EFFECT OF COLONY CONDITIONS AND REARING METHODS ON QUALITY OF DRONES IN *Apis mellifera* L.

Thesis

by

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CERTIFICATE- I

This is to certify that the thesis titled, **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 is a bonafide research work carried out by **Mr. Ojas Chauhan (H-2019-04-M)** son of Shri Gian Chauhan under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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

Place: Nauni, Solan
Dated:

Dr. Harish Kumar Sharma
Chairman
Advisory Committee

CERTIFICATE- II

This is to certify that the thesis titled, **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** submitted by **Mr. Ojas Chauhan (H-2019-04-M)** son of Shri Gian Chauhan to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173230 India in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE) ENTOMOLOGY** in the discipline of **PLANT PROTECTION** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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"Some goals are so, worthy its glorious even to fail"

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I solely claim the responsibility of all errors and omissions.

Place: Nauni, Solan
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CONTENTS

LIST OF ABBREVIATIONS

ºC	Degree Celsius
C.D.	Critical difference
df	Degree of freedom
F	F-calculated
g	Gram
mg	Milligram
µl	Microlitre
mm	Millimetre
MSS	Mean sum of squares
no.	Number
%	Per cent
P	Significance
SS	Sum of squares
Sq	Square
viz.	Videlicet

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Chapter-1

INTRODUCTION

Honeybees are the most important social insects that have benefited mankind for nutritional and medicinal purposes for thousands of years (Johannesmeier and Mostert, 2001). They play a major role in the field of agriculture by pollination and production of important substances like honey, royal jelly, bee wax, propolis, pollen and bee venom (Alburaki and Alburaki, 2008). Honeybees, along with other pollinators, are responsible for pollinating approximately 35 per cent of the world's food crops (Klein *et al.,* 2007). India has a diverse honey bee fauna with major *Apis* species like, *Apis dorsata* F., *Apis florea* F., *Apis cerana* F. and well-established exotic *Apis mellifera* L. (Atwal and Goyal, 1973)

Drones are important biological component of honey bee population although they do not forage or perform work inside the honey bee colony. They carry important genetic information which is contributed to next generation (Delaney, 2004). For passing the traits both the ability to copulate and inseminate depends upon the sperm quality (Berg *et al*., 1997). Every drone produces semen between 1.50 µL to 1.75 µL which contains around 11 million spermatozoa (Woyke, 1962). The quantity of sperms produced by drones depends upon the size and origin whether the eggs are laid from queens or by the laying workers.

Studies on drone rearing helps in breeding programmes by improving the efficacy and quality of mating (Currie, 1987). The main hinderance in bee breeding is control of parentage which is due to multiple mode of mating. The success of bee breeding programme depends upon quality of queen and drone. Drones are naturally produced during spring and summer. The populations of drones fluctuate with different seasons, it reaches highest during spring season, declines during summers and reaches to the lowest in autumn (Peng *et al*., 1992). During the spring they can usually be obtained in large quantity from any queen, because they are naturally produced at this season, but later in the year it is sometimes almost impossible to obtain any drones at all from some queens. Drones are reared in large numbers only by colonies with abundant food resources (Fukuda and Ohtani, 1977). When the colony loses its queen and is unable to rear a new queen, workers undergo ovarian development and become reproductive laying workers that can lay only unfertilized eggs that give rise to drones (Winston, 1987; Page and Erickson, 1988; Visscher, 1998). Drones are obtained out of season if conditions similar to those existing during the swarming season are created. Another way to obtain drones is to rear them from drone laying queens, which can be produced by treating virgin queens with carbon dioxide (Mackensen, 1947).

Mites that parasitize honey bees have become a global problem that threatens the survival of honey bee colonies. Tracheal and varroa mites has major contribution to the loss of honey bee colonies (Shoukry *et al.*, 2013). *Varroa destructor* cater on larval and pupal honeybees as they develop to adult stage (De Jong *et al.*, 1982). Fuch (1990) found that drones are more likely to get parasitized than worker bees and the effect of *Varroa* on drones was more devastating. Infestation of *Varroa* results in negative effects on drone weight and the spermatozoa numbers. Since the introduction of *Varroa*, effective control measures have been developed which includes the use of conventional acaricides (Stanghellini and Raybold, 2004). Acaricides used for treating colonies not only expose the mites but also eggs, larvae and adult bees. Use of Formic acid in mite control has been shown to decrease drone production and adult survival in treated colonies (Guzman *et al.*, 1999). Thus, the effects on the quality and quantity of drones for mating can be a serious concern specially in mite infested colonies and those treated with formic acid for mite management.

In India beekeeping is practiced with *A. mellifera* species as it is efficient in commercial honey production. Selective breeding is needed for production of good quality young queens under controlled conditions (Atwal and Sharma, 1970), which can be attained by adequate rearing and maintenance of healthy drones (Rhodes, 2002). Drone rearing and bee breeding has remained neglected in India. The present study is aimed to see the effect of colony conditions and rearing methods on quality and morphometric characters of drones.

Keeping in view all these aspects, the present study was carried out with following the objectives:

i) To study the effect of colony conditions on quality of drones.

ii) To study the effect of rearing methods on quality of drones.

Chapter-2

REVIEW OF LITERATURE

Drone rearing is one of the most neglected section of queen rearing (Laidlaw and Page 1997). Elements governing maturation of drones of honeybees are of great significance for the bee breeders, as quality drones are required for virgin queen bees for better future progeny (Mazeed and Mohanny, 2010). The scanty literature pertaining to various parameters and aspects of the present study entitled **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** is presented under following headings:

2.1 Drone production from *A. mellifera* virgin queens

2.2 Drone production from *A. mellifera* laying workers

2.3 Drone production from *A. mellifera* mated queens

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2.1 DRONE PRODUCTION FROM *A. mellifera* VIRGIN QUEENS

Kaftanoglu and Peng (1982) reported that virgin queen start laying eggs at the age of 14 days when treated with three treatments of carbon dioxide. The first treatment was given to a 5 to 6 - day old virgin queen and the remaining two treatments at 24-hour interval. Polaczek *et al.* (2000) induced the drone production in virgin queens of *A. mellifera carnica* with two carbon dioxide treatment for 5 minutes to produce drones which can be used to inseminate the queens using semen of their own sons.

Rinderer (1986) reported that drone production can be achieved by giving a young queen 3 minutes carbon dioxide treatment for three consecutive days. The unfertilized eggs produced by these queens are usually small as eggs are laid in worker sized cells. The semen produced is viable which can be utilized for artificial insemination.

Couvillon *et al.* (2010) examined sexual selection in *A. mellifera*. The normal sized drones were produced by mated queens while small drones were produced from virgin queen induced with carbon dioxide. They allowed a known number of normal size and small drones to compete for mating with virgin queens which revealed that small males had around half the number of matings as predicted based on their number of flights. Their study further revealed that smaller drones were less successful at mating than normal sized drones.

Virgin queen of 5 to 6 - day old when anesthetised with two carbon dioxide treatments for 15-20 minutes at an interval of 2 days results in laying of unfertilized eggs. Such queens tend to lay eggs in worker cells which results in drones of smaller size and less semen production (Abrol, 2019).

2.2 DRONE PRODUCTION FROM *A. mellifera* LAYING WORKERS

Page and Metcalf (1984) reported that queenless colony can produce more than 6000 drones whereas, queen-right colony can produce on an average 20000 drones per year. These results were confirmed by Page and Erickson (1988), who reported the production of more than 6000 drones by a single queenless colony.

Delaplane and Harbo (1987) reported that laying workers of 11-15 day old tend to produce more drones than older laying workers which were at an age group of 50- 54 days. To maintain the laying workers in the nucleus, sealed worker brood and young bees must be given on a regular basis. The colonies should also be provided with pollen and honey (Ruttner, 1976).

Harbo (1991) devised a method for producing drone populations that were genetically representative of their colonies. He discovered that laying worker drones are valuable in selective breeding since their spermatozoa genetically represent entire colony. His study further revealed that during non-breeding season, laying workers can be used to produce drones from weak colonies.

Woyciechowski (1994) studied the egg laying pattern of laying workers and inferred that laying workers starts laying more than 10 eggs per cell. Similarly, Mangum (1996) reported that laying workers tend to lay multiple eggs in worker cells with a scattered pattern.

Gencer and Firatli (2005) compared the *A. mellifera Caucasian* drones produced from mated queen and laying worker colonies on the basis of their weight at different ages, reproductive capacities and morphological characters. He reported that production of laying workers can be attained by dequeening the queen right colonies and by removing the unsealed brood which prevents the emergency rearing of queen cells. Similarly, the drone production from laying workers were carried out by Gencer and Kahya (2011) who reported that sperm traits of smaller drones produced from laying workers was minimum as compared to larger drones produced from mated queen colonies.

2.3 DRONE PRODUCTION FROM MATED *A. mellifera* QUEENS

Drone production is a universal process in mated queen colonies (Allen, 1958). The mated queen colonies regulate drone production and maintenance according to various environmental factors as drone production reaches to the maximum during spring or early summer season (Boes, 2010). A good drone producing colonies should have proper supply of nutrients for maximum production of drones (Rinderer, 1986).

Allen (1965) reported that drone production in *A. mellifera* peaks around the same time as general brood rearing activity. She also discovered that no regular colonies produce drones on more than three drone combs, with the largest area recorded being only two full brood combs (2580 sq cm).

Koeniger (1986) reported that mated queens of *A mellifera* tends to produce only drone eggs when confined to drone combs during spring and summer season.

McNaily and Schneider (1994) investigated drone production in naturally occurring colonies of *A. mellifera scutella*. He observed that drone production was concentrated in larger, flourishing and swarming colonies with a substantial proportion of worker brood.

2.4 EFFECT OF COLONY CONDITIONS AND DIFFERENT REARING METHODS ON MORPHOLOGICAL CHARACTERS OF *A. mellifera* DRONES

2.4.1 Effect of colony conditions on morphological characters of *A. mellifera* drones

Guzman *et al*. (1999) studied the effect of formic acid gel formulation on drone production and observed that formic acid treated colonies resulted in 50 per cent reduced production of drones in comparison to untreated colonies (disease free). However, the weight of emerged drones was same in healthy (2.56 ± 0.15 g) as well as formic acid treated colonies (2.56 ± 0.11 g).

Shoukry *et al*. (2013) studied the effect of different miticides including formic acid on the drones of *A. mellifera.* The drones were evaluated for forewing length and forewing width. According to them, highest mean forewing length (11.21 mm) and forewing width (3.63 mm) was observed in healthy colonies compared to the forewing length (10.95 mm) and forewing width (3.64 mm) of drones produced from the formic acid treated colonies.

2.4.2 Effect of rearing methods on morphological characters of *A. mellifera* drones.

Woyke (1978) analysed the measurements of thorax and appendages of drones which were originated from queens of *A. mellifera ligustica*, *A. mellifera adansonii*, their backcrosses and hybrids. He reported that drones of *A. mellifera ligustica* had mean thorax length of 4.42 ± 0.027 mm, thorax width of 4.59 ± 0.020 mm, forewing length of 12.00 ± 0.059 mm, forewing width of 3.96 ± 0.024 mm and hamuli number of 20.6 ± 0.38.

Berg *et al.* (1997) in their study compared drones of different body size. Large drones were reared in drone cells while small drones were reared in worker cells. They reported that small drones have reproductive disadvantage compared with large drones as the wings of large drones are bigger (12.14 mm) than those of small drones (11.29 mm).

Kumar (2002) analysed the morphological characters of drones produced from mated queen and laying worker colonies of *A. mellifera*. He observed that drones can be successfully reared in weak colonies using laying workers. The drones of laying workers had shorter mean thorax length (4.94 ± 0.06 mm) and breadth (4.89 ± 0.06 mm) as compared to normal drones with larger thorax length (5.6 ± 0.03 mm) and breadth (5.52 ± 0.03 mm).

Gencer and Firatli (2005) compared the morphological characters of drones reared in mated queen colonies and in laying worker colonies. They found that mean extent of hamuli (1.45 ± 0.01 mm), hamuli number (19.65 ± 0.54), femur length (3.02 ± 0.01 mm), tibial length (3.44 ± 0.03 mm) and metatarsus length (2.16 ± 0.01 mm) were significantly low in drones produced from laying worker colonies in comparison to extent of hamuli (1.66 ± 0.02 mm), hamuli number (21.40 ± 0.38), femur length (3.46 ± 0.02 mm), tibial length (4.01 ± 0.02 mm) and metatarsus length (2.51 ± 0.01 mm) of drones produced from mated queen colonies.

Mazeed (2011) studied the effect of presence and absence of queen on the morphology of newly emerged *Apis mellifera* drones. The colonies were divided into two parts after drone eggs were laid by the queen, one part had old queen and other was queenless. He inferred that drones produced in queenless colonies had maximum mean forewing length (12.95 ± 0.23 mm), as compared to mated queen colonies (11.71 ± 0.22mm), which was attributed to the fact that waif colonies take better care of drones than colonies with queens.

Taha and Alqarni (2013) analysed the morphometric characters of *A. mellifera jemenitica* drones from queen right colonies. They found the mean forewing length 12.33 ± 0.09 mm, forewing width 3.62 ± 0.04 mm, hindwing length 7.23 ± 0.06 mm, hindwing width 3.15 ± 0.05 mm and hamuli number 19.50 ± 0.29

Rousseau and Giovenazzo (2016) studied morphometric characters of *A. mellifera* drones from mated queen colonies. They reported that mean thorax width was 5.45 ± 60.014 mm.

Utaipanon *et al.* (2019) studied the contribution of *A. mellifera* drones produced from mated queens and laying worker colonies at drone congregation areas by measuring the forewing length and forewing breadth of drones from mated queen and laying worker colonies. They found that maximum forewing length (5.78 ± 0.26 mm) and forewing breadth (1.27 ± 0.08 mm) was recorded in drones of mated queen colonies as compared to forewing length (5.01 ± 0.21 mm) and forewing breadth (1.11 ± 0.05 mm) of drones from laying worker colonies. Their studies further revealed that 0.23 per cent of drones captured from mating area were smaller in size which suggested that drones of laying worker colonies showed minimum contribution to the drone congregation areas.

