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Department of Entomology, Kerala Agricultural University
Vellayani P.O, Thiruvananthapuram 695522, Kerala, India
Email : aae@kau.in; web: www.entomon.in

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Fat body remodeling in *Spodoptera litura* F. (Lepidoptera: Noctuidae) during postembryonic development

Vinod K. Chauhan, Narender K. Dhania, Pavani Ayinampudi, R.K. Chaitanya[#] and Aparna Dutta-Gupta[*]

Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India; [#]Centre for Animal Sciences, School of Basic and Applied Sciences, Central University of Punjab, Bathinda, 151001, India.
Email: apdgsl@uohyd.ernet.in/ aparnaduttagupta@gmail.com

ABSTRACT: During insect metamorphosis, larval structures including fat body are replaced by the adult ones. This process involves lysosomal enzyme-mediated remodeling of fat body. The objective of this study is to characterize the events leading to fat body remodeling during postembryonic development in an important agricultural pest, *Spodoptera litura*. Present study showed that the fat body undergoes significant changes in its morphology as well as histology. During the larval stage the tissue is primarily synthetic and secretory in nature and releases large amount of macromolecules including hexamerins in the heamolyph. While at pre-pupal and pupal stages it acts as a storage tissue and accumulates number of protein granules. Radiolabelling and DNA analysis studies revealed higher content of DNA in the larval fat body. The decline seen in pre-pupae corroborated well with disintegration of nuclei which were remodeled during pupal and adult stages. Further, the role of an insect morphogenetic hormone, 20-hydroxyecdysone (20E) in fat body reorganization has also been elucidated. This study enables us to understand the basic mechanism and altered micro-environment of the dynamic fat body tissue during larval-pupal-adult transition and metamorphosis.

KEY WORDS: *Spodoptera litura,* fat body, tissue remodeling, metamorphosis, 20 -hydroxyecdysone, postembryonic development

INTRODUCTION

Holometabolous insect life cycle is characterized by the presence of four distinct stages: egg, larva, pupa and adult. Larvae that are hatched out from the eggs develop (grow in size) in stages called instars followed by the dramatic transformation into quiescent, non-feeding pupal stages which then eclose into adults. As the transformation involves alterations in the feeding habits and physiology with each developmental stage, a balanced acquisition and utilization of resources is of high significance in their life cycle (Truman *et al*., 2002). Therefore, insects have developed the ability to store large quantities of protein which are called as hexamerins in their fat body, which serve as a source of amino acids and energy needed for the development of adult tissues and transformation (Haunerland *et al*., 1996).

Fat body, is a vital multi-functional tissue found in the visceral cavity of insect life stages (Telfer and

* Author for correspondence

Kunkel, 1991; Arrese *et al*., 2010; Hoshizaki *et al*., 2012). It performs diverse functions that include the maintenance of bacterial endosymbionts (Costa-Leonardo *et al*., 2013) , storage of urate during development (Park *et al*., 2013), synthesis, release and storage of a variety of macromolecules (Costa-Leonardo et al., 2013; Roma *et al*., 2013), source of humoral factors and role in immune functions (Gillespie *et al*.,1997) as well. During metamorphosis, the larval fat body being major site of biosynthetic activity, undergoes a chronologically ordered sequence of alterations and is completely remodeled by the time adult emerges (Dean *et al*.,1985; Lakshmi and Dutta-Gupta,1990; Wang and Haunerland,1992) . The histolysis and histogenesis of fat body cells during metamorphosis is preceded by quantitative changes in DNA content (Edgar and Orr-Weaver, 2001). However, owing to structural complexity and pleomorphism of the fat body, elucidation of its postembryonic remodeling has been limited so far. The distribution of stage-specific functions to various cell types of the fat body has been readily acceptable in dipterans due to the well-established underlying mechanism of adult fat body generation and precise stage-specific differences in the functions of fat body in these insects (Jansen and Borgesen, 2000). On the contrary, lepidopteran insect fat body cell types are mixed and integrated into a unified tissue thereby making it difficult to correlate a specific functional activity with a cell type (Haunerland and Shirk, 1995). Moreover, the change of function of fat body during metamorphosis was attributed to the transformation of cellular activity during the reorganization (Dean *et al*., 1985). Nevertheless, studies pertaining to fat body histology of few lepidopteran moths such as Indian meal moth, *Plodiainter punctella* (Shirk and Malone, 1989), *Heliothis zea* (Haunerland *et al*., 1990) have directed a reconsideration of the above perspective. The above studies have reported some striking differences in the fat body remodeling within the same order i.e., Lepidoptera indicating that this phenomena is not identical and thereby suggesting an independent study for every given insect.This paper documents in detail the changes that lead to the remodeling of the fat body in an important lepidopteran agricultural pest, *Spodoptera litura* F., commonly known as tobacco cutworm, during the postembryonic development. The role of major insect morphogenetic hormone, 20-hydroxy ecdysone (20E) in the process has been evaluated and discussed.

MATERIALS AND METHODS

Spodoptera litura (Noctuidae: Lepidoptera) is a polyphagous pest throughout India on economically important crops like tobacco, castor and groundnut. The insects were reared in a culture room at 70±5% relative humidity, 14:10 light and dark period. The temperature was maintained at 26±1°C. Freshly hatched larvae were fed on castor leaves. After three to four days the larvae were transferred to sterile glass vials and fed on artificial diet (Gupta et al., 2005). The pupae were collected and disinfected with 0.02% formaldehyde and kept in plastic troughs on moist sponge for adult emergence. For the current study early-last, late-last larval instars, pre-pupa, pupa and adult stages were collected (Budatha et al.,2011).

Morphological and histological studies: Morphological changes occurring in the fat body during metamorphosis were visualized under a stereo zoom binocular microscope [Olympus]. For histological studies, the fat body was dissected out in insect Ringer and fixed in Bouin's fluid. Tissue was dehydrated in series of alcohol, cleared in xylene and embedded in paraffin. Paraffin sections of 6µm thickness were cut and stained with Hiedenhain's iron alum hematoxylin eosin stain (Godwin Avwioro, 2011). Histological preparations were analyzed using Zeiss photomicroscope.

DNA extraction and estimation: DNA was extracted from fat body tissue using DNA isolation kit (Qiagen). The quality of the isolated DNA was analyzed by agarose gel electrophoresis and the concentration was estimated using NanoDrop-1000 spectrophotometer (Thermo Scientific Nanodrop 2000).

Thymidine incorporation studies: For autoradiographic studies, early-last instars were

injected with 4µCi of [H^3] thymidine and incubated for different durations. A batch of the injected larvae was incubated till they attained the pre-pupal and adult stages. The anterior and the posterior fat body were dissected out separately and fixed in Carnoy's fixative. Paraffin sections (5µm thickness) were cut and processed for autoradiography using Ilford K2 emulsion. The emulsion coated slides were developed in Kodak D 198 developer and autoradiograms were photographed under Zeiss photomicroscope, using phase optics.

Surgical procedures and hormone treatments: Thorax ligation was carried out using silk suture thread to deplete or reduce the endogenous hormone titer (Dutta-Gupta and Ashok, 1998). The larvae were anesthetized on ice, a loop of the silk thread was made and the position of the loop was adjusted behind the prolegs of the larva and the knot was tightened. The anterior part was cut and the wound was sealed using bee- wax after application of streptomycin sulphate - penicillin mixture (1:1).

Hormone 20E (Sigma, USA) was dissolved in ethanol and then diluted in insect Ringer solution (130 mMNaCl, 0.5 mMKCl, 0.1 mM $CaCl_2$) to obtain a final concentration of 80 nM per insect. The final concentration of ethanol in working 20E solution never exceeded 0.05% in any of the experiments (Arif *et al*., 2004). Experimental insects were thorax ligated 24 h prior to the hormone treatment. Control insects received equal volumes of the carrier.

Analysis of protein content, protein profile and identification of hexamerins: Fat body tissue was dissected from early-last, late-last instar larvae and pre-pupa, homogenized in insect Ringer solution to which cocktail of protease inhibitors was added. Protein content was estimated using Bradford's method (Bradford, 1976) while profiling was done using SDS-PAGE analysis (Laemmli, 1970). Identity of high molecular weight proteins present in the fat body was established using immunoblotting (Towbin *et al*., 1979) with polyclonal antibodies generated against purified larval hexamerins from the hemolymph. The antibody-bound to protein was detected using ALP conjugated anti-rabbit IgG. The visualization of the specific cross-reactivity was carried with BCIP-NBT.

Statistical analysis: All the experiments were repeated thrice and the results were expressed as mean ± SEM of three replicates. Statistical significance between control and treated groups were assessed by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls' post hoc test. Significant changes at $p<0.05$) are indicated.

