀(C3D2) X ♂(K18)	1760.80(3.24)	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	1986.00(3.30)	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	1267.80(3.10)	1295.10 (3.10)
	C3D2-O-2 ♀(C3D2) X ♂(OM)	1322.40(3.11)	
	CD _{0.05}	0.08	0.05

*Figures in parentheses are log transformed value

Individually, colonies C3D2-I-1, C3D2-I-2 and C3D2-I-3 headed by queens mated under isolation reared significantly more brood (respective brood reared being 1600.80, 1760.80 and 1986.00 cm²) than the colonies C3D2-O-1 and C3D2-O-2 having queens mated under open conditions (respective brood reared being 1267.80 and 1322.40 cm²).

4.6.3 Pollen stores

Average pollen stores, irrespective of the colonies, averaged to 106.93 cm² in colonies headed by queens mated under isolation. These colonies had pollen stores than at par with the colonies headed by queen mated under open conditions which had pollen stores of 83.70 cm² (Table 34). Differences were also non-significant among all the colonies (average pollen stores varied between 82.20 to 117.80 cm²).

Table 34. Pollen area (cm²) of colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	Pollen area(cm ²)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	102.80(1.98)*	106.93(2.00)
	C3D2-I-2 ♀(C3D2) X ♂(K18)	104.00(1.99)	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	114.00(2.02)	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	82.20(1.89)	83.70(1.90)
	C3D2-O-2 ♀(C3D2) X ♂(OM)	85.20(1.91)	
	CD _{0.05}	NS	NS

*Figures in parentheses are log transformed value

4.6.4 Honey stores

Average honey stores, irrespective of the colonies, averaged to 1436.67 grams in colonies headed by queens mated under isolation which had significantly higher stores of honey than the colonies headed by queen mated under open conditions having honey stores of 1120.00 grams (Table 35).

Table 35. Honey stores (grams) of colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	Honey stores(g)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	1290.00(3.10)*	1436.67(3.15)
	C3D2-I-2 ♀(C3D2) X ♂(K18)	1450.00(3.16)	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	1570.00(3.19)	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	1160.00(3.06)	1120.00(3.04)
	C3D2-O-2 ♀(C3D2) X ♂(OM)	1080.00(3.03)	
	CD _{0.05}	0.11	0.07

*Figures in parentheses are log transformed value

When individual colonies were compared, the colonies C3D2-I-1 to C3D2-I-3 (average honey stores varying between 1450 to 1570g/colony) headed by queens mated under isolation had significantly higher honey stores than the colony C3D2-O-2 (1080g/colony) headed by queen mated under open condition. However, the difference was non-significant among colonies C3D2-I-1 and C3D2-I-2 compared to C3D2-O-1 (average honey stores varied between 1160 to 1450g/colony).

4.6.5 24-hour natural mite fall

The data on number of mites falling within 24 hours (Table 36) on bottom board was significantly at par in colonies headed by queens mated under isolated conditions (0.47) as compared to colonies headed by queens mated under open conditions (0.50).

Individually, the 24 our mite fall was significantly at par in all the colonies (viz. C3D2-I-1 -3 and C3D2-O-1-2) and varied between 0.40 to 0.60.

Table 36. Number of mites fallen after 24 hours in colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	24 hour natural mite fall (number of mites)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	0.40	0.47
	C3D2-I-2 ♀(C3D2) X ♂(K18)	0.60	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	0.40	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	0.60	0.50
	C3D2-O-2 ♀(C3D2) X ♂(OM)	0.40	
	CD _{0.05}	NS	NS

4.6.6 Number of mites per 100 bees and per 50 brood cells

The data on number of mites per 100 bees (Table 37) revealed that in general, irrespective of colonies, the colonies headed by queens mated under isolation had infestation of adult bees (0.20 mites/100 bees) significantly at par with the colonies headed by queens mated under open conditions (0.60 mites/100 bees). Irrespective of mating conditions, the average number of mites per 100 bees was also significantly at par in all the experimental colonies (varying non-significantly between 0.00 to 0.60 mites/100 bees).

Table 37. Average number of mites per 100 bees and per 50 brood cells in colonies headed by second generation daughter queens mated under isolated mating conditions and open conditions, during May to August, 2009

Group	Colony number	Number of mites/100 bees	Group mean	Number of mites per 50 brood cells	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀(C3D2) X ♂(K18)	0.40	0.20	0.20	0.13
	C3D2-I-2 ♀(C3D2) X ♂(K18)	0.00		0.00	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	0.20		0.20	
B (Openly mated Queens)	C3D2-O-1 ♀(C3D2) X ♂(OM)	0.60	0.60	0.40	0.30
	C3D2-O-2 ♀(C3D2) X ♂(OM)	0.60		0.20	
	CD _{0.05}	NS	NS	NS	NS

The data on number of mites per 50 brood cells (Table 37) revealed that the colonies headed by queens mated under isolation had infestation of sealed brood (0.13 mites/50 brood cells) significantly at par with the colonies headed by

queens mated under open conditions (0.30 mites/50 brood cells). Irrespective of mating conditions, the average number of mites per 100 bees was also significantly at par in all the experimental colonies (varying non-significantly between 0.00 to 0.40 mites/50 brood cells).

4.6.7 Hygienic behaviour

The data on the per cent pin killed brood cleaned by honey bees after 24 hours are depicted in Table 38. The data on percentage of pin killed brood removed by honey bees after 24 hours of piercing revealed that bees of colonies headed by queens mated under isolation cleaned significantly more pin killed brood (96.80%) than colonies headed by queens mated under open conditions (92.40 %).

Table 38. Per cent pin killed brood cell cleaned by second generation honey bee colonies (daughter colonies) mated under isolated mating conditions and open conditions, from May to August, 2009

Group	Colony number	Mean cleaned cells (%)	Group mean
A (Queens mated under isolation)	C3D2-I-1 ♀ (C3D2) X ♂(K18)	96.00(9.80)*	96.80 (9.84)
	C3D2-I-2 ♀(C3D2) X ♂(K18)	97.60(9.88)	
	C3D2-I-3 ♀(C3D2) X ♂(K18)	96.80(9.84)	
B (Openly mated Queens)	C3D2-O-1 ♀ (C3D2) X ♂(OM)	92.00(9.59)	92.40 (9.61)
	C3D2-O-2 ♀(C3D2) X ♂(OM)	92.80(9.63)	
	CD _{0.05}	0.26	0.17

*Figures in parentheses are square root transformed value

Individually, the colony C3D2-I-2 headed by queens mated under isolation removed significantly higher amount (97.60%) of pin killed brood as compared to colony C3D2-O-1 (92.00 %) headed by queen mated under open conditions. However, the differences were non-significant between colonies C3D2-I-1 to C3D2-I-3 compared to the colony C3D2-O-2 (per cent cleaned brood varied between 92.80 to 97.60 %).

4.6.8 Post capping duration

The data on the post capping period of brood in the experimental colonies in the month of July are presented in Table 39. The observations revealed that the colony C3D2-I-3 had significantly short post capping duration of 272.00 h as compared to C3D2-I-1, C3D2-O-1 and C3D2-O-2 having post capping period in the range of 277.33 to 278.00 h. However, the post capping duration of C3D2-I-2 (275.67 h) was at par with C3D2-I-1. The difference was also non-significant among the colonies C3D2-I-1, C3D2-I-2, C3D2-O-1 and C3D2-O-2 (post capping period varied non-significantly between 275.67 to 278.00 h).

