MORPHOMETRIC CHANGES DURING ONTOGENETIC DEVELOPMENT OF WAX MOTH

THESIS

By

NITIKA SHARMA
(S-2010-30-16)

Submitted to

CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR – 176 062 (H.P.) INDIA

in

Partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE IN BASIC SCIENCES
(DEPARTMENT OF BIOLOGY AND ENVIRONMENTAL SCIENCES)
(BIOLOGY)

2012

Affectionately

dedicated

To

My Mom for her love ,
care and blessings that
have always followed me
Love you Mamma.....

Dr. Navneet Kumar Gupta
Professor and Head

Department of Biology & Environmental Sciences, CSK Himachal Pradesh Krishi Vishavavidyalaya, Palampur-176062 (H.P.) India

CERTIFICATE – I

This is to certify that the thesis entitled, **"Morphometric changes during ontogenetic development of wax moth"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Basic Sciences)** in the discipline of **Biology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms. Nitika Sharma (Admission No. S-2010-30-16)** daughter of **Shri Puran Chand Sharma** and **Late. Smt. Shakuntala Sharma** 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.

18-07-2012

Place : Palampur
Dated : 18July, 2012

(Dr. Navneet Kumar Gupta)
Major Advisor

CERTIFICATE – II

This is to certify that the thesis entitled, **"Morphometric changes during ontogenetic development of wax moth"** submitted by **Ms. Nitika Sharma (Admission No. S-2010-30-16)** daughter of **Shri Puran Chand Sharma** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Master of Sciences (Basic Sciences)** in the discipline of **Biology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

14.12.2012

(Dr.Navneet Kumar Gupta)
Chairperson
Advisory Committee

14.11.12

(Dr. Deepak C. Kalia)

External Examiner

14.12.12

(Dr.Anita Singh)
Member

14/12/2012

(Dr. Arun Kumar)
Member
(Dean's Nominee)

14-12-2012

Head of the Department

19.12.12

Dean, Postgraduate studies

ACKNOWLEDGEMENT

There is hardly a man in the world who does not dream, but to make a dream come true is a tough task. Looking back at my educational life, today I feel that I am at the meridian of achieving something precious in my career. As I begin to write these lines; after completion of my thesis, my heart is filled with deepest sense of gratitude. Emotions cannot be adequately expressed in words. My acknowledgments are many times more than what I am expressing here.

I would like to offer my prime heartfelt salutation at the lotus feet of the supreme Being for the unbroken health and vigour, bestowed upon me all through the span of my studies in these testing times.

With an overwhelming sense of legitimate pride and genuine obligation, I express my deep gratitude to my major advisor, Dr. Navneet Kumar Gupta, Head Department of Biology and Environmental Sciences, for his scientific acumen and impeccable guidance in the conduct of this investigation and attentive scanning of this manuscript.

Their guidance leads to boost both my mental and physical stamina in achieving this task. All this made it possible to prosecute my studies and hence my profound and revered acknowledgement to all associated in this endeavor.

I am obliged to Dr. Arun Kumar, Dr. Rani Dhanze and Dr. Anita Singh the esteemed members of my advisory committee for their valuable suggestions and healthy criticism during the course of investigation.

I wish to express my regards to all the staff members of Department of Biology and Environmental Sciences and department of Entomology for their help during the course of investigation.

I owe special debt and heartfelt gratitude to my affectionate papa, bhaiya and bhabhi for their moral support, encouragement and affection which were source of inspiration for me to work ahead and achieve my goals.

On this occasion I must remember my class mates and my friends Deepika, Nisha, Ragini and Suman for their ever willing help and unforgettable company during the course.

I am also thankful to Mr. Ajay Walia for giving final touch to the manuscript.

Perhaps it is inevitable that some error of facts, interpretation and emphasis will be found, but I trust no one will attribute these to anyone else, but myself.

Place: Palampur

Dated: 18 July, 2012

Nitika Sharma

(NITIKA SHARMA)

TABLE OF CONTENTS

ABBREVIATIONS USED

fig.	:	Figure
Cm	:	Centi meter
°C	:	Degree Celsius
mm	:	Mili-meter
et al.	:	et alii (and others)
i.e	:	id est (that is)
Viz.	:	vi delicet (namely)
Gm	:	Gram

LIST OF TABLES

LIST OF FIGURES

Department of Biology and Environmental Sciences
College of Basic Sciences
CSK Himachal Pradesh Krishi Vishvavidyalaya
Palampur-176062 (H.P.)

Title of the thesis	:Morphometric changes during Ontogenetic Development of Wax moth.
Name of the student	: **NitikaSharma**
Admission number	: S-2010-30-16
Major discipline	: Biology
Minor discipline	: Entomology
Date of thesis submission	: 18 July, 2012
Total pages of the thesis	: 28
Major Advisor	: Dr. Navneet Kumar Gupta

ABSTRACT

Wax moth includes two species out of which one is *Achroia grisella* Fab. (lesser wax moth). It is an important pest in apiculture. *A. grisella* has a scattered distribution throughout the tropical and temperate climates of the world. The wax moth species *A. grisella* (lesser wax moth) was studied for the morphometric changes during its ontogenetic development. The morphometric study of different parts of an insect's body is needed to obtain an index to distinguish different larval instars. In this study the wax moth species *A. grisella* (lesser wax moth) was reared at room temperature. Larvae of each instar were selected and length and width of the body of each instar during different developmental stages were measured. The mean body length and body width was ranged from 0.92±0.074 to 12.15±0.056 mm and 0.15±0.013 to 2.29±0.062 mm respectively. Width of head capsule ranged from 0.15±0.08 to 0.98±0.027 and its length ranged from 0.12±0.011 to 0.98±0.021 for all seven instars. Measurements of eggs, pupae and adults were also recorded. It was observed that male moths were smaller in size than the female moths. Males were found to be 4.5±0.50 mm and females were found to be 5.7±0.64 mm in length. Measurements were taken by calibrated micrometer, vernier caliper and stereo microscope Nikon SMZ 745 T and NIS elements D 3.1 software. Measurements of eggs, cocoon, pupa and adult male and female moths were also recorded. Dyar's ratio for the head capsule width for 1st to 7th larval instars was obtained and progression factor was also obtained through the mean values of Dyar's ratio.Dyar's rule was applied in the present study of *A. grisella* as values were found to be very close to Dyar's value 1.43 for other lepidopterans. Linear regression analysis revealed a significant effect of larval instars on head capsule width thus suggesting that no moult had been missed during larval development.

Nitika Sharma
(Nitika Sharma)
Student
Date:18.07.2012

18.07.20
(Navneet Kumar Gupta)
Major Advisor
Date: 18.07.2012

18.07.2012
Head of the Department

Introduction

1. INTRODUCTION

Wax Moth includes two species viz. *Achroia grisella* Fab. (lesserwax moth) and *Galleria mellonella* L. (greater wax moth). These are also used as a host for mass production of insect bio agents and a tool for scientific studies (William, 1980; Marston *et al* 1975). However, wax moths are also serious problem in apiculture as they incur substantial losses to honey bee products both in bee colonies and drawn comb stores. Besides combs, larvae of these moths also damage hiving material (Burges, 1978; Yusof *et al.*, 1995; Chang and Hsieh, 1992). They eat wax by making tunnels in comb, reducing them to mass of webbing and debris. Severe infestation in hive can lead to absconding of bee colonies.