RESULTS

Fat body reorganization during larval-pupal-adult transition:

a) *Morphological alteration in S. litura fat body during larval-pupal-adult development:*

Microscopic examination of *S. litura* fat body during larval, pre-pupal, pupal and adult stages revealed notable changes in the morphology (Fig. 1a-d). At larval stage, fat body appears as thin ribbon-like sheet being composed of large number of adipocytes (a). During the larval pupal transformation the fat body undergoes significant metamorphic changes, it gradually becomes more compact in pre-pupa (b)

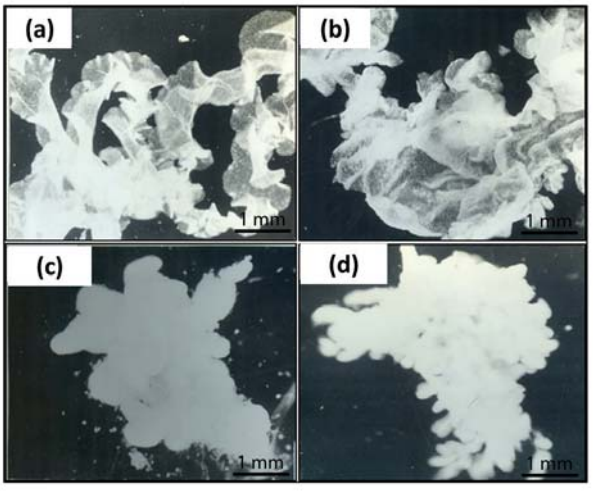

Fig.1. Progressive changes in the fat body morphology during larval-pupal-adult development. (a) Thin ribbon like sheets in late-last instar larvae; (b) Beginning of metamorphic changes where fat body cells appear more dense in pre-pupa; (c) Compact fat body in freshly molted pupa and (d) Reorganized finger lobed fat body in adult.

and it is a fairly dense structure in freshly molted pupa (c). In adult stage, fat body cells are further reorganized into compact finger-like lobular structures (d).

b) Histological changes:

Histological changes in the fat body during the postembryonic development are presented in figure 2. In the late-last larval instar, the fat body is composed of large cuboidal cells commonly known as adipocytes with centrally located nucleus. The cytoplasm consists of large number of lipid vacuoles. Based on the morphometric analysis of the lipid granules, it can be inferred that the macromolecular storage increases during development from the late-last larval instar to the pupal stage. During pre-pupal stage, the cytoplasm of the fat body cells shows accumulation of densely stained membrane bound granules interspersed with lipid vacuoles. The nuclear volume declines and the chromatin appears fairly condensed. In freshly molted pupae, the density of cytoplasmic granules increases markedly and the cell membrane becomes indistinct. In newly emerged adults, the fat body cells undergo extensive re-organization showing prominent nucleus and large number of vacuoles in the cytoplasm however the density of cytoplasmic granules declined.

c) Autoradiographic studies:

For this study, the early-last instar larvae were injected with [H^3]thymidine and incubated for varying time points i.e., 48, 72 h and several days till they attain pre-pupal, and pharate adult stages (Fig. 3). With 48 and 72 h incubation periods, the fat body cells of anterior and the posterior regions show different degree of incorporation of radioactivity in their nuclei. The anterior fat body cell nuclei show a lower level of incorporation of [H^3]-thymidine with 48 h incubation period and the intensity of labeling in the nuclei increases at 72 h (Fig. 3a and 3b). The posterior fat body cell nuclei show intense labeling within 48 h which tends to increase further at 72 h (Fig. 3c and 3d). The amount of radioactivity observed in the posterior fat body cells is much higher as compared to that of anterior fat body. This incorporation of [H^3] thymidine is primarily due to endopolyploidy in fat body which is fairly well known phenomenon in holometabolous insects.

Autoradiograms obtained with larvae incubated for long duration till pre-pupal stage, clearly show a lower degree of dispersed labeling in the anterior as well as posterior fat body cells as compared to the 48 and 72 h time points (Fig. 3e and 3f). It is interesting to note that the radioactivity although

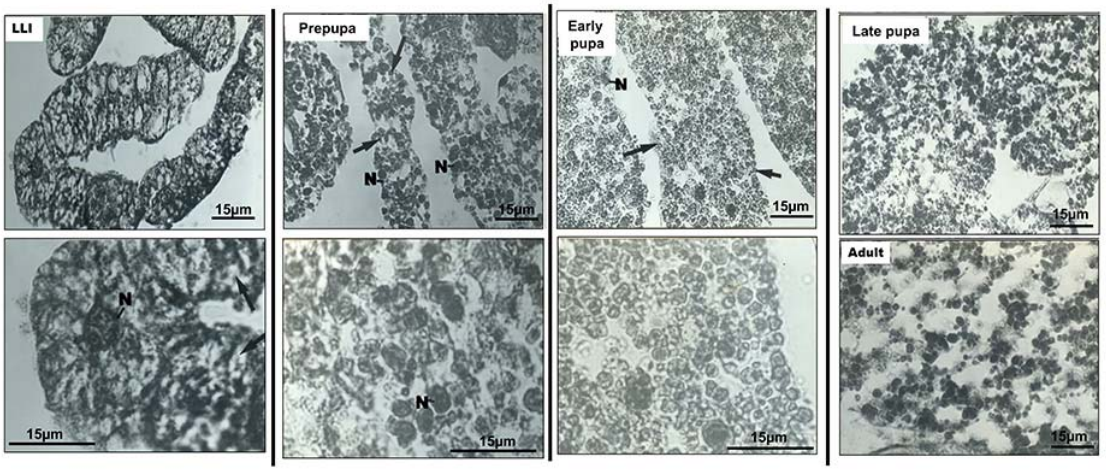

Fig.2. Cytological changes in the fat body during larval-pupal-adult transformation. Large cuboidal cells with fine granular cytoplasm with lipid vacuoles are seen in late-last instar larvae (LLI). Please note the accumulation of densely stained membrane bound granules in pre-pupal fat body. Freshly molted pupal (early pupa) fat body cells show increased density of cytoplasmic granules. Please note the disintegration of cytoplasmic granules at late-pupal (5-6 day old) stage while newly emerged adult fat body cells show large number of vacuoles and few granules in cytoplasm. (N- Nucleus, → membrane bound protein granules)

higher than anterior fat body, it was more diffused in the posterior fat body. The early-last instars injected with [H^3]thymidine and incubated till the pharate adult stage of development once again show localized labeling in the fat body cell nuclei which is once again higher in posterior fat body than anterior fat body (Fig. 3g and 3h).

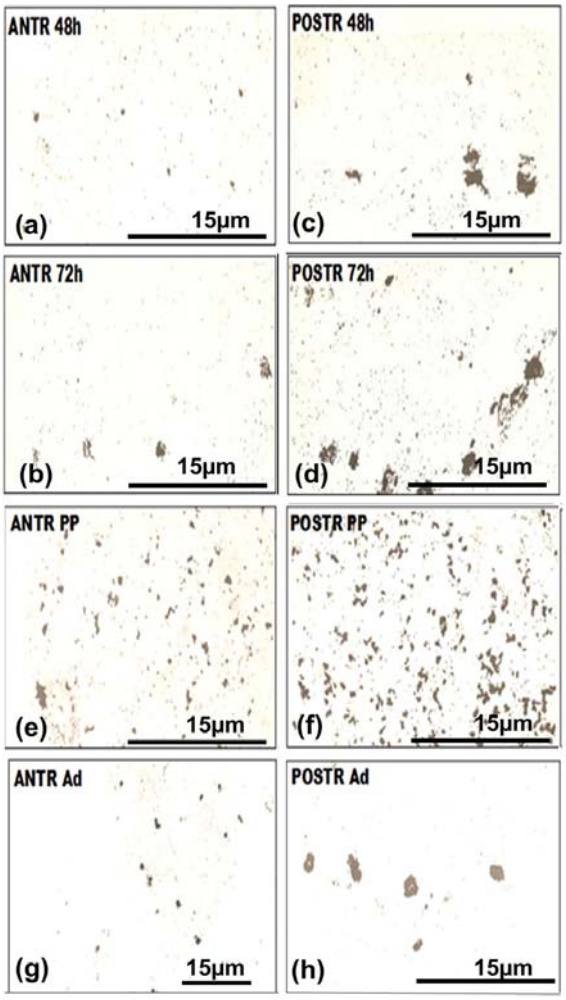

Fig. 3.Autoradiograph showing [H^3] thymidine incorporation in fat body cells of early-last instar larva for various incubation periods. (a) Incubation time 48 hours – anterior fat body cell nuclei show poor incorporation; (b) Incubation time 72 hours – showing moderate incorporation into the anterior fat body cell nuclei (which suggests synthesis of DNA in fat body cells); (c) and (d) Incubation time 48 hours and 72 hours respectively – posterior fat body cell nuclei showing higher degree of incorporation than the anterior fat body; (e) and (f) Incubation time was extended till the attainment of pre-pupal stage. Please note moderate labelling is seen in the anterior fat body cells which is dispersed type (e) as compared to the posteriorfat body where higher but dispersed labelling is observed (f); (g) and (h) Incubation time was extended till pharate adult stage of development is reached. Intense labelling of fat body cell nuclei is noticeable. Anterior: ANTR; Posterior: POSTR; Adult: Ad.

Changes in the DNA content of fat body:

a) C*hanges in the DNA content during postembryonic development:*

For this study, fat body was carefully dissected from 4thinstar larvae till adult. Total DNA content of the fat body was found to be low in the 4th instar which increased significantly in early-last instar larvae and declined thereafter during late-last instar and

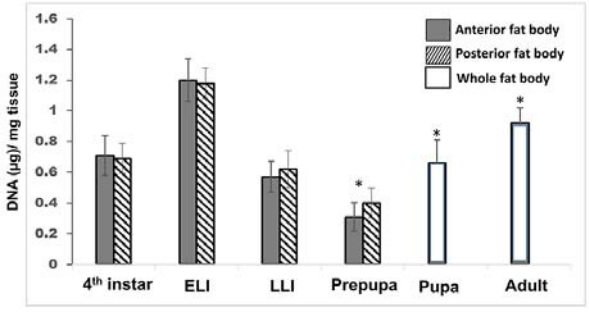

Fig. 4. DNA content in the anterior and posterior fat body during postembryonic development. DNA was isolated from the anterior and posterior fat body of different developmental stages of *S. litura* and estimated. For the pupal and adult stages, it is not possible to differentiate the anterior and posterior regions of fat body. Hence, the whole fat body was used.