Table 39. Post capping duration (in hours) of brood in colonies headed by second generation daughter queens mated under isolated mating conditions and open mating conditions, during May to August, 2009

Colony number	Post capping duration (in hours)
C3D2-I-1 ♀(C3D2) X ♂(K18)	277.33
C3D2-I-2 ♀(C3D2) X ♂(K18)	275.67
C3D2-I-3 ♀(C3D2) X ♂(K18)	272.00
C3D2-O-1 ♀(C3D2) X ♂(OM)	278.00
C3D2-O-2 ♀(C3D2) X ♂(OM)	277.67
CD _{0.05}	3.90

4.7 EFFICACY OF SUGAR DUSTING

4.7.1 Number of mites before and after sugar dusting

To know the effectiveness of sugar dusting in controlling *Varroa* mites, four sugar dustings were made during one brood cycle (June-July, 2007) in twenty colonies. Total number of mites on adult and brood per colony was worked out before start of sugar dusting as well as in the end of brood cycle. The results revealed that (Table 40) on an average there were 2451.39 ± 303.18 (mean \pm sd) mites in the colonies before dusting which increased to 4302.50 ± 749.55 mites after one brood cycle. However, the average number of mites on adult bees was 1413.06 ± 211.86 before dusting and decreased to 833.43 ± 130.32 mites at the end of the brood cycle. The mites on brood increase up to 3469.07 ± 691.72 at the end of the brood cycle from 1038.33 ± 167.03 mites present before the dusting.

Table 40. The total number of mites in the experimental colonies before and after sugar dusting

	Total number of mites	
	Before dusting	After dusting
Total number of mites in a colony	2451.39±303.18*	4302.50±749.55
Mites on adult bees	1413.06±211.86	833.43±130.32
Mites on brood	1038.33±167.03	3469.07±691.72

*Average values of 20 bee colonies

4.7.2 Percentage of mites fallen on bottom board at different time intervals

The data on percentage of mites falling within different time intervals after sugar dusting (Table 41) revealed that out of total mites falling within 24 hour of sugar dusting on an average 81.99, 82.93 and 78.96 percent felled within first hour of first (23/7/07), second(31/7/07) and third(18/8/07) dusting respectively. The percentage of mites falling within first hour in third sugar dusting were significantly lower than first and second sugar dusting. However, percentage of mites falling after 2 hours of sugar dusting varied between 4.83 to 5.86 percent(differences non significant), after 16 hours of dusting varied between 8.31 to 9.84 percent(differences non significant) and after 24 hours of dusting varied between 3.34 to 5.96 percent (differences non significant) during different dates of dusting.

Table 41. Percentage of mites fallen on bottom board at different time intervals after sugar dusting

Date \ Time	23/7/07	31/7/07	18/8/07	Mean percentage of mite fallen
1 hour after dusting	81.99 (65.41)*	82.93 (65.86)	78.96 (62.73)	81.30 (64.67)
2 hour after dusting	04.83 (11.99)	05.21 (12.81)	05.86 (13.74)	05.30 (12.85)
16 hour after dusting	09.84 (17.93)	08.31 (16.51)	09.22 (17.61)	09.13 (17.35)
24 hour after dusting	03.34 (10.12)	03.55 (10.42)	05.96 (13.84)	04.28 (11.46)

*Figures in parentheses are arc sin transformed values

CD_{0.05}

Time = 1.62
Time X Date = 2.82

In general, out of total mites falling within 24 hours of dusting on an average 81.30 per cent of mites fell within first hour of the dusting and remaining 5.30, 9.13 and 4.28 per cent of mites fell after 2, 16 and 24 hours of dusting, respectively.

Chapter-5

DISCUSSION

Improved honey production, handling qualities of bees, colony population growth, wax production and efficient pollination of specific crops may or may not be desired depending upon the specific origin of a beekeepers' profit. Generally, improved stocks which show increased production in a broad sense with reduced management costs are desired (Rinderer, 1986). Successful honey bee breeders first examine the specific need of particular region in which the stock developed is used. Based upon these general considerations most of the breeding programmes fall into two categories. In the first category the small pioneer projects mostly undertaken at university level explore ways to breed bees to avoid inbreeding, to reduce between colony variations or to maintain a desired stock and these provide genetic and breeding information which is useful for commercial breeding programmes. Such programmes have small base population of bee colonies. In the second category commercial breeding programmes are undertaken which are characterized by large number of colonies in base population (Kulincevic, 1986). The present study belongs to the first category having small base population of bee colonies.

Variation in resistance to several pathogens and pests exists in honey bees (Rothenbuhler *et. al.*, 1968). Among the various enemies, mite constitutes one of the major parasites of honey bees causing varying degree of damage (De Jong *et. al.*, 1982; Naim and Bisht, 1987). *Varroa*, which is an indigenous parasite of eastern honey bee (*A. cerana* F.) has become a major problem for bee keeping with the western honey bees (*A. mellifera* L.) almost world wide. However, in India though this mite was first reported from Himachal Pradesh on *A. mellifera* (Kumar *et. al.* 1988) yet the virulent (Korean) haplotype of *V. destructor* causing serious damage to the colonies was reported in 2004 (Gatoria *et. al.*, 2004) in Punjab and in 2005 in Himachal Pradesh (Rana *et. al.* 2005). With the reporting of this mite in Himachal Pradesh, the beekeepers lost large number of bee

colonies at such a time when virulence of mite was high. The present studies encompass information on the infestation level of the mite and screening mite tolerant colonies. In these studies no chemical treatment was given to the experimental colonies for controlling the mites. Only mechanical method of collecting phoretic mites using sugar dusting was tried to keep the mite population low. Selection of bee colonies for single character like varroa tolerance, which is genetically determined, is much easier and faster but a complex breeding including important characteristics such as honey production, mite resistance, disease resistance, temper and other desirable traits considered as group are difficult breeding challenges (Kulinčević *et. al.* 1997).

5.1 SELECTION OF COLONIES AT NAUNI, SOLAN

From the data on different aspects used for selection of *Varroa* tolerant colonies in the apiary of Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan during the build up period, it was found that the colony number C3 was superior from almost all the colonies in maintaining low level of mite infestation. This was evident from data of this colony based on 24 hour natural mite fall (3.33mites), number of mites falling after 24 hour of sugar dusting (36.67), number of mites per 100 bees (0.67), number of mites on 50 brood cells (2.33) and overall colony infestation (1.54%). During this period colony C11 also had low infestation which was at par with the colony C3 but the colony C3 was found to be superior to colony C11 with respect of maintaining significantly higher population (13641.33). During the build up period some of the other colonies viz. C1, C4, C6, C8 and C10 also had higher population of bees but despite this fact these colonies also had higher mite infestation and were considered susceptible to varroa.

Later during the honey flow period eight more colonies were added. The observations on these colonies during this period revealed that C3 and C11 in addition to the colonies C13 and C14 maintained a lower number of mites on adults (ranging between 1.33 to 2.00/100 bees), brood (ranging between 1.00 to 4.67/50 brood cells) and had lower overall colony infestation (ranging between 2.00 to 5.77 %) than almost all the other colonies. These colonies also had less

number of mites fallen naturally within 24 hour and had also had less number of mites fallen after 24 hours of sugar dusting than most of the other colonies. During the honey flow period all of these colonies (C3, C11, C13 and C14) were almost equally good in maintaining higher population of bees, brood area, pollen stores and honey stores. C1, C5 and C9 were better performing colonies in terms of population but despite having higher population these colonies were susceptible to mites and had higher infestation of varroa.