A. grisella has a scattered distribution throughout the temperate and tropical climate of the world (Hassanein *et al.*1969; Singh 1962). At high elevations, this species causes no or little damage. The adult moth has a yellow head, is solid grey to buff in color. The life cycle of lesser wax moth consists of four definite stages egg, larva, pupa and adult. The larva causes most of the damage to comb and finally adult causes further damage by mating and propagating the species. Single larvae consumed 1.36-1.57 gm. wax during their development and the progeny from single female totals upto 442-665 gm. Developmental mechanisms are usually assumed to evolve by natural selection of the morphological traits they produce. Therefore, the information on phenotypic trait is an important component of comparative studies of development. Morphometric is increasingly used for analyzing morphological variations and developmental modularity. Morphometric studies of different parts of an insect's body are needed to obtain an index to distinguish different larval instars. Determination of appropriate instar for individual sampling is a major problem as in different insects almost several instars are present at the same time and their size distribution overlap to some extent. Morphometric characters were widely used by researchers to determine different developmental stages (Fink, 1984; Stark, 1988; Holloway, 1991; Guglielmino *et al.* 2006; Kayss *et al.* 2006). Growth of insect larvae is discontinuous, with the most measurable changes in size occurring

following the moults (Chapman, 1982). Dyar was the first to suggest frequency distribution of head capsule width for instar determination more than 115 years ago (Dyar, 1890) and this has been successfully applied many times since. Morphometrics yields in analyzing inter as well as intra specific variations and ultimately help in higher classification of a taxon (Ruttner, 1988). The morphology of different developmental stages and morphometric analysis dealt under the proposed study will help in identifying the larval as well in more intensive comparative studies within the taxon. Hence, the present study was planned with the following objective:

To study morphometric changes during different developmental stages of wax moth.

Review of Literature

2. REVIEW OF LITERATURE

The available literature have been reviewed keeping in view the information available on morphometrics of lepidopterans, mainly on moths.

Milum and Geuther (1935) reported that the eggs laid by individual female of *G.mellonella* varied from 400-839. Female to male sex ratio of this moth species was 1:1.31. El Sawaf (1950). El Boroliosy *et al.* (1962) studied the life history of *A. grisella* under natural conditions in Tanta (Egypt). Copulation usually lasted for 14.5 minutes. Mated females laid an average of 298 eggs whereas unmated females an average of 36.3 eggs.

El Sawaf (1950) made observations in 1947-48 on the bionomics of *Galleria mellonella* (L.) in Egypt, where this Pyralid caused considerable losses to the wax-combs of honey bees in storage as well as in the hives.In addition to the biology of *G.mellonella* anatomy of the full grown larvae and rearing techniques adopted have been discussed in details.

In Egypt, Hassanein *et al.* (1969) conducted a study on *Achroia grisella.* Investigations over five generation of the life cycle of *A.grisella* revealed that reproduction may continue throughout the year, winter being less favourable period. Unfertilized eggs did not hatch.

A seasonal variation in duration of different stages of *G. mellonella* has been revealed by Abid *et al.* (1979). The third generation had the shortest incubation period (6.3 days) and the first had the longest (13.6 days). The highest rate of hatching (89.4 per cent) was in second generation and lowest (42.8 per cent) in the third. The longest larval period (163.7 days) was in fifth generation and shortest (37.2 days) in third. Highest and lowest larval survival recorded in second and fifth generation was 79.5 per cent and 31.5 per cent, respectively. The pupal period was longest in fifth generation (17.4 days). Adults of the fifth generation had the longest life cycle (17.4 days) and those of the third generation the shortest (11.7 days). In all the generations, females outnumbered the males. The second generation females laid the most eggs (740 per females) and the fifth generation, the least (415.4 per female).

Chang and Hsieh (1992) reported though female and male adults of *G. mellonella* were observed to be active from14°C to 41°C, moths were found to live year round and had five overlapping generations in a year. Females began to oviposit on the day of emergence. Development time was found to be shortest in the mid summer. Larvae of 5th generation overwintered, pupating in spring. Whereas, *A.grisella* had seven overlapping generations in a year. Females were observed to oviposit from the day of emergence. Eggs laid by unfertilized females of *A. grisella* did not hatch. The shortest development period was during mid summer. Larvae of fifth generation overwintered and completed pupation in spring (Chang *et al.*, 1993).

In the lesser wax moth, *Achroia grisella* (F) newly hatched larva soon penetrate into comb. Mature larvae invariably pupate in tunnel inside the damaged comb. The effective ranges of living temperatures, the high and low lethal temperatures were determined to be 20-39°C for mature larvae and 20-40°C for medium larvae. The observations were made by Chang *et al.* (1993).

Bera *et al.* (2000) made morphometric studies on progression of growth of head capsule width and body length through instars of *Helicoverpa armigera* Hubn. *Remegia archesea* Cram reared on *Cajanus cajan* showed linearity vindicating the postulates of Dyar (1890) and Przibram and Megusor (1928) respectively. Progression factor for head capsule width for *Helicoverpa armigera* did not agree exactly with that postulated by Dyar(1912). Similarly the progression factor of body length for *Helicoverpa armigera* 1.8 and *Remegia archesea* 1.4 showed variance with that given by Przibram and Megusor(1928). The results indicated that values of progression factor vary according to species.

Verma and Desh Raj (2001) conducted a study at Ranital in Kangra valley of Himachal Pradesh during 1993 and 1994 and revealed that the maximum population of wax moths, *Galleria mellonella* L. and *Achroia grisella* F. were 25 and 29 larvae per 3 colonies; and 169 and 192 larvae per 3 colonies during the first fortnight of August of the two respective years. The parasitization of the larvae of G. *mellonella* and *A. grisella* by *Apanteles galleriae* Wilk during 1993 varied from 14.29 to 40.00 and 12.50 to 41.42 per cent, respectively. The corresponding larval parasitization of the two wax moth species during 1994 varied from 14.29 to 41.38 and 8.33 to 42.19 per cent.

Chandrakar and Shrivastava (2002) maintained a culture of *M. separata* on rice cv. Kranti and then adults were released on 8 host plants: rice (Oryza sativa), maize (*Zea mays*), sorghum (*Sorghum vulgare*, finger millet (*Eleusine coracana*), barnyard millet (*Echino chloacolonum*), quack grass (*Ischaemum orogosum,* nut sedge grass (*Cyperus sp.*) and Canna grass (*Commelina benghalensis*). Morphometric studies on *M. separata* infesting the host plants revealed that the larval length and weight gains were maximum on sorghum and the number of moults was minimum (3-4) on wheat. In case of canna grass, the number of larval moults was 4 and 6 in the two generations, and larval length and weight gains were minimum. Cranium development was linear in the successive instars and remained within the range of Dyar's law. The measurements of larval weight and length exceeded the values required under Przibram theory and Dyar's law, respectively.