2.5 EFFECT OF COLONY CONDITIONS AND DIFFERENT REARING METHODS ON QUALITY CHARACTERS OF DRONES

2.5.1 Effect of colony conditions on quality characters of *A. mellifera* drones

Duay *et al.* (2002) studied the effect of *Varroa destructor* infestation during pupal stage on the sperm production in drones of *A. mellifera.* The drones from unparasitized pupal stage produced more sperms (2.5 x 10^6 to 12.8 x 10^6) in comparison to drones having infestation of one female mite per brood cell (1 x 10^6 to 13.5 x 10^6) and two female mites per brood cell (1 x 10^6 to 9.5 x 10^6). They reported that drones infested during development with one to two mites produced 24 per cent and 45 per cent fewer sperm.

Duay *et al.* (2003) observed the detailed data on individual drone weight loss, especially from the red eyed pupal stage onwards to adult stage. The decreased weight of adult drones was positively correlated to the female mite population (up to 20) attacking the brood cells. Weight loss was significant in drones which emerged from brood cells infested with single mite (250.4 mg) as compared to drones which emerged from uninfested brood cells (277.1 mg). They reported that mini-drones were found to emerge from heavily infested brood cells.

Shoukry *et al*. (2013) studied the effect of different miticides including formic acid on the drones of *A. mellifera*. Drones were measured for body weight and number of sperms in one seminal vesicle. According to them, highest mean body weight (0.211 g) and sperm count (5.39 ± 1.37 x 10^6) was recorded in healthy colonies compared to the body weight (0.205 g) and sperm count (5.32 ± 1.45 x 10^6) of drones produced from formic acid treated colonies.

Hossain *et al.* (2015) investigated the effect of formic acid, bayvarol and oxalic acid + sugar syrup on drone fertility of *A. mellifera.* They found that drones of healthy colonies had more viable sperms (94.58 %) and sperm production (1.63 µl) as compared to drones produced from formic acid treated colonies having lesser sperm production (0.96 µl) and sperm viability (88.68 %)

Omar (2017) evaluated the effect of varroa infestation on body weight from 1, 10, 17 and 21 days old drones of *A. mellifera* reared in healthy and varroa infested colonies. The 1 day old drones of healthy colonies was heaviest (259.62 mg) in comparison to one day old drones of 7 - 10 per cent *Varroa* infested colonies (224.53 mg). Thereafter the weight was found to steadily decrease with age as, 10 day old drones of healthy colonies was 227.94 mg in comparison to 185.19 mg in mite infested colonies, which further decreased in 17 day old drones of healthy (207.33 mg) and mite infested colonies (174.77 mg). Likewise, the lightest body weight was recorded for 21 day old drones of healthy (198.34 mg) and varroa infested colonies (163.04 mg).

2.5.2 Effect of rearing methods on quality characters of *A. mellifera* drones

Rinderer *et al.* (1985) worked on comparison of body weight and spermatozoa count of drones produced from the mated queen colonies of Africanized (*A. mellifera scutellata*) and European (*A. mellifera ligustica*) bees. The drones had mean weight of 194.6 mg and 220.2 mg. Whereas, the spermatozoa count was found to be 4.6 x 10^6 and 5.7 x 10^6 in Africanized (*A. mellifera s.*) and European (*A. mellifera l.*) drones, respectively.

Kumar (2002) analysed the quality of drones produced from mated queen and laying worker colonies of *A. mellifera*. He observed that drones of laying workers produced lower amount of semen (0.70 ± 0.05 µl), sperm count (2.85 ± 0.08 x 10^6), extent of full eversion per drone (54.44 ± 6.87) and weight of newly emerged drones (178.84 ± 1.63 mg) as compared to normal drones produced from mated queen colonies (1.01 ± 0.06 µl) semen, (3.29 ± 0.13 x 10^6) sperm count, (71.11 ± 4.60) extent of full eversion per drone and weight (216.11 ± 0.94 mg). He inferred that drones can be successfully reared in weak colonies using laying workers for using in inseminating virgin queenbees during non - breeding season.

Schluns *et al.* (2003) investigated the impact of the body size of the honeybee drone of *Apis mellifera* on semen development. The drones were reared in drone cells and in worker cells of same colonies. The sperm number and wing size of small and large drones were compared. The small drones had reduced wing size (13 %) and they produced significantly fewer spermatozoa 7.5 ± 0.5 million than normally sized drones 11.9 ± 1.0 million spermatozoa. They inferred that small drones produced an average of 37 per cent less spermatozoa than larger drones.

Gencer and Firatli (2005) compared the reproductive characters and live weight at different ages of drones reared in mated queen colonies and laying worker colonies. They inferred that drones from mated queen colonies had 273.8 ± 0.83 mg weight at emergence which decreased in 12 day drone 233.0 ± 0.85 mg to 222.3 ± 1.02 mg in 18 day drone. The weight at emergence was (173.5 ± 0.85 mg) in drones produced by laying worker, respectively. The weight was found to decrease in 12 day drones produced by laying workers (155.4 ± 1.17 mg), which further reduced in 18 day old drones (150.5 ± 1.24 mg). Likewise, the drones reared in mated queen colonies produced more spermatozoa (12.01×10^6) as compared to drones ($8.62 \pm 0.274 \times 10^6$) from of laying worker colonies.

Zaitoun *et al.* (2009) analysed the weight and sperm count of drones reared in mated queen and laying worker colonies of two honey bee species, *A. mellifera ligustica* and *A. mellifera syriaca.* They concluded that average weight of drones matured from worker of *A. mellifera ligustica* (181 mg) and *A. mellifera syriaca* (170 mg) was low than those produced in queenright colonies of *A. mellifera ligustica* (203 mg) and *A. mellifera syriaca* (181 mg). The average sperm count of drones obtained from workers of *A. mellifera ligustica* and *A mellifera syriaca* was 6.8 million (5.4-8.5 million) and 6.1 million (4.3-7.8 million) which was less than the drones of queenright colonies of *A. mellifera ligustica* 10.2 million (8-12.8 million) and *A. mellifera syriaca* 8.8 million (8.8-10.6 million).

Mazeed and Mohanny (2010) studied the effect of age on *A. mellifera carnica* drones. Measurements of drones were carried out at an interval from 1 to 3, 4 to 6, 7 to 9, 10 to 12 and 13 to 15 days. The results showed that initially in 1- 3 day old drones the mean weight was 201 ± 16.4 mg. Further the weight was found to increase to 212.5 ± 25.37 mg in (4 to 6 day) old drones, after that it gradually decreased from 210 ± 22.36 mg in (7 to 9 day) old drone to 193.8 ± 14.95 mg in (10 to 12 day) old drones and finally to 180 ± 7.07 mg in (13 to 15 day) old drones, respectively.

Gencer and Kahya (2011) compared the sperm traits and body weight of 18 day old small drones produced from laying worker colonies with large drones from queenright colonies of *A. mellifera.* Drones from queenright colonies were 50.40 per cent hefty (221.6 mg) than the drones from laying worker colonies (147.3 mg). The mean sperm number in drones of queenright colonies (7.32×10^6) was more than in the drones from laying worker colonies (4.42×10^6). The mean volume of sperm ejaculated by drones of queenright colonies was 53 per cent higher (1.01 µl) than the sperm ejaculated in drones of laying worker colonies (0.66 µl).

Mazeed (2011) studied the effect of absence and presence of queen on characteristics of newly emerged drones and inferred that drone produced in queenless colony had higher mean weight (225.8 ± 16.71 mg) and number of sperms (4.46 x 10^6) as compared to mean weight (210.6 ± 26.51 mg) and number of sperms (3.69 ± 0.61 x 10^6) of queen right colony. Therefore, he suggested that raising colonies should be given new drone combs and that the drones be allowed to finish their development in queenless colonies, as the resultant drones are bigger and contain more spermatozoa.

Rhodes *et al.* (2011) studied the effect of age on semen and sperm production in *A. mellifera* drones in spring, summer and autumn. Their findings revealed that 40.5 per cent of the drones examined did not release any semen. The average semen volume and spermatozoa number for 14 day old drones was 0.98 ± 0.03 µL (0.1 to 2.2 µl) and 2.83 ± 0.15 x 10^6 (0.12 to 11.52 x 10^6). Their studies further revealed that mean semen volume 1.07 ± 0.04 µL (0.2 to 3.6 µl) and spermatozoa number 3.36 ± 0.18 x 10^6 (0.18 to 13.52 x 10^6) increased in 21 day old drone whereas, in 35 day old drones the semen volume 0.73 ± 0.04 µL (0.2 to 3.0 µl) and spermatozoa number 2.83 ± 0.21 x 10^6 (0.12 to 19.13 x 10^6) gradually decreased.

Taha and Alqarni (2013) analysed the sperm quantity of *A. mellifera jemenitica* and *A. mellifera carnica* drones produced from queen right colonies. They inferred that mean body weight (190.90 ± 0.33 mg) of newly emerged drones and sperm count of 14 day old drones (9.33 ± 2886.75 x 10^6) of *A. mellifera jemenitica* were comparatively lesser than the body weight (227.22 ± 0.63 mg) of newly emerged drones and sperm count (12.67 ± 2886.75 x 10^6) of *A. mellifera carnica.* Therefore, significant positive correlations were seen between body weight and sperm number.

Abdelkader *et al.* (2014) evaluated the semen quality of 20 day old drones which were kept under laboratory, semi-field and field conditions. They concluded that drones maintained under laboratory (2.5 ± 0.1 x 10^6 spz/µl semen) and field condition (2.2 ± 0.6 x 10^6 spz/µl semen) produced lower concentration of spermatozoa as compared to those maintained at semi- field condition (3.4 ± 0.4 x 10^6 spz/µl semen). The fluctuation in temperature before semen collection resulted in the significant effect on spermatozoa count.

Rousseau *et al.* (2015) investigated the sperm quality of *A. mellifera* drones produced in queen right colonies. They studied the semen volume, sperm count and per cent eversion at the different age group of 14, 21 and 35 days. They inferred that the mean semen volume 1.01 ± 0.03 µL (0.4 µL to 2.4 µL) and average sperm count of drone was 1.80 ± 1.65 x 10^6

($0.008 – 7.77 \times 10^6$). Whereas the per cent eversion was affected by age as it increased from (58.3 %), to (65.9 %) and (80.2 %) at an age of 14, 21 and 35 days, respectively.

Rousseau and Giovenazzo (2016) analysed the quality characters of *A. mellifera* drones from mated queen colonies. They inferred that the mean weight of drones was 240.5 ± 61.1 mg, semen volume 1.09 ± 0.03 µl and sperm count per drone $3.05 \pm 61.5 \times 10^6$.

Al – Sarhan *et al.* (2018) investigated the reproductive biology of *A. mellifera jemenitica* drones. They found that average body weight (0.16 ± 0.02 g) and spermatozoa count was ($8.80 \pm 1.60 \times 10^6$) of *A. mellifera jemenitica* drones.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled, " **Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L.”** were carried out during 2021 in university apiary at the entomology experimental farm and apiculture laboratory of the Department of Entomology, College of Horticulture, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The university is situated at 33°51.607” E longitude and 1262 m amsl. Experimental work on the following aspects were undertaken:

3.1 DRONE REARING FROM DIFFERENT REARING METHODS

In present investigations, drones from virgin queen, laying workers and mated queen were reared for recording different observations. Each method was replicated in three *A. mellifera* colonies. The newly emerged drones were identified on the basis of being fuzzy and having soft abdomen. The drones were marked in morning hours on the thorax (Plate 1) using different colour markers (white, red and green) to prevent the drones from mixing with adjacent colonies having different treatments. Then after attaining the age of 10, 15 and 20 days (n= 5) drones were collected from each colony for recording observation mentioned at serial no. 3.4.1 and 3.4.2.

3.1.1 Drone production from virgin queen

Three mating nucs were made and worker bees were shaken in them from the frames of another colony. Then six-day old virgin queen of *A. mellifera* was induced by giving two carbon dioxide treatments (Plate 2) for ten minutes at an interval of two days (Mackensen, 1947). These nucs were regularly checked for egg laying after the carbon dioxide treatment. The egg laying started after 12 days of treatment and drones emerged after 24 days.

3.1.2 Drone production from laying workers

To stimulate egg production by workers, 3 colonies of *A. mellifera* were dequeened and unsealed brood was removed in order to prevent them from rearing emergency queens (Gencer and Kahya, 2011). These colonies were regularly checked on second and third day after dequeening. The observations were continued in these colonies at 2-day interval for recording initiation of egg laying. The laying workers were recognized with its black abdomen and bigger size. It took 25 days for the laying worker to lay eggs and drones emerged after 24 days (Plate 3).

3.1.3 Drone production from mated queen

Three colonies having mated queens were selected for production of drones under normal condition.

3.2 DRONE REARING IN DIFFERENT COLONY CONDITIONS

In present investigations, drones from healthy, mite infested and formic acid treated mite infested colonies were collected for recording different observations. Each experiment was replicated in three *A. mellifera* colonies.

3.2.1 Healthy colony

The drones were taken from healthy colonies of *A. mellifera* which were free from mite infestation.

3.2.2 Mite infested colony

The drones were taken from *A. mellifera* colonies which were infested only with *Varroa* mites. The mite infestation was determined by visual examination method (Ritter, 1980) in which 100 brood cells were observed with the help of a lens. The level of mite infestation was 10 per cent to 12 per cent per colony. (Plate 4)

3.2.3 Formic acid treated mite infested colony

The drones were taken after 17 days from those *A. mellifera* colonies which were infested with mite and single treatment was given with 50 ml of 60 per cent formic acid per hive by using sponge which was covered by plastic having small holes on it and kept on the bottom board of the hive (Plate 5) (Rana *et al*., 2009).

3.3 EXPERIMENTAL DETAILS

The experiments of colony conditions and rearing methods were carried out using Completely Randomized Design (Factorial) with each 9 treatments combination. Each combination was replicated thrice.