During the dearth period the mite infestation considerably increased in most of the colonies. The colonies C3, C11, C13 and C14 were again found to have low infestation of mites on adult bees and brood. These colonies also had lower overall colony infestation (6.73 to 14.95%) and less number of mites fallen within 24 hours (5.33 to 9.33 mites) than most of the other colonies. By the end of the dearth period the colonies maintaining lower population of mites viz. C3, C11, C13 and C14 survived the mite infestation and rest of other colonies either died by the end of this period or weekend so much that they eventually perished before commencement of winter period. During this period the surviving colonies were equally good in terms of population, brood area, pollen and honey stores. The colony C3 maintained higher average population (14166.00 bees), brood area (3066.50 cm²), pollen (96.00 cm²) and honey stores (2250.00 grams) than C11, C13 and C14. Even during the winter period the surviving colonies maintained lower mite infestation and were equally good in terms of population (ranging between 2098.67 to 4459.67 bees), brood area (ranging between 2098.67 to 4459.67 cm²), pollen stores (ranging between 10.00 to 21.33 cm²) and honey stores (676.66 to 866.67 grams).

Taking into consideration all these data it was evident that among all the colonies used for screening of varroa tolerance, the colonies C3, C11, C13 and C14 were tolerant to the mite. This was also evident from the fact that only these colonies were able to survive the mite attack and other colonies died. Among these selected colonies the colony C3 was superior to other colonies as this colony in addition to having lower mite infestation also maintained higher bee

population, reared more brood and had higher stores of pollen and honey especially during build up and honey flow period.

For any successful selective breeding it is essential that there are identifiable selectable differences among colonies. These differences must be due at least in part to genetic differences and from the identified superior colonies reproductive progeny is raised. Superior stocks require proper maintenance over time (Page and Laidlaw, 1992). Selection of superior stock for breeding purpose requires accurate data collection and record keeping to identify mothers of superior performing colonies which can be selected to be queen and drone mothers for next generation. In the present studies four surviving colonies from Nauni stock were selected for raising next generation queens. However, there were significant differences among even in these four colonies in terms of colony parameter and thus later the best performing colony C3 was used as mother colony to raise daughter queens.

There has been a great pressure on honey bee scientists to select and breed honey bee colonies resistant to varroa mite. Szabo and Szabo (2001) were able to select six breeder colonies from originally 32 colonies based on data collected on low mite count, absence of disease, hygienic behaviour and maintenance of high bee population. Stock improvement is not quick and it requires tedious record keeping on different aspects of the experimental colonies (Greenleaf, 1988). From the observations recorded in the present studies it is clearly indicated that even some of the strong colonies having high bee population were susceptible to varroa and failed to survive heavy mite attack. Griffes (1996) suggests that to find out most mite resistant colonies let the nature select via rule of survival of the fittest. Untreated surviving colonies which are higher producers too are then used for further breeding. In the present studies too, four surviving colonies out of the twenty, were used for further studies. In an Austrian bee institute at Lunz, an isolated apiary has been maintained without the use of any treatment against varroa since 1986 (Ruttner, 1991) and the colony losses are compensated for every year by bringing new colonies which are re-queened by daughters of

surviving colonies. This has resulted in decline of colony losses. Similar approach is possible here too.

As far as trend of growth of mites in the experimental colonies is concerned, the maximum colony infestation was during July-September, 2007 (Table 6) in most of the colonies when the colonies had quite less amount of brood. The maximum per cent colony infestation was 72.64 (C8) per cent and minimum of 6.73 (only in colony C3). Kulinčević *et. al.* (1997) have also made similar observations. They reported that increase in infestation coincided with decrease in the amount of brood in the colonies.

Selection of varroa tolerant colonies requires regular monitoring of varroa populations. Monitoring of varroa population is also important in order to identify the time when mite populations reach their economic threshold, at which time treatment is necessary to reduce mite populations in order to avoid injury to the colony. Sticky board/24 hour mite counts are thought by some of the workers to be more efficient and accurate than other invasive measures that monitor mites from adults (Sammataro *et. al.*, 2000, Sammataro *et. al.*, 2002, Branco *et. al.*, 2006). Szabo and Szabo (2003) found that 20 fallen mites per colony represent the economic threshold, however, they also pointed out that the economic threshold may vary according to geographical conditions and colony population. In the present studies it was found that the colonies in which the 24 hour mite fall was more than 13.67 during the honey flow period (Table 3) were not able to survive and died by the end of the dearth period. The colonies C3, C11, C13 and C14 which had mite count of less than 6 mites survived the mite attack and were therefore, considered varroa tolerant. It was interesting to note that during July-September, representing the dearth period, the 24 hour natural mite fall in most of the colonies declined though per cent mite infestation was more in most of the colonies. This might be related to variations in bee population during different periods of observations as stated by Szabo and Szabo (2003). Similarly Martin (1998) has also pointed out that the economic threshold value for 24 hour natural mite fall may be 20-40 in summer and 250-500 mites in winters. Thus it seems that the economic threshold values for 24-h natural mite fall depend on amount of brood and bee population of the colonies.

Sugar rolls have also been used for monitoring varroa mite populations. This monitoring technique provides an estimate of the mite-to-adult bee ratio. According to Vander Dussen (2006) sugar rolls give good indication of phoretic mite. When brood is

present number of phoretic mites, could be 20 to 30 per cent of total mites and total mite population in colony can be estimated using this criteria. However, under broodless conditions these methods give direct ratio of number of phoretic mite in a honey bee colony. As per observations of Delaplane and Hood (1999) the threshold for treatment during late-season count comes to 5 to 12.7 mites per 100 bees and during early-season a count of about 0.17 to 1 mites in an overwintered colony. In the present study the colonies C3, C11, C13 and C14 had less than 2.67 mites per 100 bees during the dearth period (Table 5) and survived the mite attack. The rest of the colonies in which number of mites per 100 bees was 6 or above died by the end of dearth period. Thus these studies support the observations of Delaplane and Hood (1999)

In addition to measuring the percentage of adult bees infested with mites, examination of brood samples has also been used in monitoring mite populations (Branco *et. al.*, 2006). An infestation of less than 5 per cent indicates sufficiently low mite number. However, a level of 25 per cent or more infested brood indicates a severe infestation which will require immediate treatment (Central Science Laboratory, 1996). In the present study, during the honey flow period the colonies C3, C11, C13 and C14 had mite infestation ranging between 2.00 to 9.33 per cent. The other colonies had mite infestation ranging between 20 to 40.67 per cent. The results revealed that colonies having infestation of less than 9.33 per cent during the honey flow period survived the mite attack, however, the colonies with more than 20 per cent of infested brood died by the end of dearth period.

5.2 CHARACTERISTICS OF HONEY BEES AFFECTING TOLERANCE TO VARROA

Hygienic and grooming behaviour as a mite defence behaviour have been linked to tolerance of honey bees to the mite (Peng *et. al.*, 1987a; Peng *et. al.*, 1987b; Rath and Drescher, 1990). Similarly growth rate of mite has also been used as one of the methods to select *Varroa* tolerant colonies (Sammataro, 1996). These three parameters have also been used for selecting varroa tolerant colonies in the present study which are discussed below.