Godina *et al.* (2002) conducted a study on fruit worm *Acrobasis vaccinii.* The objective was to determine the number of instars of cranberry fruit worm *Acrobasis vaccinii* Riley in southeastern New Brunswick based on the distribution of head capsule widths from field and laboratory observations. In 2000 head capsules from field samples were measured across their widest point. The data suggested that *A. vaccinii* exhibited five instars in 2000. In 2001, larvae were reared in the laboratory until the final molt, and head capsules were counted and measured. The results were also plotted against observed frequencies. It was found that none of the laboratory specimens exhibited more than five instars, supporting the results of the previous year.

Chandel *et al.* (2003) observed the 5 generations of Wax moths, *G.mellonella* and *A. grisella* in one year. The larvae passed through 7 instars. The duration of larval development during 4 active generations varied from 23.6 to 29.5 days in *G. mellonella* and from 25.4 to 29.0 days in *A.grisella.* The wing expanse of female and male *G. mellonella* averaged 23.9 mm and 17.8 mm respectively. Whereas in *A.grisella* 19.2 mm and 13.0 mm respectively. The males lived longer than the females. Eggs were laid either singly or in batches of 2-80 and 2-65 in *G. mellonella* and *A. grisella* respectively. The population of females outnumbered the males in all generations of both species with male to female ratio varied from 1:1.1 to 1:1.8 in *G. mellonella* and 1:1.2 to 1:1.8 in *A. grisella.*

Pal *et al.* (2003) studied the biology and morphometrics of shoot and fruit borer (*Leucinodes orbonalis*) in the laboratory for 3 generations using aubergine as food plant. The total life cycle of *L. orbonalis* varied from 26.3 to 45.9 days. Distinct differences in egg, larval and pupal periods were observed. The egg period ranged from 3.01±0.18 to 5.69±0.21 days, larval period from 11.05±0.23 to 18.34±0.51 days and pupal period from 7.22±0.14 to 12.43±0.43 days. The male to female ratio varied from 1:1.07 to 1:1.14. The average length and width, respectively, were 0.72±0.06 mm and 0.48±0.06 mm for eggs, 1.83±1.20 and 0.40±0.06 mm for first instar larva, 17.52±1.40 and 3.13±0.10 mm for full grown larvae and 10.83±0.42 and 3.47±0.18 mm for pupa. The mean body length and wingspan, respectively, were 1.07±0.05 and 2.47±0.12 cm for female and 0.94±0.05 and 2.02±0.11 cm for male.

The biology of *O. materna* on sweet orange was studied by Patel and Patel (2006) and during the study it was found that the larva passed through five instars. The average length and breadth of first, second, third, fourth and fifth larval instars were 4.32±0.42 and 0.86±0.05 mm, 11.81±0.44 and 1.26±0.15 mm, 18.8±1.28 and 2.58±0.33 mm, 35.01±2.41 and 4.15±0.15 mm, and 65.33±0.51 and 8.83±0.14 mm, respectively. The average development period of first, second, third, fourth and fifth larval instars were 2.10±0.31, 1.35±0.49, 2.50±0.51, 3.55±0.51 and 3.85±0.37 days, respectively. Total larval period was 13.25±1.25 days. The average length and breadth of pupa was 25.04±1.11 mm and 8.99±0.59 mm in males and 26.16±1.72 mm and 9.45±0.53 mm in females, respectively. The males and females measured on an average 29.84±0.94 and 30.36±1.47 mm in length and 76.07±1.82 and 76.54±1.46 mm in breadth with expanded wings, respectively. The average longevity of males and females was 28.8±3.85 and 31.1±1.66 days, respectively. The total life period was 58.00±3.97 days for males and 59.00±3.16 days for females.

Morphometric characterization of twelve geographic populations of cotton bollworm, *Helicoverpa armigera* occurring in south Indian cotton ecosystems was done at larval, pupal and adult stages over three cropping seasons by Fakrudin *et al.* (2007). Traits such as length and weight of larvae, pupa and length and width of the wing, length of fore, mid- and hind femur, male reproductive organ-length of genital capsule, valves,

and ejaculatory duct, female reproductive organ-length of appendix bursae and ductus bursae at adult stage were measured across three years. Populations significantly differed for most of the traits studied. Besides larval, pupal and adult external phenotypic traits, attributes of male reproductive organ viz., length of genital capsule, valves, and ejaculatory duct and female reproductive organ viz., length of appendix bursae and ductus bursae differed significantly among populations. Morphometric parameters of northern population of the insect varied considerably from the southern population. Information on population structure and differences based on morphometry would be useful in better understanding of population dynamics and management of this pest in cotton and other crops in south India.

Delbac *et al.* (2010) advocated that morphological measurements such as head-capsule width can be very useful and accessible tool that may be employed for classifying lepidopteran larval instars and in the management of larval pests, because their control relies upon making accurate assessment of the life history stage at which larvae has reached in various environmental conditions. Such forecasts were then used in order to estimate the timing of emergence for future adult populations. Head capsule widths from field larvae of European Grapevine Moth *Lobesia botrana* have been used to describe distributions of the five instars during three generations of the insect. The method was calibrated on a large number of individuals (N = 552) issued from insect culture and used a non-linear least-squares parameter estimation to describe the distribution of each larval instar inside each generation. The model was tested on a wild larval population (n = 3007) occurring in our experimental vineyard during two complete years. The instar class ranges and boundaries were characterized with the associated probabilities of misclassification. A final classification statistical model was developed for each instar and each generation. From this study, they concluded that size of larval head capsule increases statistically according to the generation of the year, and thus was influenced by grape phenology.

Ilijin *et al.* (2010) found that larval stage duration, relative growth rate (RGR) and mass of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) may be influenced by the components of the diet.

To determine the cotton bollworm migrating population rate in Hungary, Kezthelyi *et al.* (2011) examined the weights and the front wing morphological features of moths trapped during the maize vegetation cycle period in 2008. A definite regularity was seen in their body mass and morphological features, namely the front wing quotient (quotient of length of front wing/width of wing), modified wing loading (weight of moth/surface of front wing), and the relative thorax size (width of thorax/width of head).

Materials and Methods

3. MATERIALS AND METHOD

The present study was undertaken in the Department of Biology and Environmental Sciences, Chaudhary Sarwan Kumar Krishi Vishvavidyalaya, Palampur from August 2011 to May 2012.The details of materials and methods employed are described as below:

3.1 Maintenance of culture

The wax moth species viz. *Achroia grisella* was collected from drawn comb store of University Bee Research Station, Nagrota Bagwan, District Kangra, Himachal Pradesh and was taken to laboratory. For raising the culture in the laboratory, one sexual pair of *Achroia grisella* was released into the glass jars, mouth of the jars was protected with a muslin cloth. A sterilized cotton swab containing 30% honey solution was kept in each jar as a feed for adults. The eggs obtained were kept in petri dishes for hatching and newly emerged larvae were reared on wax which is a natural diet for the wax moth larvae. First instar larvae of less than 24hr old were picked up carefully with the help of camel brush and one larva was transferred to each vial having thin slices of wax in it. The mouth of these vials was also protected with fine muslin cloth tied tightly around the neck to check the larval escape. The larvae were allowed to develop in the vials.The fully grown larvae of each instar was distinguished by its laziness day before ecdysis. These larvae were collected separately, killed in boiling water and preserved in 70 per cent alcohol.