Plate 1: Marking of immature drones

Plate 2: Carbon dioxide treatment

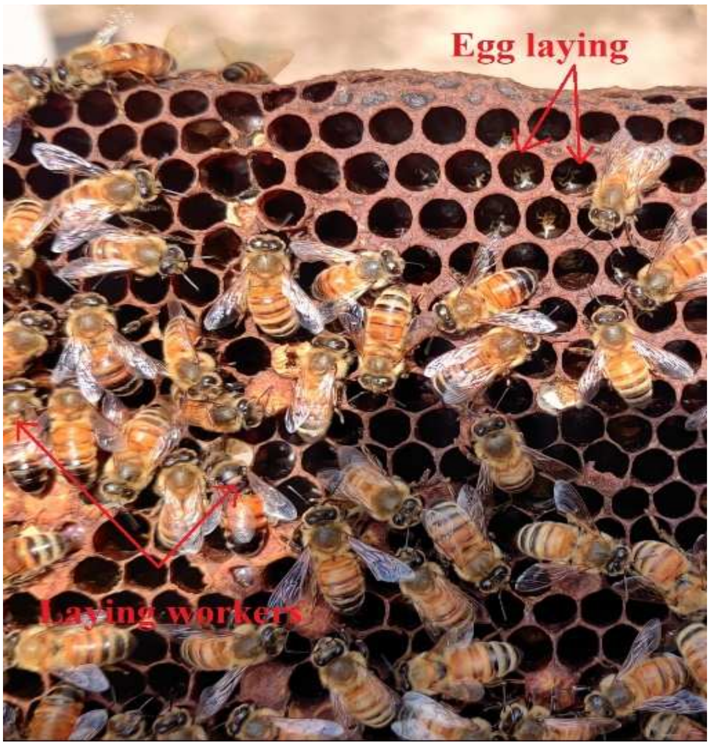


Plate 3: Laying workers

Plate 4: mite infested drone brood

Plate 5: Formic acid treatment

3.3.1 Details of treatments of colony conditions

T_1 – 10 day old drones from healthy colonies

T_2 – 15 day old drones from healthy colonies

T_3 – 20 day old drones from healthy colonies

T_4 – 10 day old drones from mite infested colonies

T_5 – 15 day old drones from mite infested colonies

T_6 – 20 day old drones from mite infested colonies

T_7 – 10 day old drones from formic acid treated colonies

T_8 – 15 day old drones from formic acid treated colonies

T_9 – 20 day old drones from formic acid treated colonies

Number of treatment combinations	**:**	**9**
Number of replication	**:**	**3**
Experimental design	**:**	**Completely Randomized Design (Factorial)**

3.3.2 Details of treatment of rearing methods

T_1 – 10 day old drones from virgin queen colonies

T_2 – 15 day old drones from virgin queen colonies

T_3 – 20 day old drones from virgin queen colonies

T_4 – 10 day old drones from laying worker colonies

T_5 – 15 day old drones from laying worker colonies

T_6 – 20 day old drones from laying worker colonies

T_7 – 10 day old drones from mated queen colonies

T_8 – 15 day old drones from mated queen colonies

T_9 – 20 day old drones from mated queen colonies

Number of treatment combinations	**:**	**9**
Number of replication	**:**	**3**
Experimental design	**:**	**Completely Randomized Design (Factorial)**

3.4 DRONE COLLECTION

Drones of *A. mellifera* from different colony conditions and rearing methods were collected at their given ages (10, 15 and 20 days old) in the spring season during 2021. About

15 drone bees per treatment combination at particular age were collected from 3 colonies in drone cages.

3.5 OBSERVATIONS RECORDED

The following observations were recorded from the drones produced by different methods and under different colony conditions.

3.5.1 Quality characters

The following quality characters were recorded: (Plate 6)

3.5.1.1 Weight of drone

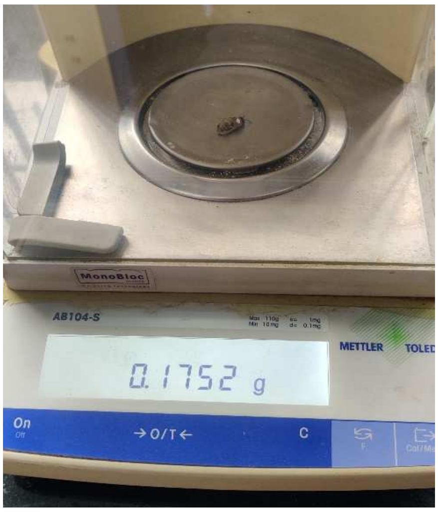
Drones were collected from the experimental colonies as per the selected days and weighed individually on electrical balance (AB104-S). The weight of drones was expressed in milligram (mg). (Plate 6.a)

3.5.1.2 Eversion per cent

According to Harbo and Williams (1987), who used manual eversion, reported that drones aged between 10-21 days are mature. Drones of 10, 15 and 20 days old were evaluated. Drones were everted by grasping the head and thorax of the drone between the thumb and forefinger, ventro-dorsally, with the abdomen facing upward and then pressure was applied along the sides of the abdomen, starting at the anterior base and working toward the posterior tip together in one steady forward motion, which resulted in eversion (Laidlaw 1977 and Cobey *et al.,* 2013). The drones which everted fully by exposing their endophallus completely with semen and a pair of yellow-orange cornua were marked “fully everted” (Plate 6.b) and those which failed to expose semen and lacked colour of coruna were marked “partially everted” (Plate 6.c). The percentage of fully everted drones of different age was calculated from these observations.

3.5.1.3 Semen volume

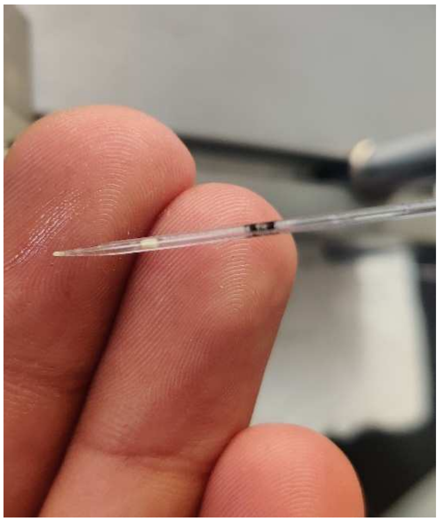
The collection of semen was done as per the procedure described by Cobey *et al.* (2013). The drones of different age groups were stimulated to evert as described under 3.4.1.2. The drones were held with their abdomen pointing upwards to keep the endophallus from falling backwards onto the fingers. Ejaculated semen was creamy and tan in colour. After assembly of the capillary tube, air space was created to separate the saline and semen



(6.a) Body weight

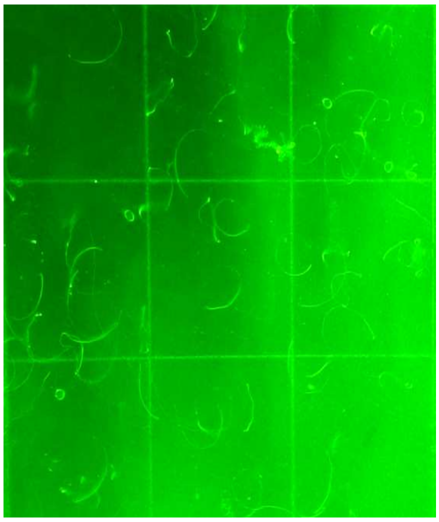
(6.b) Partial eversion

(6.c) Full eversion



(6.d) Semen volume

(6.e) Semen extraction



(6.f) Sperm cells

Plate 6: Quality characters of *A. mellifera* L. drones

column in the capillary (Plate 6.d). Then carefully semen was skimmed off the mucus layer by avoiding the collection of viscous mucus layer as it results into clogging the capillaries using Harbo syringe. The amount of semen produced per drone was measured in microlitre (µl). (Plate 6.e)

3.5.1.4 Sperm count

The sperm count was taken as per the method described by Woyke, 1979. To count the number of spermatozoa produced by each drone, improved Neubauer haemocytometer was used (Plate 6.f). Semen collected in the graduated capillary from each drone was gently mixed in 2ml of 0.85 per cent solution of sodium chloride so that sperms get mixed up in the solution. Then with the help of a pipette an aliquot of the preparation was placed in the grooved surface at the edge of cover glass. The capillary action filled each chamber with solution. Care was taken so as to avoid over-flowing of chamber by regulating the drop size. The preparation was then allowed to stand for about 5 minutes so that spermatozoa got settled on to the bottom of haemocytometer. The sperm cells were counted in 20 squares (1/16 mm^2 each) of Neubauer haemocytometer. The sperms were seen under a Stereo Electronic Microscope (Zeiss Axioskop 40) by using 150X magnification. The sperms appeared to be headless, filamentous and coiled. Then the average count of sperms in one square (n) was calculated to find total number of sperms using following formula

$$\text{Sperm count} = n \times (1.6 \times 10^5) \times \text{total volume of saline solution}$$

3.5.1 MORPHOMETRIC STUDIES

3.5.2.1 Calibration of ocular scale

The ocular scale was calibrated at 4.5 x 15X magnification and all measurements were made at this magnification. The stage micrometre had 1mm scale divided into 100 divisions of 0.001mm each.

Calibration at 4.5 x 15X magnification:

46 divisions of ocular micrometre = 100 divisions of stage micrometre
1 division of ocular micrometre = 100/46 = 2.2 divisions of stage micrometre
1 division of stage micrometre = 0.01 mm
1 division of ocular micrometre = 0.01 x 2.2 = 0.022 mm

Morphological parts of the *A. mellifera* drones were measured based on the previous work of Gencer and Firatli, 2005. The measurement of drone body length, thorax length and thorax breadth were recorded prior to manual eversion (Plate 7).

The following parameters were observed:

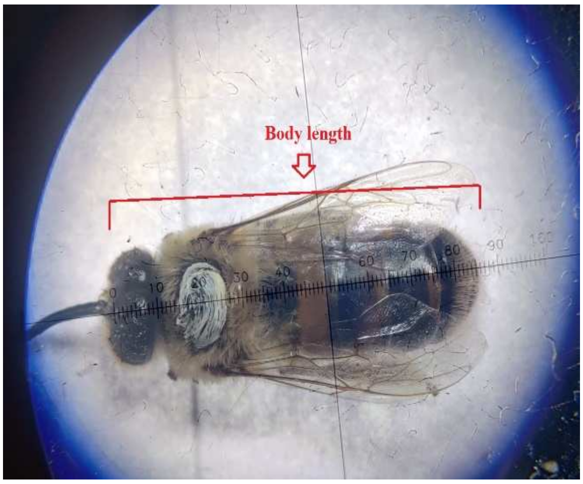
i. **Body length:** Distance between the anterior most point of head to posterior tip of the abdomen.
ii. **Thorax length:** Distance between the anterior and posterior end of thorax.
iii. **Thorax breadth:** Distance between the tips of the tegulae.
iv. **Hind leg length:** Distance from base of the coxa to tip of the arolium.
v. **Forewing length:** Distance between the articulatory point of forewing and apical tip.
vi. **Forewing breadth:** Distance in the middle of forewing where it is maximum.
vii. **Hindwing length:** Distance between the articulatory point of hindwing and its apical tip.
viii. **Hindwing breadth:** Distance in the middle of hindwing where it is maximum.
ix. **Hamuli expanse:** Distance between first hook to the last hook.
x. **Hamuli number:** Number of hooks present on the costal margin of the hindwing.

3.5.2.2 Slide preparation

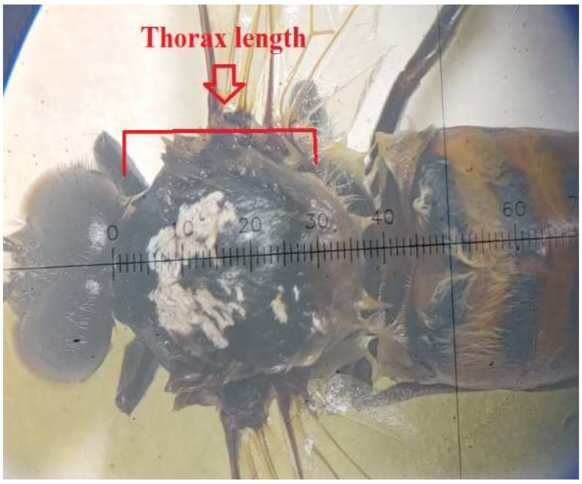
The drones after manual eversion were preserved in 70 per cent alcohol. Then the drones were carefully dissected for hind leg length, forewing length, forewing breadth, hindwing length, hindwing breadth, hamuli expanse and hamuli number. These morphological parts were mounted on the center of the slides with the help of a brush. Thereafter, a drop of glycerine was placed on the specimen and were covered with the coverslips. To avoid bubble formation the slides were kept in the oven at 45 ºC. The slides with mounted specimens were observed under stereomicroscope (Olympus SZ61) equipped with an ocular micrometer.

3.6 STATISTICAL ANALYSIS

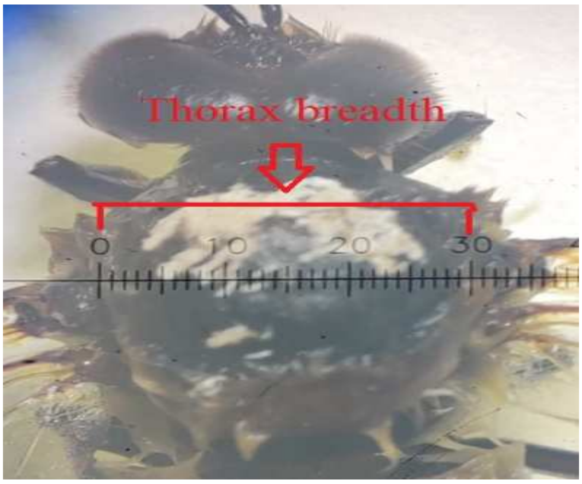
The data obtained from these investigations was significantly analysed by using MS−Excel and OPSTAT. The mean value of data was subjected to statistical analysis as described by Gomez and Gomez (1984) by applying Completely Randomized Design (CRD) Factorial.