5.2.1 Hygienic behaviour

Hygienic behaviour is one of the factors which is used while selecting the varroa tolerant colonies. This behaviour has been considered as one of the

defence mechanism towards varroa (Peng *et. al.*, 1987a; Rath and Drescher, 1990) and has been successfully used in selecting varroa tolerant colonies (Spivak and Reuter, 1998; Szabo 1998, 1999, Szabo and Szabo 2000, 2001, 2002, 2003).

The data on hygienic behaviour showed that the colonies C3, C11, C12 and C14 which were found to have low mite infestation throughout the year also showed better hygienic behaviour than most of the other colonies during build up, honey flow and dearth period. The colonies C3, C11, C12 and C14 on an average removed higher percentage of pin killed brood (ranging between 85.33 to 97.33 per cent) after 24 hours of piercing than most of the other colonies. Spivak and Reuter (1998) have compared the hygienic behaviour of selected colonies with the non selected ones. They found that hygienic colonies removed 82.9 ± 10.49 per cent killed brood as compared to only 58.9 ± 21.5 per cent by the bees from unselected stock and it was responsible for low mite count in the former case. In the present studies also selected colonies were more hygienic and had low mite count as compared to sixteen other experimental colonies.

Buchler (1994) has pointed out that removal rate of dead brood is greatly influenced by environmental factors and repeatability of single test is low. The genetic component can be demonstrated by repeated measurement. The percentage of pin killed brood removed ranged between 48.00 to 96.00, 54.67 to 97.33 and 42.67 to 97.33 per cent during build up, honey flow and dearth period, respectively, which showed the wide variability in the colonies with respect to hygienic behaviour. There were observable differences in the extent of brood removal during different periods of observations by colonies (Table 9) which support the observations of Buchler (1994).

5.2.2 Grooming behaviour

Behavioural defence of honey bees against *Varroa destructor* implies grooming behaviour as an important mechanism which enables individuals and group of bees within colony to remove dust and pollen from their bodies, to disperse pheromones and to remove ectoparasites. Grooming behaviour involves biting and licking with mouth parts and movement of the mesothoracic legs

(Boecking and Spivak, 1999). The result on damaged mites of individual colonies revealed that the colonies C3, C11, C13 and C14 had higher percentage of damaged mites (ranging between 58.33 to 69.83%) than rest of the other colonies. These colonies also had lower mite infestation than other colonies and survived the mite attack. It indicates that one of the possible cause for the tolerance of these colonies was also their ability to groom better than other colonies. These colonies were considered tolerant to varroa. In a study conducted by Ruttner and Hanel (1992) it was found that the damaged mites in varroa tolerant colonies ranged between 30 to 50 per cent. In the present study percentage of damaged mites in tolerant colonies varied between 58.33 to 69.83 per cent, indicating better grooming by the bees.

The results of grooming behaviour estimated by observing damaged mites during the month of July- August revealed that the percentage of damaged mites in all the experimental colonies varied between 24.79 to as high as 69.83 per cent. However, Boecking and Ritter (1993) found the percentage of damaged mites to vary between 0 to 39.9 per cent. These variations may be attributed to genetic make up of the colonies.

In the present study the type of damage has been categorized into six types as described by Correa-Marques *et al.* (2000). Among the different categories the most frequent type of damage observed was that of damaged legs. It was found that about 24.71 per cent of total mites had damaged legs and percentage of damaged legs varied between 11.07 to 52.67 in different honey bee colonies. Ruttner and Hannel (1992), Lodesnai *et al.* (1996), Correa-Marques *et al.* (2002) and Stanimirovic *et al.* (2005) also observed the most frequent type of damage to be that of damaged legs. It was also found that the better performing colonies C3, C11, C13 and C14 had significantly higher percentage of mites having damaged legs (43.89 to 52.67%) in comparison to rest of the colonies (11.07 to 35.95%).

5.2.3 Growth rate

The use of growth rate has also been described as one of the method to select varroa tolerant honey bee colonies (Sammataro, 1996). In the present study, the minimum growth per day was found in colony C10 ($r= 0.16$) followed by colony C3 ($r=0.01$). However, the colony C10 despite of having lower growth rate had higher population of mite throughout the period. This might be due to the fact that at higher mite population the growth rate of the mites decreases because mites in multiple infested cells do not reach their reproductive potential (De Grandi-Hoffman and Curry, 2004). Therefore, selecting the colonies on the basis of only growth rate where initial mite population has not been equalized might be controversial. The overall mean growth rate per day of the colonies was found to be $r= 1.35\pm 0.41$ which was quite higher than those found by Krauss and Page (1995). This might be due to the the varying climatic conditions as climatic conditions have been reported to influence the population growth of *Varroa* (De Jong *et. al.*, 1984; Moretto *et. al.*, 1991).

5.2.4 Bee activity

Varroa mite during the phoretic phase is attached to adult bee and defence mechanism of foraging bees also operate during this phase of parasites' life cycle. The population dynamic of mite is reported to be affected during foraging flights in several ways (Kralj and Fuchs, 2006). The parasitized workers might tend to leave the colonies resulting in rapid loss of foragers until colony remains with queen and few workers (Martin, 1997). A change in flight behaviour is also indicated by enhanced drifting from infested to less infested colonies. Observations on incoming and outgoing bees revealed that there was no significant co-relation between the bee activity with overall colony infestation during the month of March and also during September, 2007. However, during the month of June there was a significant negative correlation between the bee activity (incoming and outgoing bees) and overall mite infestation. Thus from the present studies no such generalization can be made whether mite infestation affects bee activity or not. The reason for significant co- relation during June and not during other periods remains unexplained. Whether varroa affects the

behaviour of foragers or whether such behavioural changes could benefit bees by decreasing colony infestation is unknown (Kralj and Fuchs, 2006). However, in bumble bees it has been reported that parasitized workers may stay in the field instead of returning to nest (Muller and Schmid-Hempel, 1993).

5.3 SELECTION OF HONEY BEE COLONIES FROM OTHER APIARIES

Selection of drones is a direct selection of genome generated in queens and is the most powerful method of selection. This type of selection may be useful when selecting directly for drone traits or for traits that drones or workers may have in common such as colour, insecticide or disease resistance, development time or weight, length of life etc (Laidlaw and Page, 1986). In order to find the drone mother colonies which can be used for mating of queens raised from selected colony at Nauni, Solan the colonies at different apiaries viz apiaries of migratory honey bee colonies of the university, of Bhota and that of a private beekeeper Karnal were screened for *Varroa* tolerance and higher population.

The results on selection of honey bee colonies from the migratory unit of colonies from Nauni, Solan revealed that the colony M10 was best performing colony amongst this group. Amongst the colonies at Bhota, there was no significant difference with respect to mites on adult bees. The data on screening of varroa tolerant colonies from Karnal revealed that colony K18 was superior than most of the other colonies as this colony had significantly higher population of 18888.00 bees than most of the colonies and despite this fact no mite infestation of adult bees and brood was found in this colony.

Out of the colonies screened from different apiaries, the colony K18 from the Karnal apiary was found to be best performing colony and was, therefore, selected as a drone mother colony for mating of second generation queens. Another important reason for selecting the colony from Karnal apiary was to incorporate new genetic material as the colonies of migratory unit and those at Bhota were having almost same genetic base as that of colonies at Nauni, Solan.