3.2 Diet for adults and larvae of wax moth

Since the adult and immature stages of lepidopterans consume different type of food, the lesser wax moth has been provided food as mentioned below:

- 30 per cent honey solution was used as a diet for adult moths.
- Larvae were fed upon bee wax which is natural diet for them.

3.3 Measurements:

Measurements of larval body length, width, head capsule length, width and length and width of cocoon and pupa recorded. The body length of male and female adult moth

was also recorded. Measurements were recorded under microscope by using the combination of stage and ocular micrometer. Exceeding measurements of later instars were taken directly with the help of following instruments:

- Stereo-microscope Nikon SMZ 745 T.
- NIS elements D 3.1 software
- Vernier caliper.

Daily monitoring of larvae carried out to record stage of development. Fully grown larvae of each instar (recognized by being sluggish in their activities) were randomly selected and length and width of each instar was measured. Length and width of head capsule of each instar in the widest region was measured using the above instruments

3.4 Dyar's ratio:

Dyar's ratio was calculated by dividing the width of head capsule of an instar with the width of head capsule of successive one i.e.

Dyar's ratio = Postmoult/Premoult

3.5 Statistical analysis of data

The morphometric relationship of body length with body width, head capsule length and head capsule width of different instars were estimated by evaluating the values of correlation coefficients 'r' and coefficient of determination 'r^2'(Harries and Handerson, 1938). The significance level was tested by applying the student t-test.

Results and Discussion

4. RESULTS AND DISCUSSION

The adult moth *Achroia grisella* F. belonging to the sub family Galleriinae and family Pyralidae, has yellow head and grey body colour. The body length of male and female moth were measured to be 4.5±0.50 and 5.7±0.64 mm respectively. The wing expanse was about 12.7±1.1 mm. in case of male and about 19.4±1.2 mm in females. *A. grisella* deposited 250 to 300 eggs and these eggs were laid into clusters which took 7 days to hatch into larvae. *A.grisella* took about 40-45 days from hatching to emergence of adults.

4.1 Morphometry of Eggs of *Achroia grisella*:

Eggs of *A. grisella* were yellowish in colour and averaged 0.42±0.02 mm in length and 0.30±0.01 mm in width (Table 4.1). The eggs were laid into 4-5small clusters and about 50-65eggs were there in each cluster. The egg of *A. grisella* hatched into larvae after five to eight days. It was observed that egg laying rate was at its highest in the first day after mating and diminished over the next 4-5 days.

4.2 Morphometry of larvae of *Achroia grisella*:

Larval stage took about 20 days. It was creamish in colour (Fig 4.1). They ate wax by making tunnels in it. Before moulting the larvae of lesser wax moth became sluggish. It moulted six times and passed through seven instars. The mean body lengths were 0.92, 2.07, 2.52, 4.23, 4.81, 7.34 and 12.15 mm for first, second, third, fourth, fifth, sixth and seventh larval instars respectively. The mean body width was measured to be 0.15, 0.22, 0.29, 0.67, 0.87, 1.64 and 2.29 mm for first, second, third, fourth, fifth, sixth and seventh larval instars respectively (Table 4.1).

Fig. 4.1 Larva of lesser wax moth *A.grisella*

The body length and body width showed a considerable increase with the development of larval instars. Chang *et al.* (1993) conducted study on *A.grisella* in China and observed that mean body length for 1st to 7thintars were 1.1±0.1, 4.4±0.5, 10.4±0.6, 10.6±0.4, 11.0±0.2, 11.4±0.9 and 12.3±1.1 mm respectively. Body width was measured to be 1.6±0.1, 1.1±0.1, 1.9±0.1, 1.9±0.1, 1.9±0.1, 2.1±0.2 and 2.3±0.1 mm for all seven instars of *A. grisella* respectively. These values are far more than that observed for present study. This could probably be due to the ecological and environmental conditions of China. The mean head capsule width from 1st to 7thinstar was 0.15, 0.18, 0.28, 0.38, 0.51, 0.74 and 0.98 mm respectively. The head capsule length for 1st to 7th instar were measured to be 0.12, 0.13, 0.23, 0.34, 0.42, 0.75 and 0.98 mm (Table 4.1).

Table 4.1 Measurements of different life stages of *Achroia grisella*

Stages	Head capsule width(mm)	Head capsule length(mm)	Length(mm)	Width(mm)
Egg			0.422±0.02 (0.3923-0.4461)	0.3007±0.01 (0.2846-0.3153)
Larva I	0.15±0.004 0.15-0.17)	0.12±0.010 0.11-0.14)	0.92±0.074 (0.83-1.03)	0.15±0.012 (0.14-0.17)
II	0.18±0.019 (0.16-0.22)	0.13±0.020 (0.11-0.17)	2.07±0.234 (1.57-2.36)	0.22±0.013 (0.21-0.26)
III	0.28±0.015 (0.27-0.31)	0.23±0.012 0.22-0.26)	2.52±0.123 (2.41-2.84)	0.29±0.014 (0.27-0.32)
IV	0.38±0.028 0.35-0.41)	0.34±0.026 0.31-0.38)	4.23±0.345 (3.75-4.62)	0.67±0.037 (0.62-0.73)
V	0.51±0.017 0.49-0.54)	0.42±0.021 0.38-0.45)	4.81±0.071 (4.72-4.97)	0.87±0.37 (0.83-0.95)
VI	0.74±0.028 (0.72-0.79)	0.75±0.030 0.73-0.82)	7.34±0.120 (7.12-7.52)	1.64±0.047 (1.60-1.72)
VII	0.98±0.027 (0.94-1.03)	0.98±0.021 (0.95-1.02)	12.15±0.056 (12.07-12.25)	2.29±0.062 (2.19-2.40)
Cocoon			7.8±0.74 (7.00-9.00)	2.6±0.489 (2.00-3.00)
Pupa			5.56± 0.607 (4.70-6.50)	1.66±0.061 (1.59-1.78)
Male			4.5±0.50 (4.00-5.00)	
Female			5.7±0.64 (5.00-7.00)	
Wing expanse				
Male			12.7±1.1 (11.00-14.00)	
female			19.4±1.2 (18.00-21.00)	

n = 10, Figures in parentheses are range values

4.2.1 Larval Head capsule width:

The width of head capsule in 1st to 7thinstars were 0.15-0.17 mm, 0.16-0.22 mm, 0.26-0.31 mm, 0.35-0.41 mm, 0.49-0.54 mm, 0.72-0.79 mm and 0.94-1.03 mm respectively (Table 1). Head capsule width was relatively constant within the seven instars. There was no overlapping in extremes of measurements in instars. The Dyar's ratio for subsequent instars was 1.26, 1.47, 1.35, 1.34, 1.47 and 1.3 for all the instar respectively (Table 4.2).The ratio was calculated according to the Dyar'srule.It showed that growth rate was slow in 1st and 2nd instar and faster in later instars. The progression factor was found to be 1.35 which describes the growth trends. Mohammadi *et al.* (2010) also reported almost same trends in cotton bollworm, *Helicoverpa armigera* Hubner. Their findings suggested that head capsule width could be used for the estimation of larval instar in laboratory population of *Helicoverpa armigera.* The apparent lack of increase in the growth of had capsule width of *A. grisella* for early instars showed that no moult occurred during this phase. The perfect geometric progression of head width was presented by plotting the head width against each instar. The equation $y= 0.136x - 0.081$ ($R^2 = 0.930$) gave a good fit regression line (Fig.4.2).