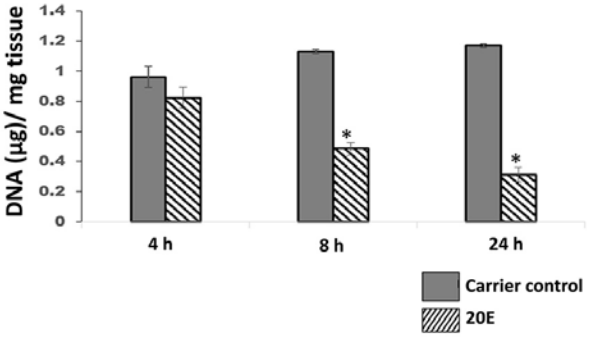

Fig. 5. Effect of 20E on the posterior fat body DNA content of ligated early-last instar larvae. 24h thorax-ligated early-last instars larvae were injected with 20E (1µg/ insect in 10µl of 10% ethanol) and incubated for 24h. The values represent mean ± standard deviation of 4 determinations. For each determination, fat body tissue was pooled from 3 insects. Significance was calculated using Student Newman-Keul's test and values were considered significant at $p<0.05$.

reaching to a fairly low value in the pre-pupal stages which is most likely due to the accumulation of hemolymph proteins in the fat body as well as remodeling seen during the pre-pupal stage and fairly evident from our histological as well as DNA synthesis studies reported above. However, it increased again during pupal development and in freshly emerged adults it was high (Fig.4).

b) ***Effect of 20E on DNA content in the posterior fat body:***

Significant reduction was observed in the DNA content of posterior fat body after 8 h of 20E administration to thorax ligated abdomens and the effect lasted till 24 h post injection (Fig. 5). Please note a moderate but gradual increase in age matched unligated control insects during this period.

Alteration in fat body protein profile and identification of hexamerins:

Results presented in figure 6 show that the fat body protein concentration increased significantly during different developmental stages (Fig. 6a) and SDS profile of proteins revealed presence of large molecular weight proteins in hemolymph of last instar larvae (Fig. 6b, lane 2). Using ammonium sulphate fractionation, hexamerins were partially purified from hemolymph of last-instar larvae of *S. litura* (Fig. 6b, lanes 2-6) and these hexamerins with molecular weight of 82-86 kDa, present in fairly pure form (Fig. 6b, lane 7) were used for the generation of polyclonal antibodies. The antisera showed selective cross reactivity with high molecular weight hexamerins (82-86 kDa) alone present in the hemolymph (Fig. 6c). SDS-PAGE profile of fat body proteins during the early- and late-larval instar and pre-pupal stages clearly show a significant increase in protein content during pre-pupal stages (data not presented). The presence of hexamerins in the fat body was also detected by western blotting (Fig. 6d). It was found to be fairly high in pre-pupal fat body when compared to early-last and late-last instar larval stages which is most likely due to sequestration of hexamerins from hemolymph at this stage and its widely reported phenomenon in various lepidopteran insects.

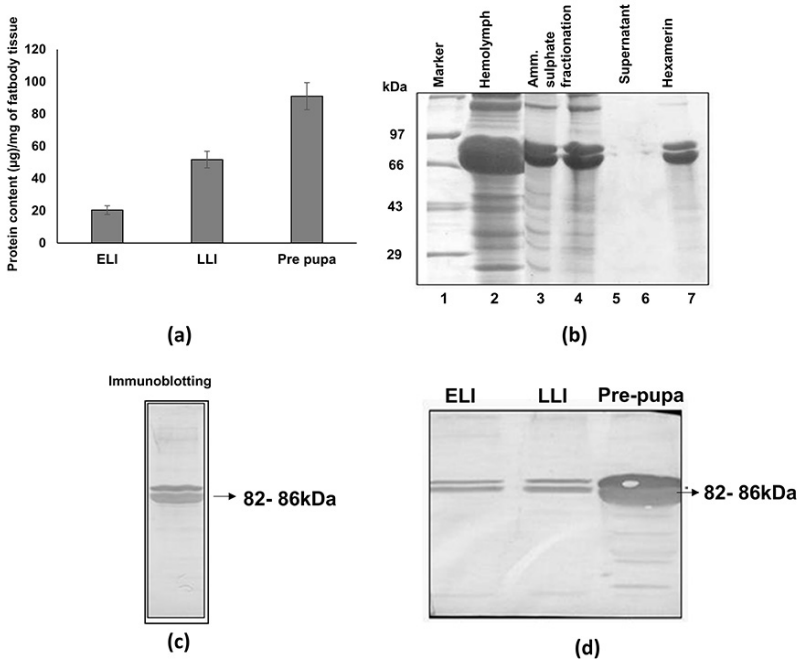

Fig. 6. Hexamerin profile in the fat body tissue during last larval and pre-pupal stages of development. Please note a significant increase in protein content of the fat body tissue from early-last to late-last instar larvae, which further increased during pre-pupal stage (a); partial purification of hexamerins from the hemolymph by ammonium sulphate fractionation (b); western blotting to show the detection of hemolymph hexamerins (c) and immuno-detection of hexamerins in the fat body of early-last (ELI), late-last (LLI) larvae and pre-pupa. Equal quantity of total protein was loaded in all the lanes (d).

DISCUSSION

Present study clearly shows that the fat body undergoes a gradual but significant alteration which is morphological as well as histological during postembryonic development of *S. litura*. The larval fat body appears fairly synthetic which releases proteins and other macromolecules synthesized by it into the hemolymph, thereafter it gradually changes into a dense structure which is primarily a storage tissue. These findings corroborate well with earlier reports of lepidopteran as well as dipteran insects (Levenbook, 1985). This study reveals considerable increase in DNA concentration of the fat body during penultimate to early-last instar larval development which is associated with DNA synthesis as shown by incorporation of [H^3] thymidine. Autoradiographic studies further suggest that this DNA synthesis occurs in the absence of nuclear division and results from polyploidy (Dean *et al*., 1985). Usually in fat body cells, this DNA synthesis often precedes the synthesis of storage proteins (hexamerins) in larval stages of holometabolous insects (Dean *et al*.,1985; Lakshmi and Dutta-Gupta,1990) and vitellogenin synthesis in adult insects (Klowden, 2013) which is stimulated by juvenile hormone (JH) (Ramaswamy *et al*.,1997). Furthermore, present autoradiographic studies show differential degree of polyploidy in anterior and posterior fat body cells, and it is higher in the posterior fat body of *S. litura* larvae. During the late-last larval instar, DNA content of the fat body declines and is most likely due to extensive increase in the protein content seen at this stage (Wang and Haunerland ,1991). During pre-pupal stage not only the DNA content of the fat body declines but one can see the fragmentation of radiolabeled nuclei, which were injected with [H^3] thymidine at early-last instar larval stage.

The onset of wandering behavior during non-feeding stage marks a switch over from larval to pupal program. At this time the JH titer drops and large quantity of ecdysteroids are produced in lepidopteran insects (Bollenbacher, 1988; Nijhout, 1998). Present results suggest that the ecdysteroids promote remodeling of the fat body and stimulate degradation of DNA which was synthesized during larval development. Furthermore, 20E injection to thorax-ligated larvae, which were deprived of endogenous hormone caused substantial decline in the DNA content of the fat body in time dependent manner, suggesting that dissociation and remodeling of the fat body cells is promoted by ecdysteroids. The DNA content of the fat body increases during the pupal development and reaches a high level in adults. Earlier studies showed that the new DNA synthesized during pupal-adult metamorphosis is primarily supported by nucleotides which are released from the larval tissue DNA (Dean *et al*., 1985). Our present autoradiographic studies also support this finding where early-last instar larvae injected with [H^3] thymidine showed incorporation of radiolabel in pupal fat body further suggesting that salvage pathway of DNA synthesis might be operative during pupal development of *S. litura*.

In *S. litura* protein content of the fat body gradually increases during postembryonic development from early-last instar larval stage to pre-pupal stage. The result corroborates well with earlier reports of other lepidopteran insects (Levenbook, 1985; Kiran Kumar *et al*., 1997). Further our electrophoretic studies reveal presence of high molecular weight hexamerin proteins (82-86 kDa) in the fat body. Previous experimental studies from our laboratory in *Corcyra cephalonica* has already demonstrated that arylphorin hexamerin (84kDa) a multifunctional protein, is expressed in tissue specific manner by the larval fat body during postembryonic development and its gene is transcriptionally regulated by 20E (Manohar *et al*., 2010; Venkat Rao *et al*., 2015). However, the hexamerins are known to be synthesized by the fat body and released immediately into the hemolymph during the active feeding phase of insect; hence they do not accumulate in the fat body cells (Burmester, 2002). Interestingly, the non-feeding pre-pupal stage fat body of *S. litura* in the present study not only shows increase in total protein content but also abundance of high molecular weight protein (82-86kDa) which cross-reacted intensely with the polyclonal antibodies generated against purified haemolymph hexamerins. Earlier studies from our as well as other groups have already demonstrated that fat body undergoes functional transition during

larval-pupal development from a synthetic to storage organ. The fat bodies of *C. cephalonica* pre-pupa as well as pupae were shown to actively incorporate remarkable amounts of injected radiolabeled hexamerins from the haemolymph (Ismail and Dutta-Gupta, 1990). Further, this uptake was shown to be mediated by plasma membrane bound receptor (KiranKumar *et al*.,1997), which gets activated under the influence of 20E (Arif *et al*.,2003).

The present study unambiguously demonstrates that the morphological alteration in fat body structure and its compaction during the larval-pupal transformation seen in *S. litura* is associated with the massive sequestration and accumulation of hexamerins in protein granules most likely act as amino acid resources for metamorphosis.