5.4 RAISING OF NEXT GENERATION QUEENS

Based on the various parameters taken together the colonies C3, C11 and C14 were found to be best performing colonies and among these colonies colony C3 was found to be better because beside being varroa tolerant it was also better than other colonies with respect to higher bee population, brood rearing and collecting food stores. Therefore for raising next generation queens the colony C3 was selected. Queens raised were allowed to mate under open condition at Nauni, Solan and successfully mated queens were further evaluated.

5.4.1 Evaluation of colonies headed by first generation queens

Many workers have selected the *Varroa* tolerant colonies on the basis of different criteria viz. 24 hour natural mite fall, shorter post capping period, grooming behaviour, hygienic behaviour etc. and were successful in raising varroa tolerant colonies from their selected colonies (Kulincevic *et. al.* 1992; Harbo and Hoopingarner, 1997; Wallner, 1997; Spivak and Reuter, 1998; Szabo, 1998, 1999; Szabo and Szabo 2000, 2001, 2002), which indicated the heritability for tolerance to varroa. According to Boigenzanh (1999) the heritability for tolerance to varroa is 0.29.

In the present study it was found that daughter colonies raised from selected colony (C3) were superior from the general stock colonies with respect to varroa tolerance and other parameters relating to colony performance. This was evident from the fact that daughter colonies had higher population of bees (10652.61), reared more brood (2752.25 cm²), had higher pollen stores (171.14 cm²) and honey stores (1275.36 g) than the general stock colonies. Moreover daughter colonies had significantly less number mites (1.07) fallen on the bottom board within 24 hours, less mite infestation of adult bees (0.75 mites/100 bees) and brood (0.25 mites/50 brood cells) in comparison to the general stock colonies. For further checking the performance of these colonies in terms of honey production, these were migrated to different places of Haryana, during the month of December for availing available honey flow. Under migratory conditions the colonies headed by daughter queens proved to be superior than the

general stock colonies as they had lower mite infestation on adult bees (0.92 mites/100 bees) and brood (0.33 mites/ 50 brood cells) and maintained higher population of bees, reared more brood and collected higher amount of food stores than the general stock colonies. The higher amount of honey was harvested from daughter colonies (ranging between 7700 to 13800 grams) in comparison to general stock colonies (ranging between 1800 to 5000 grams).

The results obtained indicated that the varroa tolerant characters along with the other colony performance traits were inherited successfully from the mother colony (C3) to the daughter colonies without compromising honey production. Similar observations have been made by Spivak and Reuter (1998) when they compared the naturally mated queens from colonies selected for hygienic behaviour with those not selected for this behaviour. Even in the present studies colonies headed by daughter queens from selected mother colony C3 were more hygienic than the general stock (Table 25). If compared with the mother colonies, the daughters cleaned dead brood to almost same level as their mother colony.

5.4.2 Post capping duration

In addition to different parameters observed in the colonies headed by daughter queen post capping duration of brood was also recorded and compared with the colonies headed by mother queens and queens of general stock. This aspect is of great significance as reproduction of the mite occurs inside the capped brood. A shorter post capping period provides less time for varroa to reproduce and provides the bee colony with some resistance to the mite (Harbo, 1992). Even with one hour reduction in capped period there is 8.7 per cent decline in varroa population (Buchler and Drescher, 1990). The post capping period has been used in testing varroa tolerant colonies by Siuda and Wilde (1995). Variations in post capping period has been found in *A. mellifera* colonies (Schousboe, 1986). In a study conducted by Harbo and Hoopingarner (1997) the post capping duration ranged between 267 to 286 hours. The results on the post capping period in present study revealed that all of the daughter colonies had significantly shorter post capping period (ranging between 269.78 to 272.89 h)

than all the general stock colonies (275.22 to 277.78 h). This might be one of the reasons for low infestation of daughter colonies in comparison to the general stock colonies. The post capping period of the mother colony MC3 (from which daughter queens were raised) was found to be 273.67 hours, which was lower than two of the general stock colonies. One of the daughter colony has post capping period at par with the mother colony MC3 and two daughter colonies had shorter post capping period than mother colony MC3 which indicates that the post capping behaviour was successfully inherited from the mother colony to the daughter colonies and also improved in two of the colonies. In general, during the month of July and August the average post capping period was significantly shorter (272.07 and 272.11 h) than in month of September (275.70). Buchler and Drescher (1990) had also reported variations in post capping period during different seasons.

5.5 RAISING AND EVALUATION OF COLONIES HEADED BY SECOND GENERATION QUEENS

For raising second generation queens the colony C3M2 was selected from the daughter colonies because this colony was found to better performer than others colonies of the same group and was also better performer than all the general stock colonies. From colony C3M2 maximum amount of honey (13800 g) was also harvested in comparison to the other daughter colonies (less than 8500 g). The second generation queens raised were subjected to mating with drones of colony selected (K 18) from Karnal apiary in an isolated mating yard and another set of queens were open mated under Nauni, conditions. The queens were mated under isolation to drones of varroa tolerant colonies to know effect of mating of these tolerant queens with desired drones having varroa tolerance capabilities.

In general the colonies heading by second general queens mated under isolated mating conditions to the desired stock of drones, had significantly more population, brood and honey stores than those headed by queens mated under open conditions to unknown population of drowse.

However, there were no significant differences in mite infestation level in colonies of both the groups. These studies thus point out that there was no effect on varroa tolerance characteristics of the colonies despite different mating conditions. The results further revealed that the colonies mated under isolation removed significantly higher percentage of pin killed brood (96.80%) compared those headed by queen mated under open conditions (92.40%). The hygienic behaviour in one of the attribute which is related to tolerance to varroa. Thus the superiority of isolated mated queens was found in terms of some of the colony performance parameters and hygienic behaviour. However, the data on post capping period revealed that only one of the colony (C3D2-I-3) headed by second generation queen mated under isolated conditions had shorter post capping period (272.00h) than the queens mated under open conditions. These observations thus revealed that type of mating the queens received, affected only some of the characters. In fact, with the exception of queen mother, the female population of a single colony consists of subfamilies of highly related super sisters (Rothenbuhler 1960, Laidlaw, 1974). Each of the subfamily is sired by one drone. The number of subfamilies is determined by the number of mates of the queen mother. However, size of each subfamily depends on proportion of total sperms in the spermatheca that came from the subfamily sire. The characteristics of different subfamilies are likely to differ due to different sires and individuals within a subfamily may vary because of segregations (Laidlaw and Page, 1986). This might possibly be the reason for variations found in the colonies headed by daughter queens mated to drones from only one mother colony and those mated under open conditions.

5.6 CHECKING MITE POPULATION USING SUGAR DUSTING

Dusting with powdered sugar is a mechanical means of mite control. The fine sugar particles interfere with the suction mechanism of the mite's ambulacrum, causing it to lose its grip on the bee's exoskeleton and fall off. Fakhimzadeh (2001a) showed 91 per cent effectiveness in mite knock-down when the sugar was applied directly to bees. The particle size of the sugar powder is comparable to that of pollen, and it was found not to obstruct the perpetually

open T2 spiracle of honey bees when examined with a scanning electron microscope, nor were they in the respiratory ducts (Fakhimzadeh 2001a). Application of powdered sugar has also been shown to be harmless to immature bees unless large amounts of sugar are applied directly to open brood cells (Aliano and Ellis 2005b).