Table 4. 2. Mean head capsule width and Dyar's ratio for different larval stages of *A. grisella*

INSTARS	HEAD WIDTH	DYAR'S RATIO
I	0.15	
II	0.19	1.26
III	0.28	1.47
IV	0.38	1.35
V	0.51	1.34
VI	0.75	1.47
VII	0.98	1.3

Progression factor = 1.35

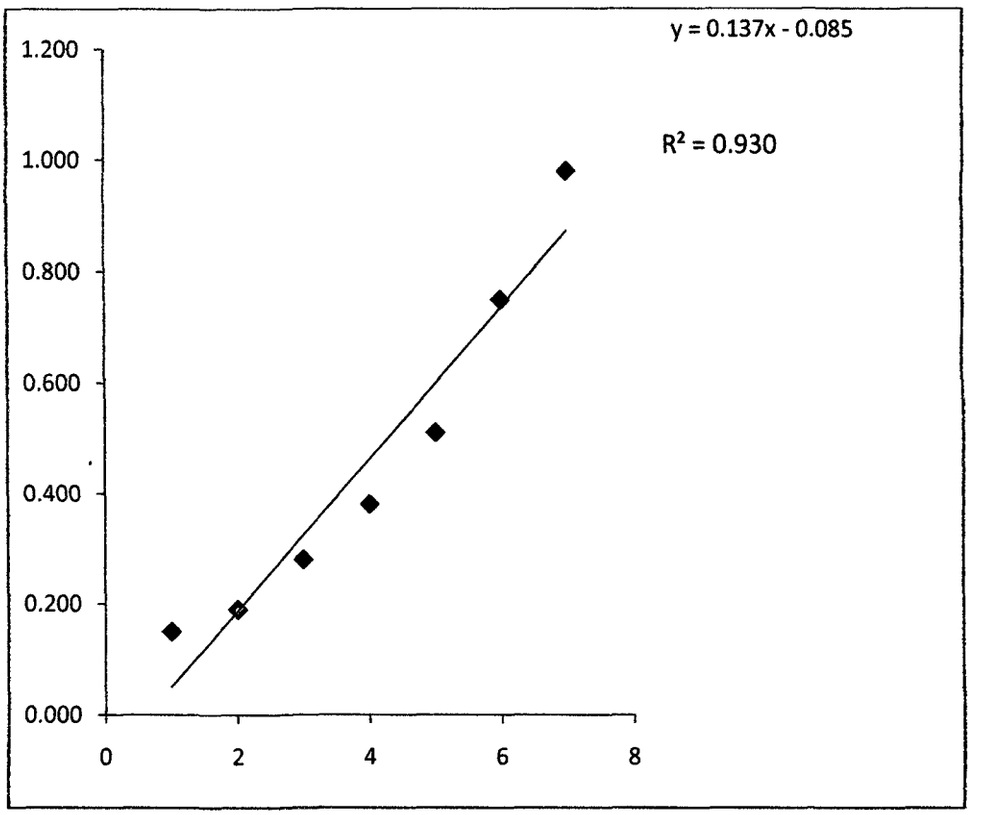

Fig. 4.2 Regression line for head capsule width

4.2.2 Statistical analysis for Body length and head capsule length of *Achroia grisella*:

The perusal of data of all the seven larval instars showed that the highest 'r' value of 0.593981 was observed in 3rd larval instar between body length and head capsule length though the correlation was not that strong between these two characters. The lowest value of 'r' was found in 1st larval instar which is 0.008214. It indicates that there was no correlation in body length and head capsule width of 1st instar (Table4.3). Relationship between head capsule length and subsequent development of larval instar was also studied and it was observed that head capsule length was increasing with the development of larva (Fig. 4.3).

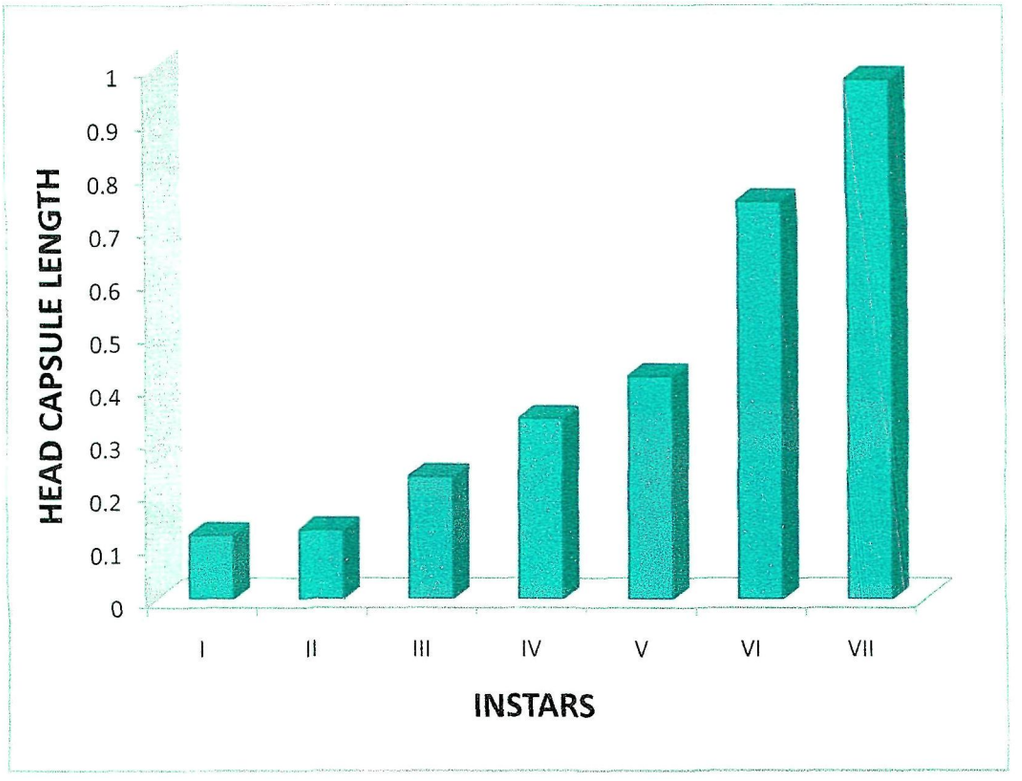

Fig. 4.3 Relationship between instars and head capsule length

Table 4.3. Correlation between body length and head capsule length

INSTAR	BODY LENGTH (mm)	HEAD LENGTH (mm)	r	r^2	t-test
I	0.92	0.12	0.008214	6.75E-05	2.166692
II	2.07	0.13	0.432012	0.186634	0.642425
III	2.52	0.23	0.593981	0.352813	0.738929
IV	4.23	0.34	0.264651	0.07004	3.435514
V	4.81	0.42	0.355402	0.126311	-0.16596
VI	7.34	0.75	0.168354	0.028343	0.668493
VII	12.15	0.98	0.418598	0.175224	-0.66531