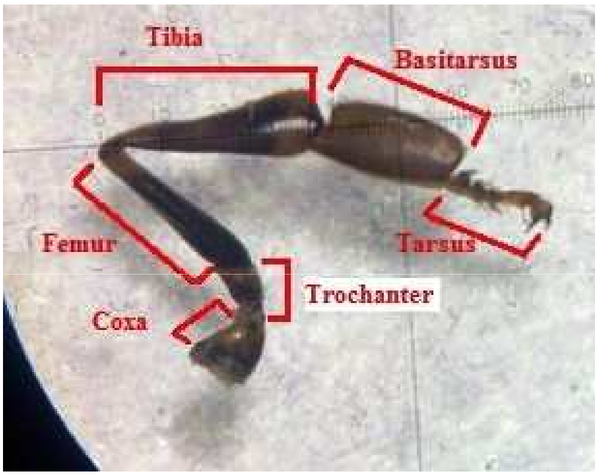
(a) Body length



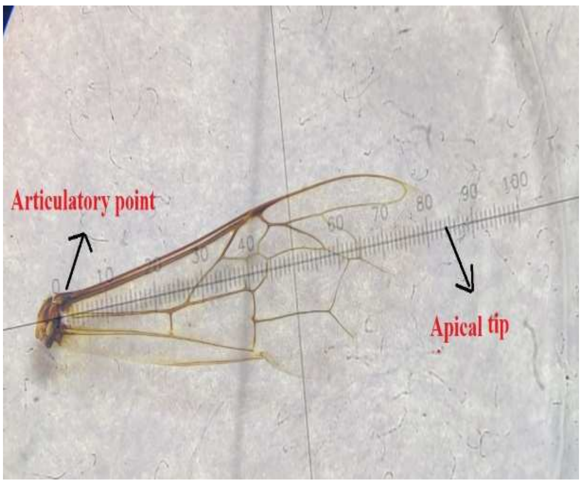
(b) Thorax length



(c) Thorax breadth

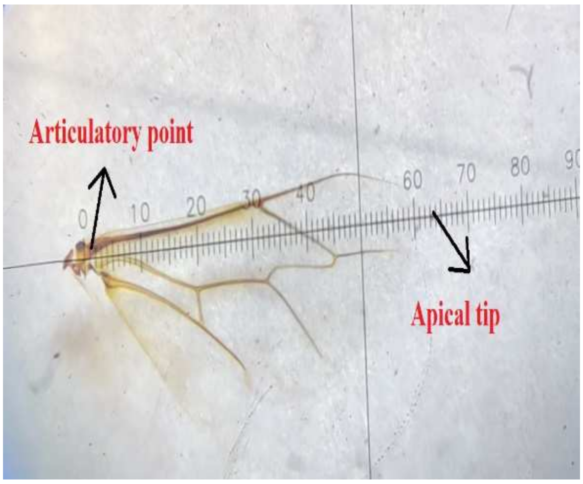


(d) Hind leg length

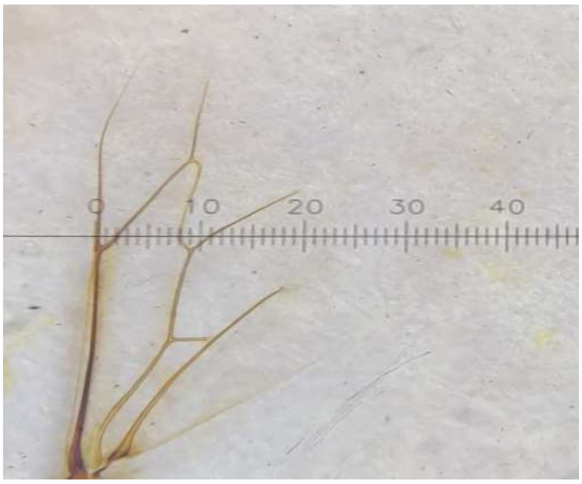


(e) Fore wing length

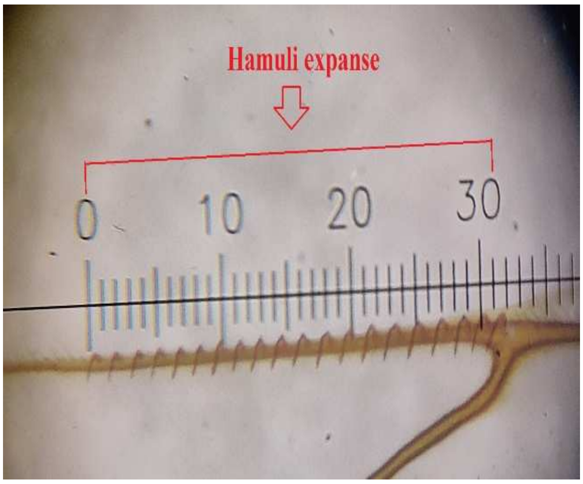
(f) Fore wing breadth



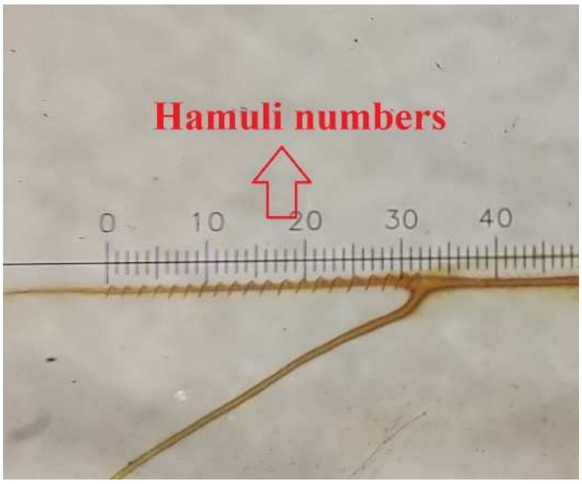
(g) Hind wing length



(h) Hind wing breadth



(i) Hamuli Expanse



(j) Hamuli number

Plate 7: Morphological characters of *A. mellifera* L. drones

Chapter-4

RESULTS AND DISCUSSION

The present investigations entitled, **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** were carried out in university apiary at the experimental farm and apiculture laboratory of the Department of Entomology, College of Horticulture, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.

4.1 EFFECT OF COLONY CONDITIONS ON QUALITY AND MORPHOMETRIC CHARACTERS OF *A. mellifera* DRONES

4.1.1 QUALITY CHARACTERS

Quality characters like drone weight, eversion per cent, semen volume and sperm count have the potential to influence the queen's reproductive success, colony survival and productivity, as well as the success of artificial insemination (Pettis *et al.*, 2016). Present investigations were conducted to evaluate the impact of colony conditions and drone age on quality characters of *A. mellifera* drones during spring of 2021. The results are discussed under the following heads.

4.1.1.1 Effect of colony conditions and drone age on the weight of *A. mellifera* drones

The data on the effect of colony conditions and drone age on the weight of drones presented in Table 1 revealed that body weight of drones produced in healthy colonies (205.35 mg) and formic acid treated colonies (203.77 mg) was statistically similar. Significantly minimum body weight (180.04 mg) was found in drones produced from mite infested colonies.

Perusal of data in Table 1 further indicate that body weight is influenced with drone age. The maximum weight was found in 10 day old drones (207.05 mg) which was followed by 15 day old drones (195.16 mg) and 20 day old drones (186.95 mg), being statistically different.

The interaction effect of colony conditions and drone age, on body weight of drones varied significantly. The highest body weight (216.20 mg) was found in 10 day old drones from healthy colonies which was followed by body weight (210.25 mg) of 10 day old drones from formic acid treated colonies. Significantly minimum body weight (170.32 mg) was found in 20 day old drones of mite infested colonies which was statistically at par with 15 day old drones from mite infested colonies (175.11 mg).

Table 1. Effect of colony conditions and drone age on the weight of *A. mellifera* drones

Colony condition	**Drone weight (mg)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	216.20	205.78	194.06	**205.35**
Mite infested	194.71	175.11	170.32	**180.04**
Formic acid treated	210.25	204.58	196.48	**203.77**
Mean	**207.05**	**195.16**	**186.95**	

CD $_{(0.05)}$

Colony conditions : 3.33
Age of drones : 3.33
Colony conditions x Age of drones : 5.77

The present observations on higher body weight of *A. mellifera* drones from healthy colonies and formic acid treated colonies in comparison to mite infested colonies are in agreement with the observations of Shoukry *et al*. (2013), who have also reported that body weight of drones from healthy colonies (0.221 g) and formic acid treated colonies (0.205 g) was statistically same. The present observations showing statistically minimum drone body (180.04 mg) weight in mite infested colonies got support from the work of Duay *et al.* (2003), who reported that drones emerging from mite infested brood cells showed significant weight loss (250.4 mg) when compared with drones emerging from uninfested brood cells (277.1 mg).

In the present studies the weight of *A. mellifera* drones decreased with age from 10 day old to 20 day old drones in healthy colonies (216.20 mg to 194.06 mg), mite infested colonies (194.71mg to 170.32 mg) and formic acid treated colonies (210.25 mg to 196.48 mg). These findings fall in the line with Mazeed and Mohanny (2010) and Omar (2017), who have also reported that body weight suffered gradual reduction with age in drones. Mazeed and Mohanny (2010) reported decrease in the body weight of drones from 210 ± 22.36 mg in 7 to 9 day old drones to 180 ± 7.07 mg in 13 to 15 day old drones. Similarly, Omar (2017) reported significant reduction in body weight from 10 day old to 21 day old drones in healthy colonies (227.94 mg to 198.34 mg) and mite infested colonies (185.19 mg to 163.04 mg). Their observations showing significant reduction in drones body weight of 10, 17 and 21 day old of healthy colonies in comparison to mite infested colonies also support the present findings, where average weight in each studied age group was significantly higher for drones from healthy colonies in comparison to formic acid treated colonies.

4.1.1.2 Effect of colony conditions and drone age on the eversion per cent of *A. mellifera* drones

The data on the effect of colony conditions and drone age on the eversion per cent of drones presented in Table 2 indicate that full eversion of drones produced in healthy colonies (77.77 %) and formic acid treated colonies (68.88 %) was statistically at par. Significantly minimum number of drones with full eversion (57.77 %) were found in mite infested colonies.

The data in Table 2 further indicate that full eversion in drones is influenced with drone age as there was significantly more number of 20 day old drones everted fully (88.88 per cent) followed by 15 day old drones (73.33 %) and 10 day old drones (42.22 %). Irrespective of colony conditions and drone age, the proportion of fully everted drones varied non-significantly from 33.33 per cent in 10 day old drones from mite infested colonies to 93.33 per cent in 20 day old drones from healthy and formic acid treated colonies.

Table 2. Effect of colony conditions and drone age on the eversion per cent of *A. mellifera* drones

Colony condition	**Eversion (per cent)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	53.33 (46.92)*	86.66 (72.29)	93.33 (81.14)	**77.77 (66.78)**
Mite infested	33.33 (35.00)	60.00 (50.76)	80.00 (68.06)	**57.77 (51.28)**
Formic acid treated	40.00 (39.23)	73.33 (59.21)	93.33 (81.14)	**68.88 (59.86)**
Mean	**42.22 (40.38)**	**73.33 (60.75)**	**88.88 (76.78)**	

*Values in parentheses are means of arc sine values

CD $_{(0.05)}$

Colony conditions	:	11.79
Age of drones	:	11.79
Colony conditions x Age of drones	:	NS

The present observations on more proportion of fully everted drones of *A. mellifera* in healthy colonies (77.77 %) in comparison to formic acid treated colonies (68.88 %) are in close proximity with the results of Hossain *et al.* (2015), who have reported that drones of formic acid treated colonies showed less full eversion (66.97 %) as compared to healthy colonies (74.28 %). The present findings indicating increase in the proportion of fully everted drones at 10 (42.22 %), 15 (73.33 %) and 20 (88.88 %) day age got support from work of Kumar (2002), whose findings also revealed increased number of fully everted drones with age. He found that drones of 12, 15 and 20 day old showed 40, 75 and 90 per cent full eversion, respectively. Later Rousseau *et al*. (2015) also reported that eversion was dependent on drone age as it increased from 58.3 per cent in 14 day old drones to 65.9 per cent in 21 day old drones.

4.1.1.3 Effect of colony conditions and drone age on the semen volume of *A. mellifera* drones

The data on the effect of colony conditions and age on the semen volume of drones presented in Table 3 indicate that higher semen volume (1.01 µl) was recorded from drones produced in healthy colonies, which was statistically similar to semen volume (0.86 µl) of drones produced from formic acid treated colonies. The low semen volume (0.66 µl) was found in drones of mite infested colonies.

Table 3. Effect of colony conditions and drone age on the semen volume of *A. mellifera* drones

Colony condition	Semen volume (µl)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	0.22	1.22	1.59	**1.01**
Mite infested	0.13	0.74	1.10	**0.66**
Formic acid treated	0.20	1.04	1.34	**0.86**
Mean	**0.18**	**1.00**	**1.34**	

CD $_{(0.05)}$

Colony conditions : 0.16
Age of drones : 0.16
Colony conditions x Age of drones : NS

The data further show that semen volume significantly increased with drone age. The maximum volume of semen was found in 20 day old drones (1.34 µl) followed by 15 day old drones (1 µl). Significantly low semen volume was found in 10 day old drones (0.18 µl). Irrespective of colony conditions and drone age, semen volume varied non-significantly from

0.13 µl in 10 day old drones from mite infested colonies to 1.59 µl in 20 day old drones from healthy colonies.

4.1.1.4 Effect of colony conditions and drone age on the sperm count of *A. mellifera* drones

The data on the effect of colony conditions and age on the sperm count of drones presented in Table 4 indicate that sperm count of drones produced in healthy colonies (1.88×10^6) and formic acid treated colonies (1.67×10^6), was statistically at par. Whereas the minimum sperm count (1.30×10^6) was found in mite infested colonies.

Perusal of data in Table 4 indicate that sperm count is influenced with drone age being significantly more in 20 day old drones (2.60×10^6) followed by 15 day old drones (1.81×10^6) and 10 day old drones (0.44×10^6). Irrespective of colony conditions and drone age, semen count varied non-significantly from 0.37×10^6 in 10 day old drones of mite infested colonies to 3.06×10^6 in 20 day old drones of healthy colonies.

Table 4. Effect of colony conditions and drone age on the sperm count of *A. mellifera* drones

Colony condition	**Sperm count/drone ($\times 10^6$)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	0.50	2.09	3.06	**1.88**
Mite infested	0.37	1.37	2.14	**1.30**
Formic acid treated	0.44	1.97	2.61	**1.67**
Mean	**0.44**	**1.81**	**2.60**	

CD $_{(0.05)}$

Colony conditions	:	0.26
Age of drones	:	0.26
Colony conditions x Age of drones	:	NS

The present observations on semen volume (1.01 µl) and sperm count (1.88×10^6) in healthy colonies are in accordance with the findings of Rhodes *et al.* (2011), who reported that average semen volume and sperm count of drones from healthy colonies ranged from 0.72 µl to 1.12 µl and 1.88×10^6 to 4.11×10^6. Similarly, the average value for semen volume and sperm count falls within the range value of semen volume (1.22 µl to 1.59 µl) and sperm count (2.09×10^6 to 3.06×10^6) recorded by Rousseau *et al.* (2015).