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Life-table of *Odoiporus longicollis* Oliver (Coleoptera: Dryophthoridae) under varying temperature ranges, an in-vitro study

Jithu U. Krishnan*, C. A. Jayaprakas, G. Ajesh, N. R. Lekshmi and D. Sree Sankar

Biopesticide laboratory, Division of Crop Protection, ICAR- Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India; Qingdao Institute of Bioenergy and Bioprocess Technology- Qingdao, Shandong, China. Email: jithuukrishnan@gmail.com

ABSTRACT: The age specific and stage specific life table of *Odoiporous longicollis* when exposed to various temperatures (ranging from 15-42ºC) reveals that there is a strong influence of temperature on the very existence of this weevil. The survivorship exhibit a parallel pattern over all the temperatures delivered and it tend to decline as the age proceeds with no indispensable mortality at any stage or age. The developmental stages such as egg, grub, pupa and the adult up to the age of two months showed highest survivor fraction and lowest apparent mortality, mortality ratio, survival ratio and K-values at lowest temperatures. The study displays its significance to determine the optimum temperature for the laboratory rearing of the pest and ensures zero mortality other than due to aging. A simulation model was also generated based on global temperature to predict the possible locations where the insect can be a major pest.

KEY WORDS: Banana weevil, *Odoiporous longicollis,* thermal response distribution model

INTRODUCTION

Life is described by successive age intervals, the number of deaths, the survivors, the rate of mortality and the expectation of further life. Life table provides an important tool in understanding the aforesaid changes within a population. It is an especially useful approach in entomology, where developmental stages are discrete and mortality rates may vary widely from one life stage to another. It is very useful to analyse the mortality of insect population to determine key factors responsible for the highest mortality within population. The construction of life tables can be used to predict models which can be compared against natural population fluctuations. In pest management, life-table is a most important analytical tool, which provides detailed information on population dynamics and generates simple but informative statistics. Agriculturally important pests demand the knowledge of life table since this can significantly contribute over the pest management strategies. A high index in mortality of a pest is questioned in its life table and this is usually the time when it is most vulnerable due to various stress. By knowing such vulnerable stages from life table, one can make timely application of insecticides for the management of pest. It can also be utilised to conserve the natural parasites and predators and to reduce environmental pollution by adding interactions of the pest with its environment. It is a kind of hardback custody system that ecologists

* Author for correspondence

often used to keep track of stage specific mortality in the population they study.

The banana pseudostem weevil (*Odoiporus longicollis* Oliver) is the most noxious pest of banana (Visalakshi *et al.*, 1989) and is a major issue –'out of control'- for banana farmers. The pest enjoys a tropical distribution throughout the equatorial belt. It spreads either by flying or through infested planting material transport. The apodus, soft, fleshy, white cream coloured grub of this weevil was the infesting stage of the insect. Grubs are voracious feeders. It is estimated that *O. longicollis* causes 10-90% yield losses in banana fields were an active inoculum exist (Padmanaban and Sathiamoorthy, 2001). In the last decade several incidence of *O. longicollis* has been reported from different parts of the world (Mohammad *et al.*, 2010; Palanichamy, 2011; Azad *et al.*, 2012; Khairmode, 2015; Srinivasa *et al.*, 2015). The collection of life table data of insects reared at different temperatures give valuable information that can be used to propose a distribution model.

MATERIALS AND METHODS

Maintenance of insect: Pupae of *O. longicollis* collected from the banana fields of Thiruvananthapuram (8.5488 °N, 76.9173 °E), were maintained in the laboratory at 60 ± 10% RH, 12:12-L: D and 26 ± 1 ºC and they were allowed to moult into adults. On emergence, the adults (20 nos. irrespective of sex) were transferred into rearing bottles (one litre plastic container) and maintain such 10 containers (n = 200). Weevils were timely fed with fresh pieces of pseudostem (50g) in every alternate day. The rearing bottles were provisioned in such a way that the physical parameters set in the BOD incubator (LABLINE-4000 097; Bangalore-India) will have a parallel reflection and no other factors interfere. The optimum humidity range (60 ± 10% RH) and day length (L: D 12:12) was set constant throughout the experiment while varying temperature ranges as 15-18, 18-21, 21-24, 24-27, 27-30, 30-33, 33-36, 36-39 and 39-42 ºC were provided.

Life-table: Instar specific and age specific life table of *O. longicollis* for varying temperature was constructed following Arshad and Parvez (2009) and Kakde *et al.* (2014). After stage based classification of grub, since the longevity of adult is higher, it was categorised into four age groups (1, 2, 3 and 4 month old) to study the life table.

Apparent mortality (q): It is the percentage of death of grub while moulting from one instar to other, and the adult at different ages.

$$q = d / l$$

where -

d = mortality of either the grub or the adult at the particular stage or period

l = number surviving of either the grub or the adult at the beginning of each interval

Survival fraction (Sx): It is the no. of individuals alive in each stage. Data obtained on apparent mortality was used for the calculation of the stage specific survival fraction (Sx) of each stage by using the equation:

Sx of a particular stage = LS/LP (It is always ≤ 1)

where -

L = average survivorship at each class

S = of subsequent stage

P = of particular stage

Mortality Survivor Ratio (MSR): It is the increase in population that would have occurred if the mortality in the stage of interest had not occurred and was calculated as follows:

MSR of particular stage =
[d* in particular stage] / [l of subsequent stage],

where -

d= death in each class

d*= cumulative death in each class

Indispensable mortality (IM): This type of mortality would not be there in case the factor(s) causing it is not allowed to operate. The equation is:

IM = Total individuals observed × MSR of particular stage

RESULTS AND DISCUSSION

Life table for *O. longicollis* was constructed and the following results were achieved for the various temperature ranges trialled. Extreme temperature responses and the median are listed in Table 1. In general, the apparent mortality curve shows that mortality is due to aging (Fig. 1). The 'J' curve warrants low mortality of *O. longicollis* in its active young stages. A noteworthy difference in the life span of *O. longicollis* with varying temperatures was observed. Lowest survival at both high (42ºC) and low (15ºC) extremes of temperature studied were with cent per cent mortality within 2-3 month after emergence (MAE). The Survival fraction curve (Fig. 2) shows the breadth of adaptation that *O. longicollis* could survive under varying ranges of temperatures trialled. Survival was found to decrease through aging. Mortality to Survivor Ratio was maximum in adult insects at 3rd and 4th month and minimum at pupa and adult stage in 1st and 2nd month of growth (Fig. 3). In the indispensable mortality curve the maximum mortality was on the adults on 3rd and 4th month respectively. Apart from the apparent mortality curve, indispensable mortality curve shows that there is indispensable death that occurs during the egg, pupae and in the 3rd MAE adults, that was due to unaffordable temperature ranges (Fig. 4). From the simulation study it was observed that, plantations with a temperature range of 24-30ºC is ideal for the optimum growth and spread of this noxious weevil.

Lu *et al.* (2002) first constructed a life table of *O. longicollis* population in artificial conditions,in his study only the survivorship and mortality was estimated in regular format and all other parameters were done in an exclusion index format for easiness. Current study by regular indexing gives clear idea on all the life table parameters including

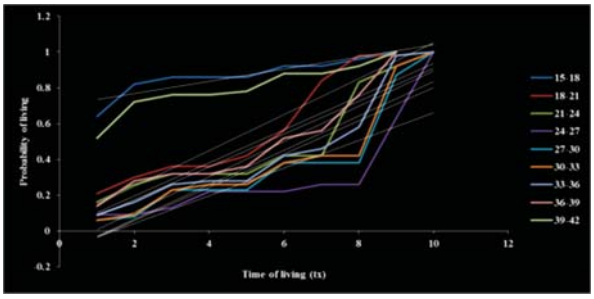

Fig. 1. Apparent mortality (qx) curve of *Odoiporus longicollis* to varying ranges of temperature

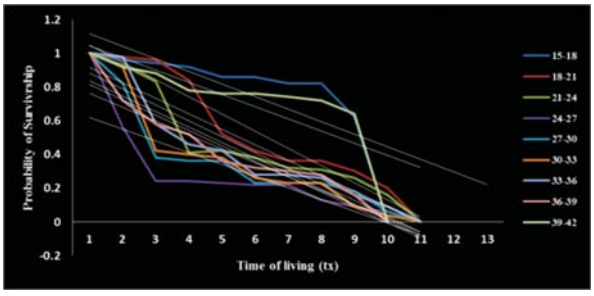

Fig. 2. Survival fraction (Sx) curve of *Odoiporus longicollis*to varying ranges of temperature.

the indispensible mortality which was not addressed by Lu *et al.* (2002). Apparent mortality of *O. longicollis* was on par with the observation documented by Christa and Shelby (2006) in *Acalymma vittatum* (Coleoptera: Chrysomelidae). Dixon and Houseweart (1982); Wittmeyer and Coudron (2001); Christa and Shelby (2006); Ali and Parvez (2009) claim the apparent mortality for a coleopteran pest will be less in the initial instar stages and pupa and this pattern was similarly followed by *O. longicollis*. Life table of *Coccinella transversalis* (Coleoptera: Coccinellidae) by Ali and Parvez (2009) also reported that in the egg stage, the apparent mortality was minimum, and in 4th instar it was high. It was clearly observed that temperature has influence the life span and cycle of *O. longicollis* with difference to apparent mortality over higher and lower ranges.