Dusting of sugar twice a month has been reported to keep mite at low level and dusting monthly keeps mite at tolerable levels. Oliver (2007) described a model in which monthly dusting keeps mite populations below moderate thresholds of 3000 mites and bimonthly dusting keeps them below 1000 mites. However, in the present studies there was no evidence of decline in mite population using sugar dusting. Even four dustings during a period of 21 days (one brood cycle) on 20 experimental colonies, failed to keep mite population low (Table 40). These studies thus support the observations made by Ellis *et al.* (2009), that the dusting sugar does not cause significant varroa control possibly due to varying reproduction rates of varroa mites under different population pressure. However, this method can be used for estimating phoretic mite population of a bee colony. By sugar dusting 81.3 per cent of the phoretic mites fell to bottom board within one hour of dusting in the present studies Oliver (2008, 2009a, 2009b) has also made similar observations.

Chapter-6

SUMMARY AND CONCLUSION

Result of the study conducted on “Screening of *Apis mellifera* L. colonies for *Varroa* tolerance and evaluation of colony performance of selected stock” during the course of present investigations are summarized here under:

6.1 SELECTION OF HONEY BEE COLONIES AT NAUNI, SOLAN

6.1.1 Colony performance and varroa tolerance

Data on screening of honey bee colonies of Dr. Y. S. Parmar University of horticulture and Forestry, Nauni, Solan during the different periods of colony development revealed that the out of the twenty colonies screened for varroa tolerance the colonies C3, C11, C13 and C14 maintained lower degree of mite infestation and were found to be more tolerant to varroa mite. Amongst these colonies the colony C3 was better performer as this colony in addition to maintaining lower mite infestation also showed better performance in terms of maintaining higher population, rearing brood and collection of pollen stores.

6.1.2 Hygienic behaviour

The data recorded on hygienic behaviour revealed that the colonies C3 and C11 possessed better hygienic behaviour than rest of the colonies. These colonies removed more than 93.33 per cent of pin killed brood during different periods of colony development.

6.1.3 Grooming behaviour

The data on grooming behaviour showed that colonies C3, C11, C13 and C14 had higher percentage of damaged mites (more than 58.33%) and, therefore, were considered tolerant to varroa mite. Amongst the different type of damage observed, it was found that maximum damage was done to the legs of mites (24.71%) followed by damage to the shield (5.44%).

6.1.4 Growth rate

The growth rate of colonies C3, C5, C10, C11 and C17 was found to be lower than those of other colonies but some of these colonies despite having lower growth rate of mites possessed higher mite infestation. Therefore, use of growth rate of mites may not be a better option for using in colony selection for varroa tolerance. The mean growth rate was found to be 1.35 ± 0.41 per cent per day.

6.2 BEE ACTIVITY

The data on incoming and outgoing bees revealed that there was no effect of mite infestation on the bee activity during the month of March and September. However, during the month of June significantly negative correlation was found between the bee activity (incoming and outgoing bees) and overall colony infestation.

6.3 SELECTION OF HONEY BEE COLONIES FROM OTHER APIARIES

The data on screening of varroa tolerant colonies from different apiaries revealed that the colony M10 was best performing colony amongst the migratory group of colonies, colony B9 was best performing colony amongst the colonies at Bhota and Colony K18 was best performing colony amongst the Karnal colonies. There was no mite infestation found in colony K18 and moreover the colony maintained higher bee population. Therefore, colony K18 was later used as drone mother colony for mating of second generation queens from the selected stock.

6.4 RAISING AND EVALUATION OF FIRST GENERATION QUEENS

From the data observed on the screening of varroa tolerant colonies the colony C3 was found to be best performing colony both in terms of *Varroa* tolerance and colony build up. Therefore, it was used for raising of first generation queens. The raised queens were allowed to open mate under Nauni, Solan conditions.

6.4.1 Colony performance and varroa tolerance

The data recorded on the performance of first generation queens quite clearly revealed that the daughter colonies raised from mother colony C3 were superior than the general stock colonies both in terms of colony performance and mite tolerance. The daughter colonies maintained significantly lower level of mite infestation than the general stock colonies.

6.4.2 Hygienic behaviour

The data on hygienic behaviour revealed that the daughter colonies removed significantly higher percentage of pin killed brood (92.57%) than the general stock colonies (69.33%) which showed that these colonies were more hygienic than the general stock colonies. This was one of the reason for low mite infestation in daughter colonies.

6.4.3 Post capping duration

Post capping duration of the daughter colonies was found to be shorter (ranging between 269.78 to 272.89 h) than all the general stock colonies (275.22 to 277.78 h) which might be one of the reason for low mite infestation in these colonies.

6.4.4 Evaluation of first generation queens under migratory conditions

The data on evaluation of first generation queens under the migratory conditions again revealed the superiority of daughter colonies over the colonies of the general stock. The daughter colonies possessed significantly higher population, brood area, pollen stores and honey stores than the general stock colonies. Moreover daughter colonies had significantly lower mite infestation than general stock colonies. Higher amount of honey was also harvested from the daughter colonies (ranging between 7700 to 13800 g) in comparison to the general stock colonies (ranging between 1800 to 5000 g).

6.5 RAISING AND EVALUATION OF SECOND GENERATION QUEENS

Amongst the different daughter colonies the colony number C3D2 was found to be superior than rest of the daughter colonies (C3D1, C3D3 and C3D4)

both in terms of colony performance and in mite tolerance. The higher amount of honey (13800 g) was also harvested from this colony in comparison to other colonies. The colony C3D2 was, therefore, selected for raising next generation queens.

Second generation queens raised were subjected to mating with the drones of colony K18 (selected from Karnal colony) in an isolated mating yard. Another set of queens from this stock was open mated under Nauri, Solan conditions to drones of unknown parentage under open conditions.

6.5.1 Colony performance and varroa tolerance

The second generation colonies which were headed by openly mated queens and those with the isolated mated queens had no significant variations in terms of varroa tolerance. However, the colonies headed by isolated mated queens were superior with respect to parameters relating to colony performance.

6.5.2 Hygienic behaviour

The colonies mated under isolation were found to possess better hygienic behaviour and removed significantly higher percentage of pin killed brood (96.80 %) than those mated under open conditions (92.40%) which showed the superiority of these colonies over the openly mated queens.

6.5.3 Post capping duration

The data on post capping duration revealed that one of the colony mated under isolation had significantly lower post capping duration (272.00 h) than the colonies headed by queens mated under open conditions (277.67 and 278.00 h).

6.6 Efficacy of sugar dusting in controlling mite population

The sugar dusting was found to be ineffective for the control of varroa mite. However, it can be used for sampling or estimating varroa infestation level of bee colonies.

Chapter-7

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ABSTRACT

The present studies were carried out for screening out *Apis mellifera* colonies tolerant to *Varroa* mites from different apiaries and to evaluate the performance of the selected stock and the colonies raised from it. In the present studies, after screening of honey bee colonies from apiary at Nauni, Solan it was found that the colonies C3, C11, C13 and C14 had lower infestation of mites and were *Varroa* tolerant. These colonies also removed significantly higher percentage (more than 93.33) of pin killed brood than other colonies and also had higher percentage (58.33) of damaged mites. The maximum type of damage which was found in mites was that of damaged legs (24.71%).