In context of semen volume from drones of formic acid treated colonies, the present findings (0.86 µl) are in close proximity with the results of Hossain *et al*. (2015), who reported that semen volume of drones from formic acid treated colonies was low (0.96 µl) while the healthy colonies showed higher semen volume (1.63µl).

The present findings revealed statistically similar sperm count in drones from healthy (1.88×10^6) and formic acid treated colonies (1.67×10^6). These observations got support from the work of Shoukry *et al*. (2013), who reported that sperm count in drones of formic acid treated colonies ($5.32 \pm 1.45 \times 10^6$) was statistically at par with drones of healthy colonies ($5.39 \pm 1.37 \times 10^6$). These observations indicate that mite management by using formic acid is resulting into production of drones similar to healthy colonies.

In the present studies, the findings reveal least sperm count (1.30×10^6) in mite infested drones. The results are in agreement with Duay *et al.* 2002, who observed that drones having infestation of one female mite per brood cell and two female mites per brood cell produces sperm in the range of (1×10^6 – 13.5×10^6) and (1×10^6 – 9.5×10^6) thereby producing 24 per cent and 45 per cent fewer sperms, respectively, in comparison to drones of healthy colonies. Similarly, Rinderer *et al.* (1998) also reported reduction in sperm count of drones from mite infested colonies (3.672×10^6) in comparison to healthy colonies (4.54×10^6). The reason for less semen production in drones of mite infested colonies could be attributed to the fact that mite infested drones have severe reduction of testis, vesicula seminalis and mucus gland volume. Moreover, mite infested drones also suffer loss of nutrients (Omar, 2017). These reasons must have led to low semen volume in drones from mite infested colonies.

The present results revealing increase in semen volume and sperm count with the age in 10 day to 20 day old drones (Table 3 and 4) are in close proximity to the observations of Rhodes *et al.* (2011), who reported that semen and sperm count released per drone depends upon drone age. According to them semen volume increased from 0.98 ± 0.03 µL in 14 days old drones to 1.07 ± 0.04 µL in 21 old drones. The respective values for sperm count were reported to be $2.83 \pm 0.15 \times 10^6$ to $3.36 \pm 0.18 \times 10^6$.

4.1.2 MORPHOLOGICAL CHARACTERS

4.1.2.1 Effect of colony conditions and drone age on the body length of *A. mellifera* drones

The data on the effect of colony conditions and drone age on body length of drones presented in Table 5 revealed that body length of drones produced in healthy colonies (15.82 mm) and formic acid treated colonies (15.66 mm) was statistically similar. Whereas, minimum body length (15.49 mm) was found in drones of mite infested colonies

The data enumerated in Table 5 further revealed that body length of drones depends upon their age. The body length significantly decreased with the age of drones and was 16.22 mm, 15.74 mm and 15.00 mm in 10, 15 and 20 day old drones, respectively. Irrespective of colony conditions and drone age, the body length varied non-significantly from 14.90 mm in 20 day old drones from mite infested colonies to 16.50 mm in 10 day old drones from healthy colonies.

Table 5. Effect of colony conditions and drone age on the body length of *A. mellifera* drones

Colony condition	**Body length (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	16.50	15.87	15.08	**15.82**
Mite infested	15.90	15.66	14.90	**15.49**
Formic acid treated	16.28	15.70	15.02	**15.66**
Mean	**16.22**	**15.74**	**15.00**	

CD $_{(0.05)}$

Colony conditions : 0.16
Age of drones : 0.16
Colony conditions x Age of drones : NS

4.1.2.2 Effect of colony conditions and drone age on the thorax length of *A. mellifera* drones

The data on the effect of colony conditions and drone age on the thorax length of drones presented in Table 6 indicated that thorax length of drones produced in healthy colonies (5.09 mm) and formic acid treated colonies (5.04 mm) was statistically at par. The thorax length (4.87 mm) in drones from mite infested colonies was significantly minimum in comparison to other two treatments.

The data further revealed that thorax length of drones depends upon their age. The thorax length significantly decreased with the age of drones and was 5.09 mm, 5.02 mm and 4.89 mm in 10, 15 and 20 day old drones, respectively. Irrespective of colony conditions and drone age, the thorax length varied non-significantly from 4.74 mm in 20 day old drones from mite infested colonies to 5.15 mm in 10 day old drones from healthy colonies.

Table 6. Effect of colony conditions and drone age on the thorax length of *A. mellifera* drones

Colony condition	**Thorax length (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	5.15	5.11	5.02	**5.09**
Mite infested	4.99	4.87	4.74	**4.87**
Formic acid treated	5.13	5.09	4.92	**5.04**
Mean	**5.09**	**5.02**	**4.89**	

CD $_{(0.05)}$

Colony conditions : 0.05

Age of drones : 0.05

Colony conditions x Age of drones : NS

4.1.2.3 Effect of colony conditions and drone age on the thorax breadth of *A. mellifera* drones

The data on the effect of colony conditions and drone age on the thorax breadth of drones presented in Table 7 revealed that thorax breadth of drones produced in healthy colonies (4.96 mm) and formic acid treated colonies (4.91 mm) was statistically similar. The thorax breadth (4.84 mm) in drones of mite infested colonies was significantly least in comparison to other two treatments.

Perusal of data in Table 7 further revealed that thorax breadth of drones depends upon their age. The thorax breadth significantly decreased with the age of drones and was 5.00 mm, 4.92 mm and 4.79 mm in 10, 15 and 20 day old drones, respectively. Irrespective of colony conditions and drone age, the thorax breadth varied non-significantly from 4.77 mm in 20 day old drones from mite infested colonies to 5.08 mm in 10 day old drones from healthy colonies.

Table 7. Effect of colony conditions and drone age on the thorax breadth of *A. mellifera* drones

Colony condition	Thorax breadth (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	5.08	4.97	4.82	**4.96**
Mite infested	4.91	4.83	4.77	**4.84**
Formic acid treated	5.01	4.94	4.78	**4.91**
Mean	**5.00**	**4.92**	**4.79**	

CD $_{(0.05)}$

Colony conditions : 0.09
Age of drones : 0.09
Colony conditions x Age of drones : NS

4.1.2.4 Effect of colony conditions and drone age on the hind leg length of *A. mellifera* drones

The data on the effect of colony conditions and drone age on the hind leg length of drones presented in Table 8 indicate that the hind leg length of drones produced in healthy colonies (12.97 mm) and formic acid treated colonies (12.96 mm) was statistically at par. The minimum hind leg length (12.78 mm) was found in drones of mite infested colonies.

Perusal of data in table 8 further indicated that the hind leg of drones was identical in 10 day (12.89 mm), 15 day (12.91 mm) and 20 day (12.91 mm) old drones. Irrespective of colony conditions and drone age, the hind leg length varied non-significantly from 12.77 mm in 15 day old drones of mite infested colonies to 13.00 mm in 15 day old drones of healthy colonies.

Table 8. Effect of colony conditions and drone age on the hind leg length of *A. mellifera* drones

Colony condition	Hind leg length (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	12.94	13.00	12.97	**12.97**
Mite infested	12.78	12.77	12.78	**12.78**
Formic acid treated	12.93	12.95	12.99	**12.96**
Mean	**12.89**	**12.91**	**12.91**	

CD $_{(0.05)}$

Colony conditions : 0.09
Age of drones : NS
Colony conditions x Age of drones : NS

The results of present investigations showing decrease in body length of drones from mite infested (15.49 mg) and formic acid treated colonies (15.66 mg) in comparison to healthy colonies (15.82 mg) got support from the work of De Jong *et al.* (1982), who reported that abdomen length reduces with mite infestation. Such observations were also recorded by Anshakova *et al.* (1978), who found that mean length of abdomen in healthy worker bees was more (1.34 ± 0.001 cm) which reduced to 1.04 ± 0.002 cm in mite infested colonies.

The present observations indicating maximum hind leg length (12.97 mm) in drones from healthy colonies in comparison to mite infested colonies (12.78 mm) are in corroboration with the work of Belaid *et al*. (2017), who reported that workers of healthy colonies had maximum tibio-tarsale length (5.21 ± 0.13 mm) as compared to *Varroa destructor* infested colonies 4.86 ± 0.28 mm.

No literature is available in relation to decrease in body length, thorax length and thorax breadth with age. The possible reason for such decrease can be explained by the fact that longitudinal diameter of sternite and tergites got reduced in workers of mite infested colonies (Belaid *et al.*, 2017).

4.1.2.5 Effect of colony conditions and drone age on the forewing length of *A. mellifera* drones

The data on the effect of colony conditions and drone age on forewing length of drones is presented in Table 9. The data revealed that drones produced in healthy colonies had maximum forewing length (12.12 mm) followed by drones of formic acid treated colonies (11.35 mm). Significantly minimum forewing length (11.00 mm) was found in drones of mite infested colonies.

The present findings further revealed that forewing length in drones of different age groups was similar in 10 day (11.56 mm), 15 day (11.47 mm) and 20 day (11.44 mm) old drones. Irrespective of colony conditions and drone age, the forewing length varied non-significantly from 10.96 mm in 20 day old drones from mite infested colonies to 12.14 mm in 10 day old drones from healthy colonies.

Table 9. Effect of colony conditions and drone age on the forewing length of *A. mellifera* drones

Colony condition	Forewing length (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	12.14	12.11	12.10	**12.12**
Mite infested	11.04	10.99	10.96	**11.00**
Formic acid treated	11.49	11.30	11.25	**11.35**
Mean	**11.56**	**11.47**	**11.44**	

CD $_{(0.05)}$

Colony conditions : 0.11
Age of drones : NS
Colony conditions x Age of drones : NS

4.1.2.6 Effect of colony conditions and drone age on the forewing breadth of *A. mellifera* drones

The data on the effect of colony conditions and drone age on forewing breadth of drones presented in Table 10 revealed that forewing breadth of drones produced in healthy colonies (3.74 mm) and formic acid treated colonies (3.69 mm) was statistically similar. The minimum forewing breadth (3.54 mm) was found in drones of mite infested colonies.

Perusal of data in Table 10 further revealed that there was no significant effect of age on forewing breadth of drones. The forewing breadth was identical for 10 day (3.68 mm), 15 day (3.65 mm) and 20 day (3.65 mm) old drones. Irrespective of colony conditions and drone age, the forewing breadth varied non-significantly from 3.53 mm in 15 and 20 day old drones from mite infested colonies to 3.74 mm in 10 day old drones from healthy colonies.

Table 10. Effect of colony conditions and drone age on the forewing breadth of *A. mellifera* drones

Colony condition	Forewing breadth (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	3.74	3.73	3.73	**3.74**
Mite infested	3.58	3.53	3.53	**3.54**
Formic acid treated	3.71	3.69	3.67	**3.69**
Mean	**3.68**	**3.65**	**3.65**	

CD $_{(0.05)}$

Colony conditions : 0.05
Age of drones : NS
Colony conditions x Age of drones : NS

The present observations revealing maximum forewing length (12.12 mm) and forewing breadth (3.74 mm) in drones from healthy colonies followed by forewing length (11.35 mm) and forewing breadth (3.69 mm) of formic acid treated colonies and minimum forewing length (11.00 mm) and forewing breadth (3.54 mm) in mite infested colonies are partly in accordance with Shoukry *et al*. (2013), who reported that forewing length (11.21 mm) and breadth (3.63 mm) of drones from healthy colonies was more in comparison to forewing length (10.95 mm) and breadth (3.64 mm) of drones from formic acid treated colonies. The observations showing least forewing length (11.00 mm) and breadth (3.54 mm) in mite infested colonies got support from the investigations of earlier work of Belaid *et al*. (2017), who reported that workers of healthy colonies had higher forewing length (8.65 ± 0.26 mm) and breadth (2.99 ± 0.06 mm) as compared to workers in *Varroa destructor* infested colonies (8.21 ± 0.51 mm) and (2.82 ± 0.08 mm). Reduction in the wings with respect to mite infestation have also been reported by other workers (De Jong *et al.,* 1982; Daly *et al.,* 1988 and Marcangeli *et al.,* 1992).

4.1.2.7 Effect of colony conditions and drone age on the hindwing length of *A. mellifera* drones

The data on the effect of colony conditions and drone age on hindwing length of drones presented in Table 11 indicate that hindwing length of drones produced in healthy colonies (7.83 mm) and formic acid treated colonies (7.74 mm) was statistically at par. Significantly minimum hindwing length (7.67 mm) was found in drones of mite infested colonies.

Table 11. Effect of colony conditions and drone age on the hindwing length of *A. mellifera* drones

Colony condition	**Hindwing length (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	7.87	7.82	7.80	**7.83**
Mite infested	7.69	7.67	7.65	**7.67**
Formic acid treated	7.77	7.73	7.73	**7.74**
Mean	**7.78**	**7.74**	**7.73**	

CD $_{(0.05)}$

Colony conditions : 0.10
Age of drones : NS
Colony conditions x Age of drones : NS

The present findings further revealed that hindwing length in drones of different age group was similar in 10 day (7.78 mm), 15 day (7.74 mm) and 20 day (7.73 mm) old drones. Irrespective of colony conditions and drone age, the hindwing length varied non-significantly from 7.65 mm in 20 day old drones from mite infested colonies to 7.87 mm in 10 day old drones from healthy colonies.

4.1.2.8 Effect of colony conditions and drone age on the hindwing breadth of *A. mellifera* drones

The data on the effect of colony conditions and drone age on hindwing breadth of drones presented in Table 12 revealed that drones produced in healthy colonies had significantly maximum hindwing breadth (3.23 mm) followed by drones of formic acid treated colonies (3.15 mm). The hindwing breadth (3.09 mm) in drones from mite infested colonies was significantly minimum in comparison to other two treatments.

Perusal of data in Table 12 further indicates that hindwing length in drones of different age groups was identical in 10 day (3.17 mm), 15 day (3.16 mm) and 20 day (3.15 mm) old drones. Irrespective of colony conditions and drone age, the hindwing breadth varied non-significantly from 3.09 mm in 15 and 20 day old drones from mite infested colonies to 3.24 mm in 10 day old drones from healthy colonies.