In this study a survival fraction curve for *O. longicollis* was plotted and observed maximum values for survival at stage 1 (egg) to 7th (Adult, 1 month old). The studies of Dixon and Houseweart (1982); Christa and Shelby (2006) and Ali and

Table 1. Life table of *Odoiporus longicollis*at varying temperature

Stage or age group (x)		Pro. survivorship (lx)			Pro. death (dx)			Pro. Mortality rate (qx)			Pro. Survival fraction (sx)			MSR		
		15-18 ℃	24-27 ℃	39-42 ℃	15-18 ℃	24-27 ℃	39-42 ℃	15-18 ℃	24-27 ℃	39-42 ℃	15-18 ℃	24-27 ℃	39-42 ℃	15-18 ℃	24-27 ℃	39-42 ℃
Egg		1.00	1.00	1.00	0.00	0.00	0.00	0.17	0.09	0.23	1.00	1.00	1.00	0.00	0.00	0.00
1st instar		0.74	0.90	0.65	0.12	0.09	0.17	0.32	0.00	0.32	0.96	0.94	0.93	0.01	0.11	0.01
2nd instar		0.61	0.90	0.52	0.22	0.19	0.36	0.12	0.10	0.14	0.84	0.94	0.82	0.03	0.11	0.02
3rd instar		0.61	0.81	0.48	0.38	0.22	0.42	0.08	0.04	0.34	0.62	0.92	0.54	0.08	0.24	0.06
4th instar		0.51	0.77	0.37	0.36	0.22	0.48	0.02	0.00	0.12	0.56	0.97	0.48	0.18	0.29	0.11
Pupa		0.51	0.77	0.37	0.42	0.29	0.48	0.17	0.08	0.62	0.56	0.95	0.42	0.52	0.30	0.31
Adult	0-1 MAE	0.34	0.71	0.32	0.61	0.00	0.52	0.42	0.00	0.82	0.42	0.95	0.39	1.20	0.40	0.98
	1-2 MAE	0.26	0.71	0.22	0.74	0.18	0.63	0.57	0.18	0.98	0.42	0.90	0.27	1.31	0.45	1.24
	2-3 MAE	0.17	0.58	0.09	1.00	0.63	1.00	1.00	0.63	1.00	0.31	0.61	0.08	2.21	1.06	2.72
	3-4 MAE	0.0	0.21	0.0	NS.	1.00	NS.	NS.	1.00	NS.	NS.	0.26	NS.	NS.	3.12	NS.
	4-5 MAE	0.0	0.00	0.0	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.	NS.

Ave.= Average, Pro.= Probable or Probability, NS. = Non Significant value, MSR= Mortality to Survivor ratio

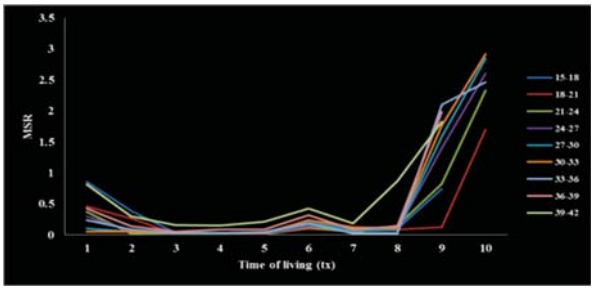

Fig. 3. Mortality to survivorship ratio of *Odoiporus longicollis*to varying ranges of temperature.

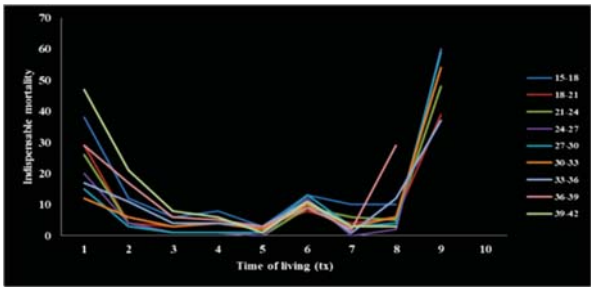

Fig. 4. Indispensable mortality curve of *Odoiporus longicollis*to varying ranges of temperature.

Parvez (2009) revealed most of the coleopteran weevils exhibit similar inverted 'J' shaped survival fraction curve, representing significant level of mortality that happens only by aging. For different temperature range the mortality exhibited a steady increase both to higher and lower ranges.

Mortality survivorship ratio (MSR) was high in adults of the age 3rd and 4th months and this support the survival fraction curve as it is exactly inverse. A similar observation was made by Dixon and Houseweart (1982) in white pine weevil, *Pissodes strobe*, and relates the MSR with survival fraction curve. MSR ratio clearly depicts the major decline in population happens by aging even in the case of higher or lower temperature stress.

By sketching an indispensible mortality curve it is clear that irrespective of sex there was no indispensable mortality for *O. longicollis* at an optimum temperature ranges such as 24-27 ºC and for 27-30 ºC. Aging was the only indispensability as far as *O. longicollis*is concerned in its optimal conditions. According to Ives (1964) "indispensible mortality only due to aging- is a status shown by a

pest, if the insect is from agricultural background". In most of the coleopteran life table significant indispensible mortality can be noticed at the egg stage due to egg viability (Ives, 1964; Dixon and Houseweart, 1982; Wittmeyer and Coudron, 2001; Christa and Shelby, 2006; Ali and Parvez, 2009), microbial infections (Lu *et al.,* 2002), and by many other abiotic futility issues (Kakde *et al.*, 2014), but in *O. longicollis* as the eggs are well protected in the pseudostem, there is no futility and no significant indispensible mortality in its egg stage.

In the current study the temperature ranges that are optimum for the survival and multiplicity of *O. longicollis* was revealed. By analysing the survivorship cure, mortality curve and indispensible mortality curve present study marks *O. longicollis* the status of a major pest. By relating the survivorship possibilities with the temperature ranges the study portraits tropical planes with an average year round temperature of 24-30 ºC with an average high humidity as the best platform for *O. longicollis* to thrive in its pest status. A detailed study regarding the biology of *O. longicollis* with varying physical parameters will be helpful for making simulation models to forecast pest incidence and for its management.

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Insect pollinators, their diversity, foraging behaviour and relative abundance on litchi, okra and sarson

Joginder Singh[1], Neelima R Kumar[2] and Anita Devi[*]

Department of Zoology, Panjab University, Chandigarh 160014, India
Email: anitakadian23@gmail.com

ABSTRACT: The study focused on the importance of the role of insects as pollinators with reference to the fruit crop *Litchi chinensis* Sonn. (Litchi) and the vegetable crops *Abelmoschus esculentus* (L.) Moench (Okra) and *Brassica campestris* (L) var.(Sarson). The studies envisaged the diversity, relative abundance, foraging rate and foraging duration of important pollinators on the target crops. The studies revealed that the diversity of insect pollinators was crop specific. Honey bees were dominating the scene and were the most efficient pollinators of most crops. The exotic honey bee *A. mellifera* outscored the other pollinators where it was present. This could be explained on the basis of domestication and migration of this bee in the field areas. It was also observed that the diversity of insect pollinators on crops studied showed definite decline, when compared to earlier studies.

KEY WORDS: Okra, litchi, sarson, insect pollinators, diversity

INTRODUCTION

It is necessary to enhance the yield of crops under cultivation and also to maintain the diversity of flora and fauna thereby assuring sustainability of agricultural productivity. For this insects are an indispensible component of sustainable agriculture, natural ecosystem balance and a pollution free environment. They provide the best free ecosystem service by way of pollination of our crop plants. The insects and the plants have a mutualistic relationship and have coevolved during the long course of evolution. The beneficial aspects of this association are immense. Pollination by insects is thought to be the main reproductive mechanism in 78% of flowering plants and is essential for maintaining plant genetic diversity. Klein *et al*. (2007) observed that 87 per cent of the leading global food crops were dependent upon animal pollination, while 13 per cent crops did not rely upon animal pollination. Thapa (2006) reported 50 species of insects visiting flowers of 17 different species of selected crops during flowering period. The visiting preferences of insects to flowers of different crops differed among the crop species and insect species as well. To increase food production the yield per unit area under cultivation has to increase. Pollinators and beekeeping are a very important bio input which can contribute greatly in this direction (Singh and Kumar, 2009; Kumar, 2002; Kumar and Kumar, 2000; Verma *et al*., 2002). A consistent pollination service is one of the key factors supporting agricultural production but land use and flowering practices also have substantial impact on pollinators.

The insect visitors of a variety of crop plants have been studied and the role of individual species

* Author for correspondence

emphasized in some instances (Free, 1993; Sihag, 1991; Kumar and Kumar, 1997a, b, 1998). Honey bees are efficient pollinators because of modification of their body parts and their behavior like hairy bodies that readily pick up pollen grains and corbiculate, legs vegetarian diet, flower visiting habits and visit to many flowers of the same species during a single trip thus affecting pollination (Delaplane *et al.,* 2000; Partap, 2003; Bhalchandra *et al*., 2014). Heard (1999) reported that in the tropics, stingless bees (Apidae: Meliponini) were the effective pollinators of several crop species and contributed to the pollination of others. Evidence is still lacking for many plant species. Although a large amount of research has been devoted to test the ability of a few non *Apis* bees as pollinators of commercially important crops (Richards, 1993, 1995a, b; Rahman and Chopra, 1994; Cane *et al*., 1996), data are inconclusive to effectively support the adoption of a series of non *Apis* pollinators in many areas of agriculture.

MATERIALS AND METHODS

Studies on *Litchi chinensis* Sonn. (Litchi) were done in the month of March-April, at Pinjore Garden, Chandigarh. *Abelmoschus esculentus* (L.) Moench (Okra) was studied in field/grooves at village Tasoli near Chandigarh in the months of June-July and studies on *Brassica campestris* L. var. Sarson were conducted during the full blooming period of crops *i.e* in the month of February-March, at village Togan near Chandigarh. For all above said crops observations were taken three times in a week for a period of five weeks

The insects visiting the flowers of the crop under study were collected by sweeping a hand net. Collections were made during the blossoming period of crop/trees every two hours between 9:00 to 5:00 hrs; few visitors observed on the bloom at any other time of the day were also captured. Collected insects were killed in a glass bottle fumigated with ethyl acetate. These were stretched on a thermocol sheet, dried and preserved in insect cabinets. The preserved insects were identified by comparison with reference collection in the entomology laboratory of the Department of Zoology, Panjab University, Chandigarh, with the help of taxonomic keys and were also got identified by taxonomists in the parent department and in the Zoology Department of Punjabi University, Patiala.

The following parameters were considered for making observations:

Pollinators' diversity was observed as the number of different species of insects visiting the crop. The insects on a particular crop were caught with a sweep net as described above.