The data on screening of colonies from other apiaries revealed that the colony M10 was the best performing colony from migratory group, B9 from colonies at Bhota and Colony K18 was best performing colony from Karnal apiary. Amongst all the colonies at different apiaries the colony K18 was found to have no infestation on adult bees and brood and was, therefore, selected as drone mother colony for mating of second generation queens.

The colony C3 from the apiary at Nauni, Solan was selected for raising first generation queens because it was found to be more superior than other selected varroa tolerant colonies. The raised colonies were open mated under Nauni conditions and were further evaluated under Nauni and migratory conditions. The results obtained revealed that daughter colonies were superior than general stock colonies both in terms of colony performance and *Varroa* tolerance. Post capping duration of the daughter colonies was also found to be shorter (269.78 to 272.89 h) than the general stock colonies (275.22 to 277.78h).

Based on the performance of the first generation daughter queens, the colony C3D2 was selected for raising second generation queens. The raised queens were subjected to mating with drones of colony K18 in an isolated mating yard and another set of queens, with drones of unknown parentage under open conditions. It was found that second generation queens irrespective of mating conditions, were equally good in maintaining lower mite infestation, however, the colonies mated under isolation were superior with respect to other parameters related to colony performance.

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Appendix-I

Anova table for Performance of the honey bee colonies used for screening for *Varroa* tolerance during build up period

Source of variance	df	Mean sum of square							
		Population	Brood area	Pollen area	Honey stores	24 hour natural mite fall	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
Colonies	11	0.047634	0.2144562	0.2258934	0.3045955	376.6742	0.196152	15.48232	22.87879
Error	24	0.0110704	0.1010173	0.1339101	0.1767306	108.2222	0.051399	1.055556	4.638889
Total	35								

Appendix-II

Anova table for Per cent brood, adult and colony infestation level in the experimental colonies during the build up period

Source of variance	df	Mean sum of square		
		Per cent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
Colonies	11	3.527417	1.134902	2.094201
Error	24	0.583519	0.082434	0.2188794
Total	35			

Appendix-III

Anova table for Performance of the honey bee colonies used for screening for *Varroa* tolerance during honey flow period

Source of variance	df	Mean sum of square							
		Population	Brood area	Pollen area	Honey stores	24 hour natural mite fall	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
Colonies	19	0.099789	0.337902	0.379715	0.150793	0.631954	0.408082	64.15702	134.6175
Error	40	0.009467	0.133353	0.459919	0.059022	0.044215	0.09423	32.45	84.5
Total	59								

Appendix-IV

Anova table for Per cent brood, adult and colony infestation level in the experimental colonies during the honey flow period

Source of variance	df	Mean sum of square		
		Percent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
Colonies	19	321.1805	2.415256	147.3392
Error	40	132.5573	0.669487	28.78367
Total	59			

Appendix-V

Anova table for Performance of the honey bee colonies used for screening for *Varroa* tolerance during dearth period

Source of variance	df	Mean sum of square								
		Population	Brood area	Pollen area	Honey stores	df	24 hour natural mite fall	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
Colonies	19	3.516362	2.352859	1.00631	2.416465	19	192.92593	0.1593082	97.192008	1221.3908
Error	60	2.068278	1.371047	0.40316	1.220776	34	59.147059	0.0856018	13.186275	257.75
Total	79					53				

Appendix-VI

Anova table for Per cent brood, adult and colony infestation level in the experimental colonies during the dearth period

Source of variance	df	Mean sum of square		
		Percent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
Colonies	19	0.2772044	3.0634407	420.687
Error	34	0.0407209	0.3372723	172.5469
Total	53			

Appendix-VII

Anova table for Performance of the honey bee colonies used for screening for *Varroa* tolerance during winter period

Source of variance	df	Mean sum of square							
		Population	Brood area	Pollen area	Honey stores	24 hour natural mite fall	Mite drop after 24 hour of sugar dusting	Number of mites per 100 bees	Number of mites per 50 brood cells
Colonies	3	0.090981	0.1044942	460.77778	0.0101477	11.555556	520.66667	28.083333	62.75
Error	8	0.0464935	0.6045639	48.666667	0.0225704	14.916667	192.08333	22.5	60
Total	11								

Appendix-VIII

Anova table for Per cent brood, adult and colony infestation level in the experimental colonies during the winter period

Source of variance	df	Mean sum of square		
		Percent brood infestation	Per cent adult bee infestation	Per cent overall colony infestation
Colonies	3	3.1163106	1.0280532	1.2845655
Error	8	6.4765993	1.0758914	2.0481638
Total	11			

Appendix-IX

Anova table for Hygienic behaviour of the experimental colonies during build up period

Source of variance	df	Mean sum of square
Colonies	11	373.4758
Error	24	27.80107
Total	35	

Appendix-X

Anova table for Hygienic behaviour of the experimental colonies during Honey flow period

Source of variance	df	Mean sum of square
Colonies	19	303.2017
Error	40	15.81698
Total	59	

Appendix-XI

Anova table for Hygienic behaviour of the experimental colonies during Death period

Source of variance	df	Mean sum of square
Colonies	19	311.95907
Error	34	21.58511
Total	53	

Appendix-XII

Anova table for damaged mites in the experimental colonies

Source of variance	df	Mean sum of square
Colonies	19	943.24
Error	80	51.029
Total	99	

Appendix-XIII

Anova table for different types of damage to body parts of *Varroa* in the experimental colonies

Source of variance	df	Mean sum of square
Colonies	19	70.501
Damaged	5	9878.6
Colonies x damaged	95	115.10
Error	480	55.185
Total	599	

Appendix-XIV

Anova table for number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of March-April 2007

Source of variance	df	Bee activity during March-April	
		Outgoing bees	Incoming bees
Colonies	11	214.45	114.26
Date	2	19.731	20.861
Colonies x date	22	3.2971	28.306
Error	72	4.5556	4.8241
Total	125		

Appendix-XV

Anova table for number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of June 2007

Source of variance	df	Bee activity during June	
		Outgoing bees	Incoming bees
Colonies	19	76.509	116.27
Date	2	10.617	4.6500
Colonies x date	38	1.1430	2.3693
Error	120	1.4722	2.0944
Total	179		

Appendix-XVI

Anova table for number of outgoing and incoming bees in the colonies screened for *Varroa* tolerance during the month of September 2007

Source of variance	df	Bee activity during September	
		Outgoing bees	Incoming bees
Colonies	13	149.20	174.47
Date	2	1.7222	3.4841
Colonies x date	26	2.5513	3.9542
Error	84	1.5397	2.1667
Total	125		

Appendix-XVII

Anova table for the migratory unit of colonies screened for *Varroa* tolerance

Source of variance	df	Mean sum of square		
		Bee population	Mites per 100 bees	Mites per 50 brood cells
Colonies	16	0.028657	5.894608	92.23039
Error	34	0.01392	1.980392	27.60784
Total	50			

Appendix-XVIII

Anova table for Bhota apiary screened for *Varroa* tolerance

Source of variance	df	Mean sum of square		
		Bee population	Mites per 100 bees	Mites per 50 brood cells
Colonies	8	0.08785	17.704	184.417
Error	18	0.03958	41.185	189.074
Total	26			

Appendix-XIX

Anova table for Karnal apiary screened for *Varroa* tolerance

Source of variance	df	Mean sum of square		
		Bee population	Mites per 100 bees	Mites per 50 brood cells
Colonies	45	0.08924	4.109	2.488
Error	92	0.01415	0.804	0.739
Total	137			