Table 12. Effect of colony conditions and drone age on the hindwing breadth of *A. mellifera* drones

Colony condition	**Hindwing breadth (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	3.24	3.23	3.22	**3.23**
Mite infested	3.11	3.09	3.09	**3.09**
Formic acid treated	3.16	3.16	3.15	**3.15**
Mean	**3.17**	**3.16**	**3.15**	

CD $_{(0.05)}$

Colony conditions : 0.05
Age of drones : NS
Colony conditions x Age of drones : NS

The present observations revealing statistically similar hindwing length (7.83 mm and 7.74 mm) and hindwing breadth (3.23 mm and 3.15 mm) in drones from healthy colonies and formic acid treated colonies and minimum hindwing length (7.67 mm) and hindwing breadth (3.09 mm) in mite infested colonies. These results are partly in agreement with the findings of Belaid *et al*. (2017), who reported that workers of healthy colonies had maximum hindwing length (6.05 ± 0.36 mm) and breadth (1.70 ± 0.12 mm) which reduced to 5.83 ± 0.22 mm and 1.57 ± 0.11 mm in *Varroa destructor* infested colonies. No information is available with respect to hindwing length and hindwing breadth of drones produced from formic acid treated colonies. Daly *et al.* (1988) reported that if mite infestation per bee is 5 to 8 per cent then fore wing length suffers 1.1% to 3.1% reduction. Earlier De Jong (1984) reported that reduced wing length in mite infested colonies is due to loss of haemolymph, as insufficient haemolymph pressure prevents the full expansion of wings. These observations could be the possible reason for reduction in length and breadth of fore and hind wings.

4.1.2.9 Effect of colony conditions and drone age on the hamuli expanse of *A. mellifera* drones

The data on the effect of colony conditions and drone age on hamuli expanse of drones presented in Table 13 indicate that colony conditions and drone age have no significant influence on the hamuli expanse of drones. The mean hamuli expanse was statistically similar in drones from healthy colonies (1.65 mm), formic acid treated colonies (1.65 mm) and mite infested colonies (1.64 mm). The data further revealed that hamuli expanse in drones of different age groups was similar in 10 day (1.64 mm), 15 day (1.64 mm) and 20 day (1.65 mm) old drones. Irrespective of colony conditions and drone age, the hamuli expanse varied non- significantly from 1.63 mm in 10 day old drones from mite infested colonies to 1.66 mm in 20 day old drone from healthy colonies.

Table 13. Effect of colony conditions and drone age on the hamuli expanse of *A. mellifera* drones

Colony condition	**Hamuli expanse (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	1.64	1.64	1.66	**1.65**
Mite infested	1.63	1.64	1.65	**1.64**
Formic acid treated	1.65	1.65	1.65	**1.65**
Mean	**1.64**	**1.64**	**1.65**	

CD $_{(0.05)}$

Colony conditions : NS
Age of drones : NS
Colony conditions x Age of drones : NS

4.1.2.10 Effect of colony conditions and drone age on the hamuli number of *A. mellifera* drones

The data on the effect of colony conditions and drone age on hamuli number of drones presented in Table 14 revealed that like hamuli expanse the hamuli number of drones varied non-significantly with colony conditions and drone age. The mean hamuli number was statistically identical in drones of healthy colonies (20.73), formic acid treated colonies (20.68) and mite infested colonies (20.71). The data further indicated that hamuli number in drones of different age groups was identical in 10 day (20.73), 15 day (20.71) and 20 day (20.68) old drones. Irrespective of colony conditions and drone age, the hamuli number varied non-significantly from 20.60 mm in 10 day old drones from formic acid treated colonies to 20.80 mm in 10 and 15 day old drones from healthy, mite infested and formic acid treated colonies.

The present findings suggest that hamuli number and hamuli expanse are not affected due to age and mite infestation or formic acid treatment. No such studies have been conducted by earlier researchers.

Table 14. Effect of colony conditions and drone age on the hamuli number of *A. mellifera* drones

Colony condition	Hamuli number (no.)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Healthy	20.80	20.73	20.66	**20.73**
Mite infested	20.80	20.60	20.73	**20.71**
Formic acid treated	20.60	20.80	20.66	**20.68**
Mean	**20.73**	**20.71**	**20.68**	

CD $_{(0.05)}$

Colony conditions : NS
Age of drones : NS
Colony conditions x Age of drones : NS

4.2 EFFECT OF REARING METHODS ON QUALITY AND MORPHOMETRIC CHARACTERS OF *A. mellifera* DRONES

4.2.1 QUALITY CHARACTERS

Present investigations were conducted to evaluate the impact of rearing methods and age on quality characters of *A. mellifera* drones during spring of 2021. The results are discussed under the following heads.

4.2.1.1 Effect of rearing methods and drone age on the weight of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the weight of drones presented in Table 15 revealed that drone produced in mated queen colonies of *A. mellifera* were having more body weight (208.88 mg) which was followed by the drones produced in virgin queen colonies (188.04 mg). The minimum body weight (153.50 mg) was found in drones produced from laying worker colonies.

The data in Table 15 further indicate that body weight significantly decreases with age of drones and was recorded 194.99 mg, 182.80 mg and 172.60 mg in 10, 15 and 20 day old drones, respectively.

The data further revealed that interaction between rearing methods and drone age of drones varied significantly. The highest body weight (219.50 mg) was found in 10 day old drones from mated queen colonies which was followed by body weight (208.49 mg) of 15 day old drones from mated queen colonies. Significantly lowest drone weight (143.98 mg) was observed in 20 day old laying worker drones.

Table 15. Effect of rearing methods and drone age on the weight of *A. mellifera* drones

Rearing method	**Drone weight (mg)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	202.32	186.57	175.18	**188.02**
Laying worker drones	163.16	153.34	143.98	**153.50**
Mated queen drones	219.50	208.49	198.64	**208.88**
Mean	**194.99**	**182.80**	**172.60**	

CD $_{(0.05)}$

Rearing methods : 2.18
Age of drones : 2.18
Rearing methods x Age of drones : 3.78

The present findings showing more body weight in drones produced by mated queens in comparison to drones produced from laying workers are in agreement with observations of Gencer and Kahya (2011), who reported maximum body weight (221.6 mg) in drones produced in colonies with mated queen in comparison to drones produced from eggs laid by laying workers (147.3 mg). The present findings showing significant lower body weight (188.02 mg) of drones produced from virgin queen colonies are in line with the findings of Rinderer (1986), who stated that drones produced from virgin queens are smaller as queen tends to lay eggs in worker cells. This could be possible reason for reduction in weight and size of drones from virgin queens, however no such observations were recorded in present investigations.

The results of present investigations revealing decrease in body weight of drones with age from 10 day old to 20 day old drones in virgin queen colonies (202.32 mg to 175.18 mg), laying worker colonies (163 mg to 143.98 mg) and mated queen colonies (219.50 mg to 198.64 mg) are in agreement with Gencer and Firatli (2005) and Mazeed and Mohanny (2010), who have also reported that body weight suffered gradual reduction with age in drones. Gencer and Firatli (2005) reported significant reduction in body weight from emergence to 18 day old drones in queen right colonies (273.8 ± 0.83 mg to 222.3 ± 1.02 mg) and laying worker colonies (173.5 ± 0.85 mg to 150.5 ± 1.24 mg). Later Mazeed and Mohanny (2010) also reported decrease in the body weight of drones from 210 ± 22.36 mg in 7 to 9 day old drones to 180 ± 7.07 mg in 13 to 15 day old drones. The possible reason for reduction in body weight could be the reduction in length of testes in drones from young to mature stages as suggested by Mazeed and Mohanny (2010). Laidlaw (1977) has also concluded that the drone weight is reduced at maturity since the abdomen of newly released drones is huge because the testes are packed with spermatozoa. The testes shrink and become amorphous as the spermatozoa travels to seminal vesicles.

4.2.1.2 Effect of rearing methods and drone age on the eversion per cent of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the eversion per cent of drones presented in Table 16 revealed that full eversion of drones produced in mated queen colonies (71.11 %) and virgin queen colonies (64.44 %) was statistically similar. Significantly least number of fully everted drones (53.33 %) were found in the laying worker colonies.

The data in Table 16 further indicate that full eversion is influenced with drone age as there was significantly more number of 20 day old drones (86.66 %) everted fully followed by 15 day old drones (66.66 %) and 10 day old drones (35.55 %). Irrespective of rearing methods and drone age, the proportion of fully everted drones varied non-significantly from 26.66 per cent in 10 day old drones from laying worker colonies to 93.33 per cent in 20 day old drones from mated queen colonies.

Table 16. Effect of rearing methods and drone age on the eversion per cent of *A. mellifera* drones

Rearing method	Eversion (per cent)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	33.33 (35.00)*	73.33 (59.21)	86.66 (72.29)	**64.44 (55.50)**
Laying worker drones	26.66 (30.78)	53.33 (47.29)	80.00 (63.43)	**53.33 (47.17)**
Mated queen drones	46.66 (43.07)	73.33 (59.21)	93.33 (81.14)	**71.11 (61.14)**
Mean	**35.55 (36.29)**	**66.66 (55.24)**	**86.66 (72.29)**	

*Values in parentheses are means of arc sine values

CD $_{(0.05)}$

Rearing methods : 10.11
Age of drones : 10.11
Rearing methods x Age of drones : NS

The present observations on more number of fully everted drones of *A. mellifera* from mated queen colonies (71.11 %) in comparison to laying worker (53.33 %) are in accordance with the observation of Kumar (2002), who reported that percentage of fully everted drones (71.11 %) from mated queen colonies was more in comparison to drones of laying worker colonies (54.44 %). The drones in present findings produced from virgin queen colonies showed comparatively less eversion per cent (64.44 %) than from mated queen colonies. There is no such report to support this statement, however, it found support from the work of Gencer and Kahya (2011), who reported that reproductive success of drones depends upon body size. In the present investigations, the body size of drones from mated queen was highest followed by body weight of drones from virgin queens and laying workers.

The present findings indicating increase in fully everted drones with age got support from work of Kumar (2002), who reported that per cent of fully everted drones increases with age. He found that drones of 12, 15 and 20 day old showed 40, 75 and 90 per cent full eversion, respectively. Later Rousseau *et al*. (2015) also reported that eversion was dependent on drone age as it increased from 58.3 per cent in 14 day old to 65.9 per cent in 21 day old.

4.2.1.3 Effect of rearing methods and drone age on the semen volume of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the semen volume of drones presented in Table 17 indicate that semen volume of drones produced in mated queen colonies (1.00 µl) and virgin queen colonies (0.88 µl) was statistically similar. The least semen volume (0.60 µl) was found in drones from laying worker colonies.

The data further show that semen volume significantly increased with drone age. The maximum volume of semen was found in 20 day old drones (1.30 µl) followed by 15 day old drones (0.98 µl). The minimum semen volume (0.20 µl) was found in 10 day old drones. Interaction effect of rearing methods and age with regard to semen volume in drones varied non-significantly from 0.16 µl in 10 day old drones from laying worker colonies to 1.56 µl in 20 day old drones from mated queen colonies.

Table 17. Effect of rearing methods and drone age on the semen volume of *A. mellifera* drones

Rearing method	Semen volume (µl)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	0.16	1.01	1.48	**0.88**
Laying worker drones	0.17	0.75	0.87	**0.60**
Mated queen drones	0.26	1.19	1.56	**1.00**
Mean	**0.20**	**0.98**	**1.30**	

CD $_{(0.05)}$

Rearing methods : 0.21
Age of drones : 0.21
Rearing methods x Age of drones : NS

4.2.1.4 Effect of rearing methods and drone age on the sperm count of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the sperm count of drones presented in Table 18 indicate that sperm count of drones produced from mated queen colonies (1.99 x 10^6) and virgin queen colonies (1.86 x 10^6) was statistically at par. Significantly the least sperm count (1.44 x 10^6) was found in laying worker colonies.

The data further indicate that sperm count is influenced with drone age being significantly higher in 20 day old drones (2.92 x 10^6) followed by 15 day old drones (1.95 x 10^6) and 10 day old drones (0.42 x 10^6). Interaction effect of rearing methods and age with regard to semen count in drones varied non-significantly from 0.31 x 10^6 in 10 day old drones from laying worker colonies to 3.21 x 10^6 in 20 day old drones from mated queen colonies.

Table 18. Effect of rearing methods and drone age on the sperm count of *A. mellifera* drones

Rearing method	Sperm count/drone ($x10^6$)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	0.41	2.16	3.00	**1.86**
Laying worker drones	0.31	1.45	2.56	**1.44**
Mated queen drones	0.54	2.23	3.21	**1.99**
Mean	**0.42**	**1.95**	**2.92**	

CD $_{(0.05)}$

Rearing methods : 0.36
Age of drones : 0.36
Rearing methods x Age of drones : NS

The present observations on semen volume and sperm count in mated queen colonies (1.00 µl and 1.99 x 10^6) than laying worker colonies (0.60 µl and 1.44 x 10^6) are in agreement with the work of Kumar (2002), who observed that drones from mated queens produced higher semen volume (1.01 ± 0.06 µl) and sperm count (3.29 ± 0.13 x 10^6) as compared to drones of laying workers which produced lesser amount of semen volume (0.70 ± 0.05 µl) and sperm count (2.85 ± 0.08 x 10^6). Later, Gencer and Firatli (2005) reported that average sperm count (4.57 x 10^6) of drones from mated queen colonies were higher in comparison to drones from laying worker colonies (3.61 x 10^6). Similarly, Gencer and Kahya (2011) also reported that average sperm number in drones of queen right colonies (7.320 x 10^6) were higher than the drones from laying worker colonies (4.425 x 10^6) and the mean volume of semen ejaculated from drones of mated queen colonies (1.01 µl) was 53 per cent higher than the drones of laying worker colonies (0.66 µl). Rinderer (1986) stated that drones produced from virgin queens are smaller as queen tends to lay eggs in worker cells which results in smaller size with less semen production. This could be the possible reason for reduction in sperm count of drones from virgin queens, however no such observations were recorded in present investigations.

The present results revealing increase in semen volume and sperm count with the age in 10 day to 20 day old drones in all rearing methods (Table 17 and 18) are in close proximity to the observations of Rhodes *et al.* (2011), who reported that semen and sperm count released per drone depends upon drone age. According to them semen volume increased from 0.98 ± 0.03 µL in 14 days old drones to 1.07 ± 0.04 µL in 21 day old drones. The respective values for sperm count were reported to be 2.83 ± 0.15 x 10^6 to 3.36 ± 0.18 x 10^6. These observations suggest that the body weight at maturity stage of drones has impact on the semen volume and sperm count. Positive correlation between body size and sperm number has also been found by Berg *et al.* (1997).