Relative abundance from five randomly selected areas of 1mx1m size was taken in case of field crops and 5 equal sized branches in case of fruit crops. The number of insects of each species visiting the flower were recorded for 5 minutes in the selected areas and observations were taken three times in a day between 09:00-11:00hrs, 12:00-02:00hrs, 3:00-5:00hrs during the full bloom of the crop.

Foraging behaviour was assessed by recording Foraging rate and Foraging duration. Foraging rate was determined by recording the number of flowers visited per minute by each type of insect. Observations were recorded between 09:00-11:00 h, 12:00-02:00 h, 3:00-5:00 h and were repeated five times during each interval. Foraging duration as the time spent by each insect species on one flower (in seconds) was recorded with the help of a stopwatch. Observations were recorded three times a day viz., 09:00-11:00 h, 12:00-02:00 h, 3:00-5:00 h and repeated five times during each period.

Data pertaining to relative abundance, foraging rate, foraging duration were statistically analysed using factorial randomized block design.

RESULTS AND DISCUSSION

The litchi fruit crop, *Litchi chinensis* Sonn, is a medium sized, round topped, evergreen subtropical tree bearing pendent clusters of rosy pink fruits. The aromatic succulent flesh around the seed forms the relished edible part. India is now second largest producer of litchi being next only to China. The

plant bears three types of flowers male, female and bisexual. The flowers require transfer of pollen by insects. The inflorescence was observed to be visited by nine species of insects. The little honey bee *Apis florea* was the most abundant pollinator (6.26/m of branch/5min.). Scelionid bee (3.93/m of branch/5min.) *Episyrphus balteatus* (3.2/m of branch/5min.) and *A. cerana* (1.53/m of branch/5min.) were the other important visitors observed during the present investigation. *Pieris canidia* and *Coccinella septumpunctata* were infrequent visitors (Table 1 and 2). It was observed that *Episyrphus balteatus* visited maximum number of flowers per minute (11.93±0.42) followed closely by the native honey bees. It was interesting to note that the European honey bee showed relatively less number of visits (9.86± 0.50) as compared to the native honey bees (Table 3). Time spent per flower was also highest in case of *Episyrphus* (Table 4).

Table 1. Diversity of insect pollinators on *Litchi chinensis* Sonn. (Litchi)

S. No.	Name of Insect	Order	Family
1.	*Episyrphus balteatus*	Diptera	Syrphidae
2.	*Apis florea*	Hymenoptera	Apidae
3.	*Apis cerana*	Hymenoptera	Apidae
4.	*Pieris canidia*	Lepidoptera	Pieridae
5.	*C. septumpunctata*	Coleoptera	Coccinellidae
6.	*Scelionid bee*	Hymenoptera	Scelionidae
7.	*Apis. Mellifera*	Hymenoptera	Apidae
8.	*Apis dorsata*	Hymenoptera	Apidae
9.	*Eristalis sp.*	Diptera	Syrphidae

Abelmoschus esculentus (L.) Moench, Okra (Bhindi) is grown throughout the tropical and warm temperature regions of the world for its fibrous pods full of seeds, which when picked young are eaten as vegetables. Results of investigations carried out on *Abelmoschus esculentus* showed that the crop was visited by ten species of insects (Table 5 and 6). There are very few reports available on the pollination requirements and pollinators of Okra. The data available suggested that though the flowers were self fertile, there was improvement in seed and fruit set as a result of cross pollination by insects (Njoya *et al*., 2005). Sharma (2004) in his studies conducted in Himachal Pradesh observed *Ceratina sexmaculatus*, *Megachile* sp., *Xylocopa* sp and *Bombus* sp. to be foraging on Okra bloom. Njoya *et al*. (2005) have, however, reported that though *Xylocopa* visited Okra bloom, it did not contribute to pollination. High foraging rates were exhibited by *A. cerana* (16.0 flowers/min) and *Papilio demoleus* (15.73 flowers/ min) during the present study (Table 7). These species were therefore important for the pollination of Okra. *Megachile* sp. and *Halictus* sp. were rated as efficient pollinators by Njoya *et al*. (2005). The native honey

Table 2. Relative abundance (number of insects/m^2/5min.) of pollinators on Litchi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Episyrphus balteatus*	7.2±7.98	0.8±0.84	1.6±2.61	3.2±3.49	1.663
2	*Apis florea*	5.4±4.67	11.0±7.04	2.4±1.52	6.26±4.37	3.254
3	*Apis cerana*	2.2±1.48	1.6±1.14	0.8±1.79	1.53±0.70	0.795
4	*Pieris canidia*	0.8±1.10	0.2±0.45	0.2±0.45	0.4±0.35	0.207
5	*Coccinella septumpunctata*	2.0±1.41	0.2±0.45	0.00±0.00	0.73±1.10	0.379
6	*Scelionid bee*	5.0±2.55	3.6±2.19	3.2±3.03	3.93±0.95	2.043
7	*Apis. mellifera*	0.2±0.45	0.4±0.55	0.00±0.00	0.2±0.20	0.103
8	*Apis dorsata*	0.2±0.45	1.0±1.41	1.2±1.30	0.8±0.53	0.415
9	*Eristalis sp.*	0.00±0.00	0.60±0.89	0.2±0.45	0.26±0.31	0.135
Mean		2.55	2.15	1.06	1.92	

F ($p \leq 0.001$) for number of insects: Significant and F ($p \leq 0.001$) for day hours: Significant

Table 3. Foraging rate (number of flowers visited/minute) of pollinators on Litchi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Episyrphus balteatus*	11.8±2.28	12.4±3.91	11.6±1.82	11.93±0.42	1.303
2	*Apis florea*	11.8±5.22	11.0±2.12	10.2±1.64	11.0±0.80	1.201
3	*Apis cerana*	11.4±3.44	12.4±1.67	11.0±3.16	11.6±0.72	1.267
4	*Pieris canidia*	7.0±3.74	5.4±1.67	10.6±4.62	7.66±2.66	0.836
5	*Coccinella septumpunctata*	1.0±0.00	1.2±0.45	1.2±0.45	1.13±0.12	0.123
6	*Scelionid bee*	6.8±2.95	7.2±1.10	8.0±1.22	7.33±0.61	0.800
7	*Apis. mellifera*	9.8±2.17	10.4±2.79	9.4±2.51	9.86±0.50	1.077
8	*Apis dorsata*	14.8±2.17	10.4±5.57	10.2±5.26	11.66±2.72	1.274
9	*Eristalis sp.*	11.2±1.64	10.0±1.73	9.4±1.14	10.20±0.92	1.114
	Mean	9.51	8.89	9.07	9.16	

Table 4. Foraging Duration (time spent in seconds/flower) of pollinators on Litchi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Episyrphus balteatus*	19.4±11.92	13.0±3.54	21.0±9.30	17.80±4.23	42.694
2	*Apis florea*	5.0±2.74	2.4±0.55	3.6±1.82	3.66±1.30	207.639
3	*Apis cerana*	6.6±2.97	5.0±2.55	7.4±1.14	6.33±1.22	120
4	*Pieris canidia*	8.0±4.95	11.4±2.61	11.6±6.35	10.33±2.02	73.568
5	*Coccinella septumpunctata*	14.8±8.41	22.4±9.02	13.8±6.72	17.00±4.70	44.703
6	*Scelionid bee*	6.8±5.54	6.4±2.88	10.0±5.43	7.73±1.97	98.313
7	*Apis. mellifera*	4.2±2.17	5.8±1.30	9.0±2.12	6.33±2.44	120.050
8	*Apis dorsata*	14.0±5.15	10.2±4.97	10.4±6.43	11.53±2.14	65.911
9	*Eristalis sp.*	3.6±2.07	2.4±1.14	5.2±2.59	3.73±1.40	203.742
	Mean	9.16	8.78	10.22	9.39	

Table 5. Diversity of insect pollinators on Okra/Bhindi

S. No.	Name of Insect	Order	Family
1.	*Eristalis* sp.	Diptera	Syrphidae
2.	*Pieris canidia*	Lepidoptera	Pieridae
3.	*Papilio demoleus*	Lepidoptera	Papilionidae
4.	*R. flavolineatum*	Hymenoptera	Eumenidae
5.	*Polistes hebraeus*	Hymenoptera	Vespidae
6.	*Apis dorsata*	Hymenoptera	Apidae
7.	*Apis cerana*	Hymenoptera	Apidae
8.	*Apis florea*	Hymenoptera	Apidae
9.	*Apis. Mellifera*	Hymenoptera	Apidae
10.	*Megachile* sp.	Hymenoptera	Megachilidae

bee species spent highest time per visit on Okra bloom (Table 8).