Appendix-XXI

Anova table for post capping duration of brood in honey bee colonies selected for *Varroa* tolerance during different months (2008)

Source of variance	df	Mean sum of square
Colony	8	93.694
Month	2	117.37
Colony x month	16	5.8704
Error	54	3.6420
Total	80	

Appendix-XXII

Anova table for performance of colonies headed by daughter queens from the selected stock in comparison to mother colonies and colonies from the general stock, under migratory conditions

Source of variance	df	Mean sum of square					
		Population	Brood area	Pollen area	Honey stores	Mites per 100 bees	Mites per 50 brood cells
Colonies	9	0.040842	0.065955	0.082468	0.055405	8.033333	3.707407
Between groups	2	0.145682	0.225128	0.226346	0.156571	34.58056	15.46111
Within group A	2	0.003306	0.001711	0.005458	0.008485	0.777778	0.111111
Within group B	3	0.010533	0.030143	0.081394	0.022203	0.305556	0.222222
Within group C	2	0.019001	0.024742	0.017212	0.050959	0.333333	0.777778
Error	20	0.003947	0.021511	0.022233	0.043318	0.9	0.266667
Total	29						

Appendix-XXIV

Anova table for post capping duration (in hours) of brood in colonies headed by second generation daughter queens mated under isolated mating conditions and open mating conditions

Source of variance	df	Mean sum of square
Colonies	4	0.000046016
Error	10	0.000011382
Total	14	

Appendix-XXV

Anova table for percentage of mites fallen on bottom board at different time intervals after sugar dusting

Source of variance	df	Mean sum of square
Time	3	0.00025389
Date	4	6.2570
Time x date	6	34.549
Error	144	13.240
Total	157	

Appendix-XXVI

Correlation among different parameters during the month of March, 2007

	Populat ion	Brood area	Honey stores	Pollen area	24hour natural mite fall	Mites fallen after sugar dusting	Mites/1 00 bees	Mites per 50 brood cells	Total mite in the colony	Incomi ng bees	Outgoi ng bees	Over all colony infestat ion
Population	1.0000											
Brood area	0.6205 *	1.0000										
Honey stores	0.1639	-0.1494	1.0000									
Pollen area	0.6627 *	0.6984 *	0.3528	1.0000								
24hour natural mite fall	0.6145 *	-0.0137	0.2679	0.0620	1.0000							
Mites fallen after sugar dusting	0.4615	0.0133	0.3279	0.1143	0.9089 *	1.0000						
Mites/100 bees	-0.0327	-0.4570	0.5132	-0.0249	0.4949	0.6492 *	1.0000					
Mites per 50 brood cells	0.3553	-0.2862	0.1170	-0.3362	0.6479 *	0.4870	0.3403	1.0000				
Total mite in the colony	0.8191 *	0.5797 *	0.1482	0.3914	0.6014 *	0.5400	0.1313	0.5600	1.0000			
Incoming bees	-0.2791	0.1681	-0.5028	-0.2513	0.0070	0.1681	-0.0229	-0.3404	-0.1152	1.0000		
Outgoing bees	-0.0880	0.0365	-0.7650	-0.3055	0.1742	0.1704	-0.1563	-0.1321	-0.1600	0.7609 *	1.0000	
Over all colony infestation	0.4113	-0.0782	0.2855	-0.1161	0.6721 *	0.6490 *	0.5402	0.8910 *	0.7443 *	-0.2006	-0.2305	1.0000

*Significant correlation at 10 df

Appendix-XXVII

Correlation among different parameters during the month of June, 2007

	Population	Brood area	Honey stores	Pollen area	24hour natural mite fall	Mites fallen after sugar dusting	Mites/100 bees	Mites per 50 brood cells	Total mite in the colony	Incoming bees	Outgoing bees	Over all colony infestation
Population	1.0000											
Brood area	0.3002	1.0000										
Honey stores	0.5644*	-0.1159	1.0000									
Pollen area	0.2196	-0.0526	0.1561	1.0000								
24hour natural mite fall	0.1163	-0.1708	0.0424	-0.3526	1.0000							
Mites fallen after sugar dusting	0.1728	-0.2894	0.0328	-0.2873	0.5468*	1.0000						
Mites/100 bees	-0.3094	-0.5096*	-0.3674	-0.0813	0.5195*	0.4950*	1.0000					
Mites per 50 brood cells	-0.4525*	-0.3437	-0.3598	-0.1794	0.4501*	0.3980	0.6640*	1.0000				
Total mite in the colony	0.2266	0.0628	-0.0894	0.0343	0.6203*	0.4991*	0.6156*	0.6313*	1.0000			
Incoming bees	0.5670*	0.1090	0.4062	0.1215	-0.3157	-0.1335	-0.6461*	-0.7475*	-0.5746*	1.0000		
Outgoing bees	0.3984	0.2640	0.1508	-0.0673	-0.4239	-0.2866	-0.5715*	-0.8373*	-0.6356*	0.8155*	1.0000	
Over all colony infestation	-0.5005*	-0.2207	-0.4604*	-0.1275	0.4331	0.2947	0.7974*	0.8636*	0.7066*	-0.8868*	-0.8090*	1.0000

*Significant correlation at 18 df

Appendix-XXVIII

Correlation among different parameters during the month of September, 2007

	Population	Brood area	Honey stores	Pollen area	24hour natural mite fall	Mites fallen after sugar dusting	Mites/100 bees	Mites per 50 brood cells	Total mite in the colony	Incoming bees	Outgoing bees	Over all colony infestation
Population	1.0000											
Brood area	0.9080*	1.0000										
Honey stores	0.6009*	0.5375*	1.0000									
Pollen area	0.4229	0.3788	0.1069	1.0000								
24hour natural mite fall	0.1413	0.1608	-0.0285	-0.1948	1.0000							
Mites fallen after sugar dusting	0.3041	0.0848	0.4634	-0.3776	0.0631	1.0000						
Mites/100 bees	-0.1582	-0.2605	-0.3669	-0.2972	0.2743	0.1912	1.0000					
Mites per 50 brood cells	-0.2201	-0.3406	0.1509	-0.3061	-0.1237	0.2291	0.4984	1.0000				
Total mite in the colony	0.5281	0.4479	0.2742	0.0405	0.2029	0.2286	0.5812*	0.5646*	1.0000			
Incoming bees	0.6127*	0.7351*	0.2343	0.3927	-0.1323	-0.2942	-0.4914	-0.4781	0.1155	1.0000		
Outgoing bees	0.6024*	0.7137*	0.2376	0.3916	-0.1362	-0.3172	-0.4780	-0.4575	0.1235	0.9941*	1.0000	
Over all colony infestation	-0.1801	-0.1703	-0.0925	-0.3156	0.1051	0.0380	0.6508*	0.8648*	0.7002*	-0.3464	-0.3475	1.0000

*Significant correlation at 12 df

CURRICULUM VITAE

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M. Sc. (Entomology)	1 st	Dr. Y.S. Parmar, University of Horticulture and Forestry, Nauni, Solan.	2005

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Scholarship/ Stipend/ Fellowship, any during the study period	:	Ph.D. Scholarship during the year 2007-2008, worked as SRF in the project entitled "Development of e-courses for B.Sc. Horticulture degree programme" from December 2008 to October 2009.

(Ritesh Sharma)