4.2.2 MORPHOLOGICAL CHARACTERS

4.2.2.1 Effect of rearing methods and drone age on the body length of *A. mellifera* drones

The data on the effect of rearing methods and drone age on body length of drones presented in Table 19 indicate that drones produced in mated queen colonies had significantly maximum body length (15.86 mm) which was followed by body length (15.10 mm) of drones produced from virgin queen colonies. Significantly minimum body length (12.61 mm) was found in drones from laying worker colonies.

The data enumerated in Table 5 further revealed that body length of drones depends upon age. The body length showed a significant decrease from 10 day (15.03 mm), to 15 day (14.53 mm) and 20 day (14.02 mm) old drones. Irrespective of rearing methods and drone age, the body length varied non-significantly from 12.25 mm in 20 day old drones from laying worker colonies to 16.41 mm in 10 day old drones from mated queen colonies.

Table 19. Effect of rearing methods and drone age on the body length of *A. mellifera* drones

Rearing method	**Body length (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	15.66	15.12	14.53	**15.10**
Laying worker drones	13.02	12.56	12.25	**12.61**
Mated queen drones	16.41	15.90	15.28	**15.86**
Mean	**15.03**	**14.53**	**14.02**	

CD $_{(0.05)}$

Rearing methods : 0.26
Age of drones : 0.26
Rearing methods x Age of drones : NS

4.2.2.2 Effect of rearing methods and drone age on the thorax length of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the thorax length of drones presented in Table 20 indicate that drones produced in mated queen colonies had significantly maximum thorax length (5.18 mm) which was followed by thorax length (5.04

mm) of drones produced from virgin queen colonies. Minimum thorax length (4.84 mm) was found in drones from laying worker colonies.

The data further indicate that thorax length of drones depends upon their age. The thorax length significantly decreased with age of drones and was recorded 5.09 mm, 5.02 mm and 4.95 mm in 10, 15 and 20 day old drones, respectively. Irrespective of rearing methods and drone age, the thorax length varied non-significantly from 4.78 mm in 20 day old drones from laying worker colonies to 5.26 mm in 10 day old drones from mated queen colonies.

Table 20. Effect of rearing methods and drone age on the thorax length of *A. mellifera* drones

Rearing method	Thorax length (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	5.11	5.04	4.98	**5.04**
Laying worker drones	4.91	4.85	4.78	**4.84**
Mated queen drones	5.26	5.18	5.10	**5.18**
Mean	**5.09**	**5.02**	**4.95**	

CD $_{(0.05)}$

Rearing methods	:	0.04
Age of drones	:	0.04
Rearing methods x Age of drones	:	NS

4.2.2.3 Effect of rearing methods and drone age on the thorax breadth of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the thorax breadth of drones presented in Table 21 revealed that drones produced in mated queen colonies had significantly maximum thorax breadth (5.10 mm) which was followed by thorax breadth (4.98 mm) of drones produced from virgin queen colonies. The thorax breadth (4.79 mm) of drones from laying worker colonies was significantly minimum in comparison to other two treatments.

The data further indicate that thorax breadth of drones depends upon their age. The thorax breadth significantly decreased with age of drones and was recorded 5.03 mm, 4.96 mm and 4.89 mm in 10, 15 and 20 day old drones, respectively. Irrespective of rearing methods and drone age, the thorax breadth varied non-significantly from 4.72 mm in 20 day old drones from laying worker colonies to 5.18 mm in 10 day old drones from mated queen colonies.

Table 21. Effect of rearing methods and drone age on the thorax breadth of *A. mellifera* drones

Rearing method	Thorax breadth (mm)			
	10 day old	15 day old	20 day old	Mean
Virgin queen drones	5.06	4.98	4.92	**4.98**
Laying worker drones	4.85	4.80	4.72	**4.79**
Mated queen drones	5.18	5.10	5.03	**5.10**
Mean	**5.03**	**4.96**	**4.89**	

CD $_{(0.05)}$

Rearing methods : 0.04
Age of drones : 0.04
Rearing methods x Age of drones : NS

4.2.2.4 Effect of rearing methods and drone age on the hind leg length of *A. mellifera* drones

The data on the effect of rearing methods and drone age on the hind leg length of drones presented in Table 22 revealed that drones produced in mated queen colonies had significantly greater hind leg length (12.94 mm) which was followed by hind leg length (12.31 mm) of drones produced from virgin queen colonies. Significantly least hind leg length (10.46 mm) was found in drones from laying worker colonies.

Perusal of data in Table 22 further revealed that the hind leg length of drones was identical in 10 (11.89 mm), 15 (11.91 mm) and 20 (11.90 mm) day old drones. Irrespective of rearing methods and drone age, the hind leg length varied non-significantly from 10.45 mm in 10 and 15 day old drones from laying worker colonies to 12.96 mm in 15 day old drones from mated queen colonies.

Table 22. Effect of rearing methods and drone age on the hind leg length of *A. mellifera* drones

Rearing method	Hind leg length (mm)			
	10 day old	15 day old	20 day old	Mean
Virgin queen drones	12.30	12.33	12.29	**12.31**
Laying worker drones	10.45	10.45	10.47	**10.46**
Mated queen drones	12.91	12.96	12.94	**12.94**
Mean	**11.89**	**11.91**	**11.90**	

CD $_{(0.05)}$

Rearing methods : 0.14
Age of drones : NS
Rearing methods x Age of drones : NS

The results of present investigations showing decrease in thorax length and thorax breadth of drones from laying worker colonies (4.84 mm and 4.79 mm) in comparison to mated queen colonies (5.18 mm and 5.10 mm) are in corroboration with the findings of Kumar (2002), who reported that drones from laying worker colonies had minimum thorax length (4.94 ± 0.06 mm) and breadth (4.89 ± 0.06 mm) as compared to thorax length (5.6 ± 0.03 mm) and breadth (5.52 ± 0.03 mm) of drones from mated queen colonies.

The present observations indicating maximum hind leg length (12.94 mm) in drones from mated queen colonies in comparison to laying worker colonies (10.46 mm) are in agreement with the work of Gencer and Firatli (2005), who reported that drones emerged from mated queen colonies were having significantly maximum femur length (3.46 ± 0.020 mm), tibial length (4.01 ± 0.026 mm) and metatarsus length (2.51 ± 0.017 mm) as compared to femur length (3.02 ± 0.019 mm), tibial length (3.44 ± 0.030 mm) and metatarsus length (2.16 ± 0.019 mm) of drones emerged from laying worker colonies.

4.2.2.5 Effect of rearing methods and drone age on the forewing length of *A. mellifera* drones

The data on the effect of rearing methods and drone age on forewing length of drones is presented in Table 23. The data revealed that drones produced in mated queen colonies had significantly maximum forewing length (12.08 mm) followed by drones from virgin queen colonies (11.46 mm). Significantly least forewing length (10.03 mm) was found in drones from laying worker colonies.

Table 23. Effect of rearing methods and drone age on the forewing length of *A. mellifera* drones

Rearing method	**Forewing length (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	11.55	11.47	11.36	**11.46**
Laying worker drones	10.07	10.02	10.00	**10.03**
Mated queen drones	12.10	12.09	12.04	**12.08**
Mean	**11.24**	**11.19**	**11.13**	

CD $_{(0.05)}$

Rearing methods : 0.17
Age of drones : NS
Rearing methods x Age of drones : NS

The present findings further revealed that forewing length in drones of different age groups did not vary significantly in 10 day (11.24 mm), 15 day (11.19 mm) and 20 day (11.13 mm) old drones. Irrespective of rearing methods and drone age, the forewing length varied non-significantly from 10.00 mm in 20 day old drones from laying worker colonies to 12.10 mm in 10 day old drones from mated queen colonies.

4.2.2.6 Effect of rearing methods and drone age on the forewing breadth of *A. mellifera* drones

The data on the effect of rearing methods and drone age on forewing breadth of drones is presented in Table 24. The data further revealed that drones produced in mated queen colonies had significantly maximum forewing breadth (3.74 mm) followed by drones from virgin queen colonies (3.64 mm). The forewing breadth (3.28 mm) in drones from laying worker colonies was significantly minimum in comparison to other two treatments.

The present findings further revealed that there was no significant effect of age on forewing breadth of drones. The forewing breadth was similar for 10 day (3.56 mm), 15 day (3.55 mm) and 20 day (3.54 mm) old drones. Irrespective of rearing methods and drone age, the forewing length varied non-significantly from 3.28 mm in 15 and 20 day old drones from laying worker colonies to 3.76 mm in 10 day old drones from mated queen colonies.

Table 24. Effect of rearing methods and drone age on the forewing breadth of *A. mellifera* drones

Rearing method	Forewing breadth (mm)			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	3.65	3.64	3.63	**3.64**
Laying worker drones	3.29	3.28	3.28	**3.28**
Mated queen drones	3.76	3.73	3.72	**3.74**
Mean	**3.56**	**3.55**	**3.54**	

CD $_{(0.05)}$

Rearing methods : 0.05
Age of drones : NS
Rearing methods x Age of drones : NS

The present observations revealed maximum forewing length (12.08 mm) and forewing breadth (3.74 mm) in drones from mated queen colonies and minimum forewing length (10.00 mm) and forewing breadth (3.28 mm) in drones from laying worker colonies got support from the work of Utaipanon *et al.* (2019), who found that forewing length (5.01 ±

0.21 mm) and breadth (1.11 ± 0.05 mm) were minimum in drones produced by laying workers in comparison to drones from mated queen colonies being 5.78 ± 0.26 mm and 1.27 ± 0.08 mm, respectively. The observations on forewing length and forewing breadth in drones from mated queen colonies are in the range of those reported by Woyke (1978) and Taha and Alqarni (2013). Woyke (1978) reported that drones from mated queen colonies have an average forewing length of 12.00 ± 0.05 mm and forewing breadth of 3.96 ± 0.02 mm. Similarly, Taha and Alqarni (2013) reported that drones produced from mated queen colonies have an average forewing length of 12.33 ± 0.09 mm and forewing width of 3.62 ± 0.04 mm.

4.2.2.7 Effect of rearing methods and drone age on the hindwing length of *A. mellifera* drones

The data on the effect of rearing methods and drone age on hindwing length of drones presented in Table 25 indicate that drones produced in mated queen colonies had significantly maximum hindwing length (7.75 mm) which was followed by hindwing length in drones produced from virgin queen colonies (7.55 mm). The minimum hindwing length (6.95 mm) was found in drones from laying worker colonies.

The present findings further revealed that hindwing length in drones of different age group was identical in 10 day (7.43 mm), 15 day (7.42 mm) and 20 day (7.41 mm) old drones respectively. Irrespective of rearing methods and drone age, the hindwing length varied non-significantly from 6.94 mm in 20 day old drones from laying worker colonies to 7.77 mm in 10 day old drones from mated queen colonies.

Table 25. Effect of rearing methods and drone age on the hindwing length of *A. mellifera* drones

Rearing method	Hindwing length (mm)			
	10 day old	15 day old	20 day old	Mean
Virgin queen drones	7.56	7.55	7.55	**7.55**
Laying worker drones	6.97	6.95	6.94	**6.95**
Mated queen drones	7.77	7.76	7.73	**7.75**
Mean	**7.43**	**7.42**	**7.41**	

CD $_{(0.05)}$

Rearing methods : 0.06
Age of drones : NS
Rearing methods x Age of drones : NS

4.1.2.8 Effect of rearing methods and drone age on the hindwing breadth of *A. mellifera* drones

The data on the effect of rearing methods and drone age on hindwing breadth of drones is presented in Table 26. The data showed that drones produced in mated queen colonies had significantly maximum hindwing breadth (3.20 mm) followed by drones from virgin queen colonies (3.02 mm). The minimum hindwing breadth (2.60 mm) was found in drones from laying worker colonies.

The present findings further revealed that hindwing breadth in drones of different age groups was similar in 10 day (2.94 mm), 15 day (2.94 mm) and 20 day (2.94 mm) old drones. Irrespective of rearing methods and drone age, the hindwing breadth varied non-significantly in from 2.59 mm in 20 day old drones from laying worker colonies to 3.21 mm in 10 day old drones from mated queen colonies.

Table 26. Effect of rearing methods and drone age on the hindwing breadth of *A. mellifera* drones

Rearing method	Hindwing breadth (mm)			
	10 day old	15 day old	20 day old	Mean
Virgin queen drones	3.01	3.03	3.03	**3.02**
Laying worker drones	2.61	2.61	2.59	**2.60**
Mated queen drones	3.21	3.20	3.20	**3.20**
Mean	**2.94**	**2.94**	**2.94**	

CD $_{(0.05)}$

Rearing methods : 0.04
Age of drones : NS
Rearing methods x Age of drones : NS

The hindwing length (7.55 mm) and hindwing breadth (3.20 mm) of drones from mated queen colonies in present observations are in agreement with findings of Taha and Alqarni (2013), who reported that drones produced from mated queen colonies have mean hindwing length and hindwing breadth ranging from 7.23 ± 0.06 mm and 3.15 ± 0.05 mm, respectively.

4.2.2.9 Effect of rearing methods and drone age on the hamuli expanse of *A. mellifera* drones

The data on the effect of rearing methods and drone age on hamuli expanse of drones presented in Table 27 further revealed that drones produced in mated queen colonies had significantly maximum hamuli expanse (1.65 mm) followed by drones from virgin queen colonies (1.58 mm). The minimum hamuli expanse (1.45 mm) was found in drones from laying worker colonies.

The data further revealed that hamuli expanse of drones did not vary significantly in different age groups. The hamuli expanse was identical in 10 day (1.55 mm), 15 day (1.56 mm) and 20 day (1.56 mm) old drones. Irrespective of rearing methods and drone age, the hamuli expanse did not show significant change as hamuli expanse ranged from 1.44 mm in 15 day old drones from laying worker colonies to 1.66 mm in 20 day old drones of mated queen colonies, respectively.