4.2.3 Statistical analysis for Body length and head capsule width of *Achroia grisella*:

The morphometric relationship between body length and head capsule width of all the larval instars were also estimated by evaluating the values of correlation coefficient 'r', coefficient of determination 'r^2' and significance level was tested by applying t-test at 5% level. Thus, the following results were obtained from the statistical analysis of data regarding the morphometry of body length and head capsule width. It has been observed that there was a positive correlation between all the larval instars except the 4^{th}, 5^{th} and 6^{th} larval instars. Highest 'r' value of 0.6670488 was observed in 2^{nd} instar between body length and head capsule width. The 3^{rd} larval instar was also found to be positively correlated with 'r' value 0.625442 (Table 4.4). Correlation was found to be almost zero in 4^{th}, 5^{th} and 6^{th} instars. It was also observed that with the development of larva, head capsule width was also increasing (Fig.4.4, 4.5).

Table 4.4 Correlation between body length and head capsule width

INSTARS	BODY LENGTH (mm)	HEAD WIDTH (mm)	r	r^2	t-test
I	0.92	0.15	0.379525	0.144039	4.899666
II	2.07	0.18	0.667049	0.444954	2.368873
III	2.52	0.28	0.625442	0.391178	1.643574
IV	4.23	0.38	0.199709	0.039884	3.634975
V	4.81	0.51	0.188489	0.035528	0.74296
VI	7.34	0.74	0.103448	0.010701	0.783407
VII	12.15	0.98	0.558648	0.312088	-1.31431

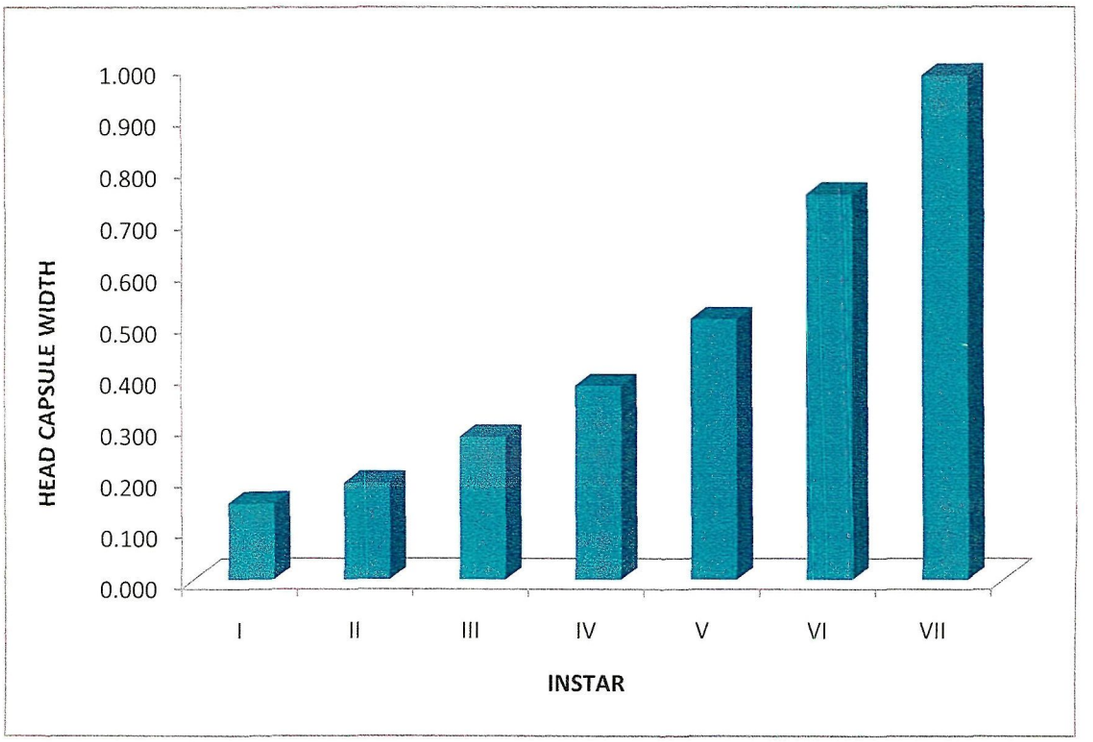

Fig. 4.4 Relationship between instars and head capsule width

Fig. 4.5 Head capsule of larva of A. grisella

4.2.4 Statistical analysis of Body length and Body width of larvae of *A. grisella*:

The perusal of data showed that the highest 'r' value of 0.985382 and the lowest value of 0.1806 were found to be in 5th and 1st instars. The values were found to be significantly correlated at 5%.The body length and body width of 6th and 7th larval instar of *A. grisella* was also correlated having 'r' value 0.916383 and 0.953128 respectively. The larval body length and body width showed a positive allometric growth (Fig.4.4).

Table 4.5 Correlation between body length and body width of *A. grisella*

INSTAR	BODY LENGTH (mm)	BODY WIDTH(mm)	r	r^2	t-test
I	0.92	0.15	0.1806	0.03262	2.7434
II	2.07	0.22	0.868728	0.754689	3.740174
III	2.52	0.29	0.6032	0.363851	1.791155
IV	4.23	0.67	0.55136	0.303998	3.098637
V	4.81	0.87	0.985382	0.970977	-9.74145
VI	7.34	1.64	0.916383	0.839758	-2.30701
VII	12.15	2.29	0.953128	0.988453	2.743477

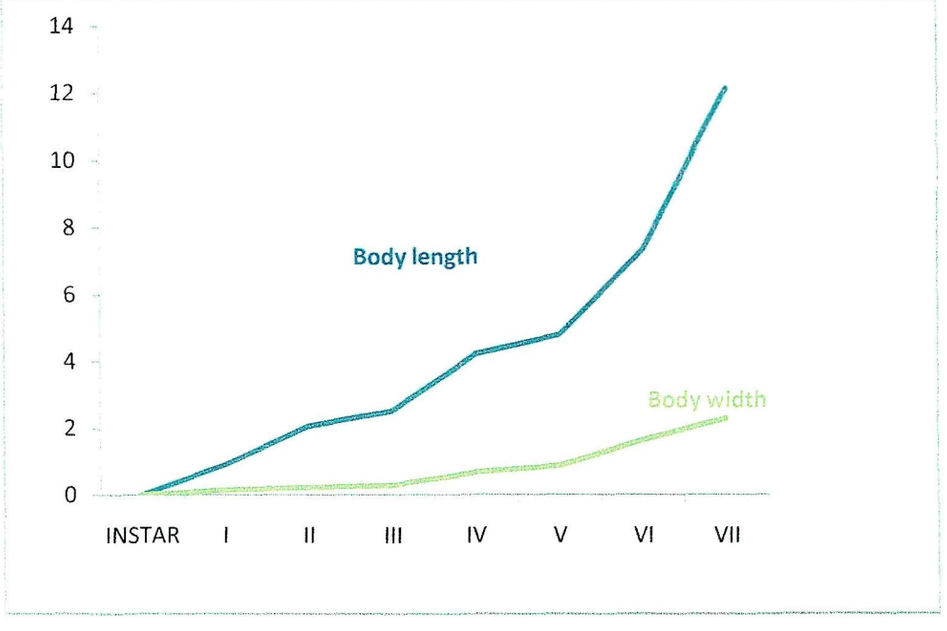

Fig. 4.6 Relationship between instars and body length-body width

4.3 Morphomerty of cocoon:

After feeding for about 10 days larvae stopped feeding, became sluggish and started cocoon formation. The cocoon of *A. grisella* was observed to be white in colour, densely covered with frass. The cocoon is made up of a coarser silk that is papery in texture and very strong. The silk is made up from the material excavated from the surface on which the larva pupates. The length and breadth of cocoon was measured to be 7.8±0.74 mm and 2.6±0.48 mm, respectively (Table 4.1).