Brassica campestris L. var. Sarson is a typical winter season crop of the sub tropical to temperate regions. It is cultivated for its seeds that yield oil and leaves that are used as vegetable. It is a major source of nectar for honey bees. Reports on the pollinator diversity of *Brassica* in India are well spread over a long period of time and provide valuable information on insect pollinators decline particularly under the changed agro forest scenario following advent of *A. mellifera* (Singh and Kumar, 2003; Singh and Kumar, 2007). During the present studies on pollinating species of *Brassica campestris*, the crop was observed to be visited by

Table 6. Relative abundance (number of insects/m^2/5min.) of pollinators on Okra/Bhindi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		0900-1100	1200-1400	1500-1700		
1	*Eristalis* sp.	1.2±2.17	1.0±1.41	0.8±0.84	1.0±0.20	1.420
2	*Pieris canidia*	0.4±0.55	0.4±0.89	0.2±0.45	0.33±0.12	0.468
3	*Papilio demoleus*	1.2±1.30	0.2±0.45	0.2±0.45	0.53±0.58	0.752
4	*R. flavolineatum*	0.8±1.30	0.4±0.89	0.6±1.34	0.6±0.20	0.852
5	*Polistes hebraeus*	2.8±1.92	1.0±1.73	0.6±0.89	1.46±1.17	2.073
6	*Apis dorsata*	0.4±0.89	1.2±1.30	0.4±0.55	0.66±0.46	0.937
7	*Apis cerana*	0.6±1.34	1.4±1.67	1.0±1.73	1.0±0.40	1.420
8	*Apis florea*	0.2±0.45	0.2±0.45	1.6±1.14	0.66±0.81	0.937
9	*A.mellifera*	0.2±0.45	0.4±0.89	0.6±0.89	0.40±0.20	0.537
10	*Megachile* sp.	0.4 ±0.89	0.4±0.89	0.4±0.89	0.40±0.00	0.568
	Mean	0.82	0.66	0.64	0.70	

F (p=0.222) for number of insects: insignificant, F (p=0.270) for day hours: insignificant, PSs- Performance Score=Nij/Nj x S

Table 7. Foraging rate (number of flowers visited/minute) of pollinators on Okra/Bhindi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		0900-1100	1200-1400	1500-1700		
1	*Eristalis* sp.	4.4±2.07	4.0±1.22	7.0±2.24	5.13±1.63	0.603
2	*Pieris canidia*	14.0±6.67	17.4±1.95	15.4±6.88	15.60±1.71	1.835
3	*Papilio demoleus*	19.4±1.34	10.8±3.35	17.0±6.32	15.73±4.44	1.851
4	*R. flavolineatum*	3.4±1.14	4.2±1.92	4.2±1.92	3.93±0.46	0.462
5	*Polistes hebraeus*	6.0±2.45	6.4±2.70	4.4±2.30	5.60±1.06	0.659
6	*Apis dorsata*	4.8±3.11	5.4±2.79	6.8±3.42	5.66±1.03	0.666
7	*Apis cerana*	12.6±5.27	18.6±2.61	16.8±2.28	16.0±3.08	1.883
8	*Apis florea*	5.2±2.95	5.0±2.24	7.0±2.65	5.73±1.10	0.674
9	*A.mellifera*	10.0±3.61	8.4±4.51	9.6±3.21	9.33±0.83	1.098
10	*Megachile* sp.	2.0±1.73	2.6±2.07	2.2±1.30	2.26±0.31	0.265
	Mean	8.18	8.28	9.04	8.49	

Table 8. Foraging duration (time spent in seconds/flower) of pollinators on Okra/Bhindi

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		0900-1100	1200-1400	1500-1700		
1	*Eristalis* sp.	11.8±7.40	13.2±5.07	7.8±6.02	10.93±2.80	78.956
2	*Pieris canidia*	8.4±5.94	8.4±4.16	6.8±4.55	7.86±0.92	109.796
3	*Papilio demoleus*	2.4±1.67	4.6±3.03	2.0±1.00	3.00±1.40	287.666
4	*R. flavolineatum*	10.0±3.81	4.8±2.39	6.4±3.65	7.06±4.81	122.237
5	*Polistes hebraeus*	8.2±2.86	5.8±2.49	8.2±2.86	7.40±1.39	116.621
6	*Apis dorsata*	12.8±5.17	13.2±4.32	7.0±5.10	11.00±3.47	78.454
7	*Apis cerana*	9.8±6.65	8.4±4.34	16.0±7.71	11.40±4.04	75.701
8	*Apis florea*	11.6±6.23	10.4±1.67	11.8±2.86	11.26±0.76	76.642
9	*A.mellifera*	13.8±7.01	9.0±3.87	15.2±4.15	12.66±3.25	68.167
10	*Megachile* sp.	2.4±1.14	3.0±2.92	5.8±2.28	3.73±1.81	231.367
	Mean	9.12	8.08	8.70	8.63	

eight species of insects (Table 9). It is important to note that *A. mellifera* outnumbered all other species during the present study and was higher in abundance (6.4 bees/m^2/5 min.) as compared to the native honey bees (1.80, 1.66 and 1.80 bees/m^2/5min for *A. dorsata*, *A. cerana* and *A. florea* respectively) (Table 10). Similar observations were made by Kumar and Kumar. (1998) on related toria crop. In their studies *A. mellifera* predominated the wild bees and made 58.94% of total visits, whereas *A. ilerda*, *H. catullus*, one solitary bee and *H. spendidulus* constituted 20.40, 11.92, 4.88 and 3.80% of total bees respectively (Kumar and Kumar. 1998). Wild bees were conspicuous by their absence during the present studies while dipterans were present.

Balachandran *et al*. (2014) observed that *Apis dorsata* had highest visitations on *Utricularias*

Table 9. Diversity of pollinators on *Brassica campestris*

S. No.	Name of Insect	Order	Family
1.	*Apis dorsata*	Hymenoptera	Apidae
2.	*Apis cerana*	Hymenoptera	Apidae
3.	*Apis florea*	Hymenoptera	Apidae
4.	*Apis. Mellifera*	Hymenoptera	Apidae
5.	*Eristalis sp.*	Diptera	Syrphidae
6.	*Episyrphus balteatus*	Diptera	Syrphidae
7.	*Pieris canidia*	Lepidoptera	Pieridae
8.	*Junonia almanac*	Lepidoptera	Nymphalidae

Table 10. Relative Abundance (Number of insects/m^2/5min.) of pollinators on Sarson

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Apis dorsata*	3.8±1.64	0.2±0.45	1.4±0.89	1.80±1.86	1.168
2	*Apis cerana*	2.8±1.10	1.8±1.30	0.4±0.89	1.66±1.48	1.077
3	*Apis florea*	2.2±1.30	0.00±0.0	0.2±0.45	0.80±1.26	0.519
4	*Apis. mellifera*	0.4±0.8	9.2±2.79	9.6±7.86	6.4±6.66	4.155
5	*Eristalis sp.*	0.4±0.5	0.00±0.0	0.2±0.45	0.20±0.41	0.129
6	*Episyrphus balteatus*	0.4±0.89	1.2±1.1	1.0±1.22	0.86±1.06	0.558
7	*Pieris canidia*	0.4±0.55	0.8±0.84	0.00±0.00	0.40±0.63	0.259
8	*Junonia almana*	0.4±0.8	0.00±0.0	0.2±0.4	0.20±0.56	0.129
	Mean	1.35	1.65	1.62	1.54	

F ($p \leq 0.001$) number of insects : significant, F ($p \leq 0.001$) for day hours : significant

Table 11. Foraging rate (Number of flowers visited/minute) of pollinators on Sarson

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Apis dorsata*	20.8±2.68	14.2±1.30	14.8±2.17	16.6±3.66	1.679
2	*Apis cerana*	19.2±2.28	14.8±2.68	14.6±2.61	16.2±3.21	1.634
3	*Apis florea*	2.6±1.52	2.8±1.10	3.6±2.88	3.0±1.88	0.303
4	*Apis. mellifera*	14.2±1.79	12.8±1.92	14.4±2.30	13.8±2.00	1.396
5	*Eristalis sp.*	13.8±2.49	9.6±7.44	13.4±3.78	12.26±5.04	0.1240
6	*Episyrphus balteatus*	2.0±0.71	2.0±0.71	2.0±0.71	2.0±0.65	0.202
7	*Pieris canidia*	7.4±4.10	12.0±1.00	10.2±1.30	9.86±3.06	0.997
8	*Junonia almana*	5.0±5.79	4.4±2.88	6.6±4.88	5.33±4.43	0.539
	Mean	10.62	9.07	9.95	9.88	

F ($p \leq 0.001$) number of insects : significant, F ($p \leq 0.001$) for day hours : significant

Table 12. Foraging duration (Time spent in seconds/flower) of pollinators on Sarson

Sl No.	Name of insect	Time of observation In hours			Grand mean	(PSs) = Nij/NjxS
		9-11AM	12-02PM	3-5PM		
1	*Apis dorsata*	2.6±1.52	4.2±3.35	1.8±0.84	2.86±2.26	251.132
2	*Apis cerana*	6.4±7.70	10.6±6.43	7.4±7.80	8.13±7.03	88.344
3	*Apis florea*	20.4±14.26	17.0±4.47	46.0±15.97	27.8±17.78	25.835
4	*Apis. mellifera*	1.4±0.55	2.2±0.84	1.8±0.84	1.8±0.77	399.022
5	*Eristalis sp.*	2.4±0.55	2.2±1.64	9.8±1.48	4.8±3.85	149.633
6	*Episyrphus balteatus*	40.2±13.33	30.4±16.12	44.2±18.95	38.2±17.15	18.772
7	*Pieris canidia*	3.8±1.79	2.4±0.89	6.8±7.46	4.33±4.54	165.875
8	*Junonia almana*	1.2±0.45	2.2±2.17	2.0±1.73	1.8±1.56	399.022
Mean		9.8	8.9	14.97	11.22	

F ($p \leq 0.001$) number of insects : significant, F ($p \leq 0.001$) for day hours : significant

i*mpatiens* and *Flacourtia indica*, whereas *Trigona* preferred *Eriocaulons* especially in the absence of *A. mellifera.* A significant finding during the present studies was that the native honey bee *A. dorsata* and *A. cerana* were better performer than *A. mellifera* with respect to foraging rate (Table 11and 12). Further *A. dorsata* and *A. cerana* are cold hardy (Verma *et al.* 2002) and were therefore observed to become active on these winter season flowers earlier in the day (9:00-11:00hrs) as compared to *A. mellifera* which started foraging comparatively later (12:00-2:00 hrs). However the exotic honey bee *A. mellifera* outscored the native bees in pollinating efficiency on the basis of abundance.

The area around Chandigarh particularly, the *Brassica* fields are extensively exploited for honey harvesting by bee keepers who migrate *A. mellifera* colonies to the plains for this purpose. This accounts for the high population of *A. mellifera* observed in this crop. Similar trend is also available in the studies of Kumar and Kumar. (1997a). According to them *A. mellifera* was the most abundant visitor to toria bloom in the mid hills. Based on pollination indices, they reported *A. mellifera* followed by *A. ilerida* to be the most efficient pollinator on toria bloom.