Table 27. Effect of rearing methods and drone age on the hamuli expanse of *A. mellifera* drones

Rearing method	**Hamuli expanse (mm)**			
	10 day old	**15 day old**	**20 day old**	**Mean**
Virgin queen drones	1.57	1.59	1.59	**1.58**
Laying worker drones	1.45	1.44	1.45	**1.45**
Mated queen drones	1.65	1.65	1.66	**1.65**
Mean	**1.55**	**1.56**	**1.56**	

CD $_{(0.05)}$

Rearing methods : 0.02
Age of drones : NS
Rearing methods x Age of drones : NS

4.2.2.10 Effect of rearing methods and drone age on the hamuli number of *A. mellifera* drones

The data on the effect of rearing methods and drone age on hamuli number of drones presented in Table 28 further revealed that drones produced in mated queen colonies had significantly more hamuli number (20.91) followed by drones produced from virgin queen colonies (19.97). The hamuli number (19.04) in drones from laying worker colonies was significantly least in comparison to other two treatments.

The data further revealed that hamuli number of drones did not vary significantly in 10 day (19.95), 15 day (19.97) and 20 day (20.00) old drones. Irrespective of rearing methods and drone age, the hamuli number did not show significant change as hamuli number ranged from 19.00 in 15 day old drones from laying worker colonies to 20.93 in 15 and 20 day old drones of mated queen colonies, respectively.

Table 28. Effect of rearing methods and drone age on the hamuli number of *A. mellifera* drones

Rearing method	Hamuli number (no.)			
	10 day old	15 day old	20 day old	Mean
Virgin queen drones	19.93	20.00	20.00	**19.97**
Laying worker drones	19.06	19.00	19.06	**19.04**
Mated queen drones	20.86	20.93	20.93	**20.91**
Mean	**19.95**	**19.97**	**20.00**	

CD $_{(0.05)}$

Rearing methods : 0.22
Age of drones : NS
Rearing methods x Age of drones : NS

The present studies indicate that larger hamuli expanse (1.65 mm) and more hamuli number (20.91) were recorded in drones from mated queen colonies as comparison to least hamuli expanse (1.45 mm) and lesser hamuli number (19.04) of drones from laying worker colonies. These findings got support from the work of Gencer and Firatli (2005), who reported that drones emerged from mated queen colonies were having significantly larger extent of hamuli (1.66 ± 0.02 mm) and hamuli number (21.40 ± 0.38) in comparison to mean extent of hamuli (1.45 ± 0.01 mm) and hamuli number (19.65 ± 0.54) of drones emerged from laying worker colonies. The hamuli number and hamuli expanse of drones from mated queen colonies were in the range of following workers. Woyke (1978) reported that drones from mated queen colonies had hamuli expanse of 20.6 ± 0.38 mm. Likewise Taha and Alqarni (2013) reported that drones produced from mated queen colonies have mean hamuli number of 19.50 ± 0.29.

Chapter-5

SUMMARY AND CONCLUSION

The salient findings of the present investigations conducted on **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** are summarized as under:

1. The observations on quality characters from *A. mellifera* drones produced in different colony conditions revealed that maximum body weight (205.35 mg), higher eversion (77.77 %), higher semen volume (1.01 µl) and sperm count (1.88 x 10^6) were recorded in drones from healthy colonies which were statistically similar with drones of formic acid treated colonies. Drones from mite infested colonies had minimum body weight (180.04 mg), lowest eversion (57.77 %), semen volume (0.66 µl) and sperm count (1.30 x 10^6) compared to drones from healthy and formic acid treated.
2. All the morphometric characters *viz.* body length, thorax length, thorax breadth, hind leg length, forewing length, forewing breadth, hindwing length and hindwing breadth were found to be maximum in drones obtained from healthy colonies. The morphometric characters of drones from formic acid treated colonies were generally at par with drones of healthy colonies. However, all these characters were minimum in drones obtained from mite infested colonies. Irrespective of colony conditions, the hamuli expanse and hamuli number in drones varied non significantly in all three conditions.
3. The effect of different age groups on the quality of drones from different colony conditions and rearing methods revealed that body weight of drones tends to decrease with age. The eversion per cent, semen volume and sperm count also tend to increase with age.
4. The effect of different age groups on the morphometric characters from different colony conditions and rearing methods revealed that body length, thorax length, thorax breadth decreased with the age of drones. However, hind leg length; forewing length and breadth; hindwing length and breadth; hamuli expanse and hamuli number varied non significantly with age.
5. The observations of quality characters from *A. mellifera* drones in different rearing methods revealed that maximum body weight (208.88 mg), higher eversion (71.11 %),

higher semen volume (1.00 µl) and sperm count (1.99×10^6) were recorded in drones produced by mated queens, which were statistically similar with drones from virgin queens. Drones from laying worker colonies had minimum body weight (153.50 mg), eversion (53.33 %), semen volume (0.60 µl) and sperm count (1.44×10^6) compared to drones from mated and virgin queen colonies.

6. Body length, thorax length, thorax breadth, hind leg length, forewing length, forewing breadth, hindwing length, hindwing breadth, hamuli expanse and hamuli number were found to be maximum in drones from mated queen colonies followed by that of virgin queen drones and minimum in drones from laying worker colonies.

7. The effect of different age groups on the quality of drones produced by different rearing methods revealed that body weight of drones decreased with age. Maximum body weight (194.99 mg) was recorded in 10 day old drones and minimum in 20 day old drones (172.60 mg). The eversion per cent, semen volume and sperm count increased with age. The maximum eversion (86.66 %), semen volume (1.30) and sperm count (2.92×10^6) were recorded in 20 day old drones and minimum in 10 day old drones.

CONCLUSION

- Production of drones with heavier weight (205.35 mg and 208.88 mg), highest eversion (73.33 % and 71.11 %), semen volume (1.01 µl and 1.00 µl) and sperm count (1.88×10^6 and 1.99×10^6) in healthy and mated queen colonies suggest that drone production should be taken up only from such colonies.
- Drones aged between 15 and 20 days produced maximum semen volume (1.00 µl and 1.34 µl) along with highest eversion (73.33 % and 88.88 %), which indicate that drones get mature at the age of 15 to 20 days.
- The same quality and quantitative characteristics of drones from healthy and formic acid treated colonies revealed that formic acid treatment for the management of mites does not affect the quality of drones produced in such colonies. Besides, management of *Varroa* is essential for production of quality drones in honey bee colonies.
- Present study revealed that the quality and morphometry of drones produced by laying workers was inferior in comparison to drones produced by virgin and mated colonies. However, the semen volume (0.60 µl) was enough for collection and for its use in artificial insemination.

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APPENDIX- I

Analysis of Variance (ANOVA) for body weight of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	3,616.990	1,808.495
Factor B	2	1,838.242	919.121
Intraction A X B	4	187.333	46.833
Error	18	200.939	11.163
Total	26	5,843.503	

Analysis of Variance (ANOVA) for eversion per cent of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	1,085.796	542.898
Factor B	2	5,989.914	2,994.957
Intraction A X B	4	180.639	45.160
Error	18	2,515.805	139.767
Total	26	9,772.154	

Analysis of Variance (ANOVA) for semen volume of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.566	0.283
Factor B	2	6.408	3.204
Intraction A X B	4	0.164	0.041
Error	18	0.510	0.028
Total	26	7.649	

Analysis of Variance (ANOVA) for sperm count of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	1.599	0.800
Factor B	2	21.615	10.807
Intraction A X B	4	0.566	0.142
Error	18	1.259	0.070
Total	26	25.040	

Analysis of Variance (ANOVA) for body length of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.488	0.244
Factor B	2	6.845	3.423
Intraction A X B	4	0.190	0.048
Error	18	0.493	0.027
Total	26	8.017	

Analysis of Variance (ANOVA) for thorax length of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.249	0.125
Factor B	2	0.176	0.088
Intraction A X B	4	0.015	0.004
Error	18	0.047	0.003
Total	26	0.488	

Analysis of Variance (ANOVA) for thorax breadth of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.065	0.033
Factor B	2	0.195	0.098
Intraction A X B	4	0.013	0.003
Error	18	0.146	0.008
Total	26	0.420	

Analysis of Variance (ANOVA) for hind leg length of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.215	0.107
Factor B	2	0.004	0.002
Intraction A X B	4	0.007	0.002
Error	18	0.165	0.009
Total	26	0.391	

Analysis of Variance (ANOVA) for forewing length of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	5.897	2.948
Factor B	2	0.071	0.036
Intraction A X B	4	0.039	0.010
Error	18	0.234	0.013
Total	26	6.241	

Analysis of Variance (ANOVA) for forewing breadth of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.182	0.091
Factor B	2	0.004	0.002
Intraction A X B	4	0.002	0.000
Error	18	0.049	0.003
Total	26	0.237	

Analysis of Variance (ANOVA) for hindwing length of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.113	0.057
Factor B	2	0.012	0.006
Intraction A X B	4	0.001	0.000
Error	18	0.214	0.012
Total	26	0.340	

Analysis of Variance (ANOVA) for hindwing breadth of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.082	0.041
Factor B	2	0.001	0.001
Intraction A X B	4	0.000	0.000
Error	18	0.048	0.003
Total	26	0.131	

Analysis of Variance (ANOVA) for hamuli expanse of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.000	0.000
Factor B	2	0.001	0.000
Intraction A X B	4	0.001	0.000
Error	18	0.012	0.001
Total	26	0.014	

Analysis of Variance (ANOVA) for hamuli number of drones in different colony conditions

Source of Variation	DF	SS	MSS
Factor A	2	0.008	0.004
Factor B	2	0.008	0.004
Intraction A X B	4	0.143	0.036
Error	18	2.747	0.153
Total	26	2.906	

Analysis of Variance (ANOVA) for body weight of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	14,081.958	7,040.979
Factor B	2	2,262.192	1,131.096
Intraction A X B	4	57.102	14.276
Error	18	86.204	4.789
Total	26	16,487.455	

Analysis of Variance (ANOVA) for eversion per cent of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	889.238	444.619
Factor B	2	5,837.025	2,918.512
Intraction A X B	4	99.056	24.764
Error	18	1,848.043	102.669
Total	26	8,673.362	

Analysis of Variance (ANOVA) for semen volume of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	0.779	0.389
Factor B	2	5.819	2.909
Intraction A X B	4	0.377	0.094
Error	18	0.850	0.047
Total	26	7.825	

Analysis of Variance (ANOVA) for sperm count of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	1.474	0.737
Factor B	2	28.663	14.331
Intraction A X B	4	0.362	0.091
Error	18	2.398	0.133
Total	26	32.896	

Analysis of Variance (ANOVA) for body length of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	52.114	26.057
Factor B	2	4.602	2.301
Intraction A X B	4	0.146	0.036
Error	18	1.282	0.071
Total	26	58.144	

Analysis of Variance (ANOVA) for thorax length of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	0.497	0.249
Factor B	2	0.090	0.045
Intraction A X B	4	0.001	0.000
Error	18	0.041	0.002
Total	26	0.629	

Analysis of Variance (ANOVA) for thorax breadth of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	0.443	0.222
Factor B	2	0.090	0.045
Intraction A X B	4	0.001	0.000
Error	18	0.028	0.002
Total	26	0.563	

Analysis of Variance (ANOVA) for hind leg length of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	29.969	14.984
Factor B	2	0.004	0.002
Intraction A X B	4	0.004	0.001
Error	18	0.382	0.021
Total	26	30.358	

Analysis of Variance (ANOVA) for forewing length of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	19.854	9.927
Factor B	2	0.052	0.026
Intraction A X B	4	0.020	0.005
Error	18	0.537	0.030
Total	26	20.463	

Analysis of Variance (ANOVA) for forewing breadth of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	1.025	0.513
Factor B	2	0.003	0.001
Intraction A X B	4	0.001	0.000
Error	18	0.056	0.003
Total	26	1.086	

Analysis of Variance (ANOVA) for hindwing length of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	3.119	1.560
Factor B	2	0.003	0.001
Intraction A X B	4	0.001	0.000
Error	18	0.079	0.004
Total	26	3.202	

Analysis of Variance (ANOVA) for hindwing breadth of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	1.685	0.843
Factor B	2	0.000	0.000
Intraction A X B	4	0.002	0.000
Error	18	0.038	0.002
Total	26	1.725	

Analysis of Variance (ANOVA) for hamuli expanse of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	0.195	0.098
Factor B	2	0.000	0.000
Intraction A X B	4	0.000	0.000
Error	18	0.008	0.000
Total	26	0.204	

Analysis of Variance (ANOVA) for hamuli number of drones in different rearing methods

Source of Variation	DF	SS	MSS
Factor A	2	15.679	7.839
Factor B	2	0.008	0.004
Intraction A X B	4	0.019	0.005
Error	18	0.880	0.049
Total	26	16.586	

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ABSTRACT

The present investigations entitled **"Effect of colony conditions and rearing methods on quality of drones in *Apis mellifera* L."** were carried out during 2021 in university apiary at the entomology experimental farm of the Department of Entomology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. *A. mellifera* drone honeybees were reared under different colony conditions (healthy, mite infested and formic acid treated) and rearing methods (drones from virgin queens, laying workers and mated queens) for quality characters (body weight, eversion per cent, semen volume and sperm count) and morphometric characters (body length, thorax length, thorax breadth, hind leg length, forewing length, forewing breadth, hindwing length, hindwing breadth, hamuli expanse and hamuli number). Measurements were carried out from drones at an age group of 10, 15 and 20 days. The observations on quality and morphometric characters in different colony conditions revealed that maximum body weight (205.35 mg), eversion (77.77 %), semen volume (1.01 µl), sperm count (1.88×10^6), body length (15.82 mm), thorax length (5.09 mm), thorax breadth (4.96 mm), hind leg length (12.97 mm), forewing length (12.12 mm), forewing breadth (3.74 mm), hindwing length (7.83 mm) and hindwing breadth (3.23 mm) were recorded from drones produced by healthy colonies which were generally at par with drones of formic acid treated colonies. Further, the observations on quality and morphometric characters in different rearing methods revealed that maximum body weight (208.88 mg), eversion (71.11 %), semen volume (1.00 µl), sperm count (1.99×10^6), body length (15.86 mm), thorax length (5.18), thorax breadth (5.10 mm), hind leg length (12.94 mm), forewing length (12.08 mm), forewing breadth (3.74 mm), hindwing length (7.75 mm), hindwing breadth (3.20 mm), hamuli expanse (1.65 mm) and hamuli number (20.91) were recorded in drones produced from mated queen colonies. The present study suggests that drone production should be taken from mated queens and healthy colonies. Further, the study indicate that drones get mature at the age of 15 and 20 days. The formic acid treatment for the management of mites does not affect the quality of drones produced in such colonies and management of *Varroa* is essential for production of quality drones in honey bee colonies.

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