4.4 Morphometry of Pupa:

Pupa was broad anteriorly and tapering posteriorly. The colour of the pupa was found to be brownish white initially and later it changed to dark brown when adult was about to emerge. The mean length and width of pupa of *A. grisella* was measured to be 5.56±0.607 and 1.66±0.061 mm respectively (Table 4.1). Pupa developed and hatched within 8-9 days when temperature conditions were favourable during the months of September and October, but it was observed that it took three months to emerge out of the cocoon and to become an adult moth when temperature conditions were low from January to March 2012 (Appendix II).

Summary and Conclusions

5. SUMMARY AND CONCLUSIONS

In the present investigation entitled 'morphometric changes during ontogenetic development of wax moth, the wax moth species *Achroia grisella,* which is an important pest of apiculture, was studied for its morphometric changes during different developmental stages. The various findings of the present study are summarized as below:

1. Morphometry of larva revealed that the growth of each instar was increasing in a constant manner.
2. Measurements recorded for its body length and body width showed that the growth rate was a little slow during early instar but it was faster in case of later instars.
3. The mean body length and body width of larvae ranged from 0.92±0.074 to 12.15±0.056 mm and 0.15±0.013 to 2.29±0.062 mm for I, II, III, IV, V, VI and VII larval instar respectively.
4. Length and width of head capsule also showed a positive relation with the development of larva. The mean head capsule width ranged from 0.15±0.08 and 0.98±0.027 mm. and the mean head capsule length was found to be 0.12±0.011 and 0.98±0.021 mm. for 1st to 7th instar larvae.
5. The regression line plotted for head width against each instar showed that no moult had been accidently missed throughout the development.
6. The Dyar's ratio for all seven instars of *A. grisella* was calculated to be 1.26, 1.47, 1.35, 1.34, 1.47 and 1.3 respectively and progression factor was 1.35.Dyar's rule for the head capsule was also applied in case of *A. grisella* as values were found to be very close to Dyar's value 1.43 for other lepidopterans.
7. The mean length and breadth of cocoon was measured to be 7.8±0.74 and 2.6±0.48 mm.
8. The mean length and width of pupa of *A. grisella* was measured to be 5.56±0.607 and 1.66±0.061 mm respectively. It is observed that the colour of pupa was brown initially but later it changed to dark brown when adult was about to emerge out of the cocoon.

9. The male moths were smaller than the female moths. The body length of male and female adult moth was measured to be 4.5±0.50 and 5.7±0.64 respectively.
10. It had been observed during the study that all the stages of development were affected to a large extent by temperature variations. It was observed that pupa took almost 3 months to become an adult when temperature conditions were not favourable during the months of January to march, 2012 (Appendix II). While cool temperature slowed down the development, warm temperature accelerated it.

Literature Cited

LITERATURE CITED

Abid MK, EI Shaaraawy MF and EIYarsery MK.1979.The Biolology of greater wax moth, *Galleria mellonella* (Lepidoptera Galleridae) in Iraq. *Bulletin of Natural History Research Centre*7(3): 365-373

Bera S, Bandyopadhyay B and Paul SK. 2000. Morphometric studies on larvae of *Helicoverpa armigera* hubn. and *Remegia archesea* Cram. (Noctuidae Lepidoptera) infesting pigeon pea (*Cajanus cajan*). *Journal of Interacademicia (India)* 4(3): 426-429

Burges HD. 1978. Control of wax moths. Physical, chemical and biological methods. *Bee World* 59: 129-138

Chapman RF. 1978. The insect structure and function. *Hodder and Stoughton Publication* p 819

Chang CP and Hsieh FK. 1992. Morphology and bionomics of *Galleria mellonella* L. *Chinese Journal of Entomology* 12(2): 121-129

Chang CP, Hsieh FK and Hsu LR. 1993. Primery investigation on morphology and bionomics of lesser wax moth (*Achroia grisella*). *Chinese Journal of Entomology* 13(3): 219-227

Chandrakar HK and Shrivastava SK. 2002. Morphometric variation in *Mythimia separate* (Walker) on some host plants. *Journal of Applied Zoological Research* 13(2/3): 135-138

Chandel YS, Sharma S and Verma KS. 2003. Comparative biology of greater wax moth *Galleria mellonella* L and lesser wax moth *Achroia grisella. Pest Management and Economic Zoology* 11(1): 69-74

Delbac L, Lecharpentier P and Thiery D. 2010. Larval instars determination for European grape vine moth (Lepidoptera; Tortricidae) based on the frequency distribution of head capsule width. *Crop- protection* 29(6): 623-630

Dyar HG. 1890. The number of moults of lepidopterous larvae. *Psyche* 5: 420-422

El Boroliosy FM, Wafa AK and Allam HM. 1962.Studies on the life history of *Achroia grisella. Bulletin de la societe* 56: 257-265

El Swaf SK. 1950. The life history of the greater wax moth Galleria mellonella in Egypt with special reference to the morphology of the mature larvae. *Bulletin de la Societe fouad premier d Entomolgie* 34: 247-297

FakrudinB, Krishnareddy VK, Patil KB and Kuruvinashetty BV. 2004. Morphometric differences between pyrethroid resistant and susceptible populations of cotton bollworm *Helicoverpa armigera. Resistant Pest Management Newsletters* 13(2): 18-20

Fink JJ. 1984. Errors in instar determination of may flies and stone flies (Plectoptera) using the simple frequency, Janetschek Cassie and Dyar's law methods. *Fresh Water Biology* 13: 347-36

Godina J, Maltaisa P and Gaudetc S. 2002. Head capsule width as an indicator for larvae of fruitworm (Lepidoptera: pyralidae) in Southeastern New Brunswick *Journal of Econolmic Entomology* 95(6): 1308-1313

Guglielmino A, Buckel C and Moya- Raygoza G. 2006. Description of the larval instars of *Gonatopus bartlettiolmi* (Hymenoptera: Dryinidae). *Zootaxa*1226: 51-60

Harries FH and Henderson CF. 1938. Growth of insects with reference to progression factors for successive growth stages. *Annal of Entomological Society America* 31: 557-572

Hassanein M, Ibrahim MM, Banby MA and Arousy AFM. 1969. Ecological and Biological studies on the lesser wax moth, *Achroia grisella* Fab. (Lepidoptera: Gelleridae). *Bulletin Societe Entomologique d' Egypte* 53: 573-579

Holloway BA. 1991. Identification of third instar larvae of flystrike and carrion associated blowflies in New Zealand (Diptera calliphoridae). *New Zealand Entomologist*14: 24-28

IlijinL, Pericmataruga V, Radojicic R, Lazarevic J, Nenadovic V, Vlahovic M and Mrdakovic M. 2010. Effects of cadmium on protocerebral neuroscretory neurons and fitness components in *Lymentria dispar* L. *Folia Biologica Krakow* 58(1/2): 91-99

Kayss S, Ohi M and Kinzelbach R. 2006. Identification of larval instars of Phthirapteran ectoparasite *campanulotes bidentatus* compare by setal pattern. *Entomology Zeitschrift*. 53: 86-90

Keszthelyi S, Szentpeteri J and Pal-Fam F. 2011.Morphometrical and front wing abrasion analysis of Hungarian cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) population. *Biologia Bratislava* 66(2): 340-348

Marston N, Campbell B and Boldt PE. 1975. Mass producing eggs of greater wax moth, *Galleria mellonella* L. *Technical Bulletin United States Department of Agriculture*: p 1510

Milum VG and Geuther HW. 1935. Observation on the biology of greater wax moth,*Galleria mellonella* L. *Journal of Economic Entomology* 28**:** 576-578

Pal A, Shukla BC and Pal A. 2003. Biology and morphometry of Brinjal shoot and fruit borer in Chhatisgarh. *JNKVV Research Journal* 37(1): 63-67

Patel MM and Patel RK. 2006. Biology of fruit sucking moth *othreis maternal* L. on sweet orange. *International journal of Agricultural Sciences* 2(1): 118-121

Ruttner F. 1988. Biogeography and taxonomy of honeybees. Biogeography and taxonomy of honeybees. Springer- Verlag, London. p 284

Singh S. 1962. Beekeeping in India. Indian Council of Agricultural Research, New Dehli: p 214

Stark JD. 1988. Hind-tibia length: a convenient parameter for instar differentiation in leptoceridae (Tricoptera). *New Zealand Entomologist* 11: 76-78

Verma SC and Desh Raj. 2001. Incidence of wax moth (*Galleria mellonella*) and (*Achroia grisella)* and their parasitization by *Apanteles galleriae* in *Apis cerana* in Kangra valley. *Himachal Journal of Agriculture Research* 26(1/2): 44-49

William JL.1980. Insects: Lepidoptera (Moths). In: *Honey Bee Pest Predators and Diseases*: p 106-127

Yusof MR, Ibrahim R and Kevan PG. 1995. Current status of pests and diseases of honey bee *Apis cerana* F. in peninsular Malaysia. In: *The Asiatic hive Bee*: p 185-190

Appendices

Appendix-I

Ambient climatological parameters recorded during the course of study at CSK HPKV, Palampur

Standard week	Temperature ^{0}C		Relative humidity %	Rainfall (mm)
	Max	Min		
1 Jan. (2011)	15.7	2.9	58.7	5.8
2	17.1	4.7	53.5	0.0
3	12.0	2.4	68.2	58.2
4	16.1	3.5	63.0	0.0
5	18.1	6.5	59.4	1.0
6	19.1	7.6	57.3	23.0
7	14.3	6.5	77.8	108.8
8	16.8	5.9	67.3	1.0
9	15.1	6.1	76.5	38.8
10	19.5	7.6	54.9	7.7
11	24.4	11.2	54.0	0.0
12	24.8	11.7	49.1	0.0
13	25.9	12.2	50.1	5.4
14	22.7	10.5	57.3	5.0
15	24.4	12.6	64.3	5.6
16	24.0	11.8	59.6	80.1
17	28.7	17.0	55.3	0.0
18	31.6	19.2	62.1	10.0
19	30.4	18.8	58.4	2.6
20	33.5	20.9	67.9	0.1
21	31.0	18.1	71.2	13.9
22	28.6	17.5	66.1	6.1

Appendix-II

Ambient climatological parameters recorded during the course of study for the year 2011-12 at CSK HPKV, Palampur

Standard week	Temperature ^{0}C		Relative humidity %	Rainfall (mm)
	Max	Min		
40 (Oct. 2011)	25.5	15.8	86.5	32.2
41	27.1	14.4	72.5	0.0
42	25.9	12.5	64.5	0.0
43	24.2	11.7	63.5	2.6
44	23.4	12.6	74.0	1.8
45	23.6	11.5	79.5	0.0
46	23.0	10.1	79.0	0.0
47	22.8	9.7	81.0	0.0
48	20.7	7.1	76.5	0.0
49	21.9	8.7	88.0	6.0
50	18.5	6.6	85.5	0.0
51	17.9	3.6	88.0	0.0
52	18.1	3.8	67.5	0.0
1 Jan. (2012)	17.1	4.6	66.8	1.7
2	11.6	1.7	65.7	10.4
3	10.1	2.8	84.4	15.7
4	15.0	3.5	63.2	0.0
5	16.3	3.0	59.1	0.1
6	14.3	3.2	66.6	4.6
7	13.9	4.8	71.7	5.1
8	18.2	7.6	69.1	1.5
9	19.8	8.0	47.6	0.0
10	18.9	6.7	53.0	2.9
11	20.8	8.4	53.1	1.7
12	25.5	11.0	53.6	0.0
13	25.9	13.5	45.2	0.0
14	28.3	14.8	39.6	0.5
15	25.0	12.2	61.4	2.6
16	25.5	13.8	63.0	1.0
17	26.2	12.9	54.2	3.8
18	26.9	14.4	45.2	0.0
19	31.1	16.7	21.0	4.8
20	30.5	18.0	35.0	0.0
21	32.8	19.5	29.0	0.1
22	35.5	21.6	28.2	0.0

Brief Biodata of student

Name	:	Nitika Sharma
Father's Name	:	Shri. PuranChand Sharma
Mother's Name	:	Late Smt. Shakuntala Sharma
Date of Birth	:	07 FEBRUARY, 1988
Permanent Address	:	Vill. GURETH, P.O. DURGATHI, Tehsil BHARMOUR, Distt. CHAMBA (Himachal Pradesh) 176324

Academic Qualifications:

Examination passed	Year	School/Board/ University	Marks (%)	Division	Major Subjects
10th	2003	Himachal Pradesh Board of School Education Dharamshala	81.42	First	English, Hindi, Maths, SST, Science, Sanskrit, IT
10+2	2005	Himachal Pradesh Board of School Education Dharamshala	69.00	First	English, Biology, Physics, Chemistry, Information Technology
B.Sc.	2008	GNDU AMRITSAR (PUNJAB), INDIA	64.87	First	English, Chemistry, Botany, Zoology, Punjab History Culture
M.Sc. Biology	2012	CSK Himachal Pradesh Agriculture University, Palampur (H.P.), India	70.4	First	Major Discipline: BIOLOGY Minor Discipline: ENTOMOLOGY