The studies revealed that the diversity of insect pollinators was crop specific. Honey bees were dominating the scene and were the most efficient pollinators of most crops. The exotic honey bee *A. mellifera* outscored the other pollinators where it was present. This could be explained on the basis of domestication and migration of this bee in the field areas. It was also observed that the diversity of insect pollinators on crops studied showed definite decline, when compared to earlier studies.

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Population dynamics of two species of leafhoppers of the genus *Empoasca* Walsh, 1862 (Hemiptera:Auchenorhyncha: Cicadellidae) on soybean in Rajasthan and their morphological characterization

A. K. Meena, R. Nagar, R. Swaminathan* and C. A. Viraktamath[1]

Department of Entomology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan313001 India; [1]ICAR Project on Insect Biosystematics, GKVK, Bengaluru, Karnataka, India.
E mail: udaiswami57@gmail.com; akmeenaentomology@gmail.com

ABSTRACT: Population dynamics of jassids infesting soybean studies in Rajasthan, India revealed that the jassids comprised of two species in the genus *Empoasca* [*Empoasca terminalis* Distant and *Empoasca spirosa* Dworakowska & Viraktamath]. The highest mean population of jassids was recorded during last week of August in 2015 (43.50 jassids/5 plants) that evinced a significant positive correlation with the mean atmospheric temperature (r = 0.58). During *kharif* 2016, the maximum population was recorded in the third week of September (30.50 jassids/5 plants) that exhibited a significant positive correlation with the mean atmospheric temperature (r = 0.62), but significant negative correlation with the mean relative humidity (r = - 0.71). Morphological characterization of the two species of *Empoasca* is given, besides reporting the occurrence of both leafhoppers for the first time on soybean from Rajasthan. A key to distinguish these two species has also been presented.

KEY WORDS: Soybean, *Empoasca terminalis, E. spirosa*

INTRODUCTION

Soybean is a major oilseed crop in India and is grown in the states of Madhya Pradesh, Maharashtra, Karnataka, Uttar Pradesh, Rajasthan, Tamil Nadu, Andhra Pradesh and Uttarakhand. About 275 insect species have been recorded infesting soybean in India; among these, defoliators and sap– sucking insects are the major constraints to soybean production (Raju *et al*., 2013). Among the sap feeders, jassids cause considerable damage. The members of the family Cicadellidae, commonly known as leafhoppers, cicadellids or jassids contain more than 22,600 described species (Dietrich, 2004). The fundamental features that define the family Cicadellidae are that these are small wedge shaped insects, distinguished by the presence of one or more rows of spines extending the length of hind tibiae. The tribe Empoascini under the subfamily Typhlocybinae comprises 88 described genera and 1300 described species and is well represented in the temperate and tropical regions worldwide (Yang Liu *et al*., 2014). These insects lack cross-veins in the subapical region of the fore wings and the longitudinal veins are usually indistinct in the basal region, hindwing with all longitudinal

* Author for correspondence

veins ending at the submarginal vein and the submarginal vein reaching but not exceeding the vein R+MP (Zhang, 1990).

The genus *Empoasca* is one of the most speciuse and economically important genus of the family Cicadellidae (Southern and Dietrich, 2010). It was established by Walsh in 1862 and currently comprises about 400 species grouped in 11 subgenera (Oman *et al*., 1990). Several species of *Empoasca* are relevant pests to agricultural crops such as potato, alfalfa, beans, citrus or grapes (Baspinar, 1994; Lamp *et al*., 1994, 2011; Egwurube *et al*., 2005; Kaplan *et al*., 2008; Naseri *et al*., 2009). As defended by Poos and Wheeler (1943), information on the identity, distribution, and host plants are of great significance to outline appropriate control measures against those species that act as pests. They occur on all types of vegetation and usually feed on the leaves. They inflict direct damage by sucking sap, causing stippling, cupping, puckering and bronzing of the leaves which ultimately fall off. The indirect damage is caused by transmitting the pathogens of various mycoplasmal/viral diseases of plants. Population dynamics of two species of jassids belonging to the genus *Empoasca* as pests of soybean was studied with their morphological characters, and male genitalia for identifying up to species level.

MATERIALS AND METHODS

A field experiment was undertaken at the Instructional Farm, Rajasthan College of Agriculture, MPUAT, Udaipur during *kharif*, 2015 and 2016. Soybean JS-335, the variety recommended for the zone, was sown in plots of size 4m x 3m maintaining 30 cm row to row and 10 cm plant to plant spacing and replicated six times. Population of jassids was recorded from five randomly selected and tagged plants in each replication by Vortis Suction Sampler. All the observations were taken during early hours of the day (6 to 8 am) at weekly intervals. The prevailing abiotic conditions of the atmosphere were recorded from the meteorological observatory of the farm to work out the correlation coefficients between the pest populations and the abiotic factors of the environment as per standard methodology (Gomez and Gomez, 1984).

The morphological terminology given by Dietrich (2005) was followed to describe the morphology of leafhoppers. For mounting and preparation of male genitalia slides the procedure suggested by Knight (1965) was followed. The abdomen was detached from the thoracic region under the stereozoom binocular microscope with the help of sharp micro needles (minute) by pressing at the junction of thorax and abdomen. The detached abdomen was then transferred with the help of camel hair brush carefully to the cavity block containing a few milliliters of freshly prepared 10 per cent KOH and kept them over night at room temperature to facilitate digestion of soft tissues. The period varies depending upon the specimen whether freshly collected or old and also if the leafhopper was starved or well fed at the time of death. The abdomen was removed from KOH solution and transferred to a glass cavity dish containing distilled water and with the help of a pair of blunt needles the digested soft tissues were gently pressed out. After repeated washings in distilled water the abdomen was transferred to a glass slide containing one or two drops of glycerin for genitalia dissection, which was made under Stereozoom Binocular Microscope. The above said treatment facilitates the entire abdomen to become completely transparent and permitted the study of genitalia. All slide preparations were examined under the stereozoom binocular microscope. Digital photographs of specimens and their body parts were taken with the help of Stemi 2000 C Stereozoom Binoculars of Carl Zeiss make. The software installed in the binoculars used for linear measurements was Axio Vision L.E. 4.8; besides, the graph paper method was also employed. The line diagram of both species of *Empoasca* depicting male genitalia as given by Ramakrishna (1980) has been adapted with some modifications in the structure as evident in the species collected by us. The terminology used for studying the characters of the leafhoppers was as per suggestions given by Evans (1947), Kramer (1950) and Blocker and Triplehorn (1985) for describing different parts of the body.

RESULTS AND DISCUSSION

Population dynamics of jassids in soybean

Incidence of jassids on soybean initiated on 26th July and continued up to 11th October during the first year in *kharif* 2015; while in 2016, the infestation was delayed and commenced from 14th August that continued up to 9th October. The population gradually reached the peak on 30th August in 1st year with a mean population of 43.50 jassids/5 plants, when the mean atmospheric temperature was 27.10 °C, mean relative humidity 69.79 per cent and no rainfall was recorded. During *kharif* 2016 the peak period of infestation was recorded on 18th September with a mean population of 30.50 jassids/5 plants, when the mean atmospheric temperature was 27.32 °C, mean relative humidity 62.86 per cent without rainfall. The population of jassids showed a significant positive correlation (r = 0.58) with the mean atmospheric temperature in 2015; whereas, it evinced a non significant correlation with relative humidity and total rainfall. Likewise, mean population evinced a significant positive correlation (r = 0.62) with the mean atmospheric temperature and significant negative correlation with relative humidity (r = -0.71) in 2016.

Earlier, among the sucking insects, whitefly (*Bemisia tabaci* Gennedius) and jassid (*Empoasca kerri* Pruthi) were reported as the key sucking pest of the crop. Their population was observed maximum (13.70/plant) at 28 ^{0}C temperature having a negative correlation with rainfall, morning and evening temperature; while, sunshine influenced the pest population positively. In case of jassid negative correlation was noted with rainy days and maximum temperature and a positive correlation with minimum temperature and sunshine (Alam and Patidar, 2014). Netam *et al.* (2013) studied five insect species, viz., Girdle beetle, *Obereopsis brevis*; tobacco caterpillar, *Spodoptera litura*; green semilooper, *Chrysodeixis acuta*; jassids, *E. kerri*; and white flies, *B.tabaci* were recorded as the major pests on soybean variety JS 93-05 causing damage at various stages of the crop. All these insects made their first appearance on the crop to a greater or lesser extent in the last week of July. The peak density of sucking pests was observed during third week of September with 4.4 sucking pests/plant and seasonal mean of 3.62 white flies and jassids per plant. Sutaria *et al.* (2010) studied the impact of different weather factors on the pest incidence and found no significant correlation of weather parameter with the activity of *E. kerri* in soybean. Although positive correlation was observed between the pest population and the minimum temperature, morning and evening relative humidity and sunshine hours, while the maximum temperature, rain and rainy days were negatively correlated.

Species of jassids

Two species of jassids belonging to the genus *Empoasca*, namely, *E. (Distantasca) terminalis* and *E. (Empoasca) spirosa* were observed.

1. *Empoasca* (*Distantasca*) *terminalis* Distant, 1918

Empoasca terminalis Distant, 1918, Fauna Brit. Ind., 7:92

Distantasca terminalis (Distant). Dworakowska, 1972, Bull. Acad. Polon. Sci.,Ser. Sci. Biol., 20 (1) : 25

Empoasca (D.) terminalis (Distant). Dworakowska and Viraktamath, 1975, Bull.Acad. Polon. Sci. Ser.Biol., 23 (8): 529

Earlier, Nasruddin *et al*. (2014) observed *E. terminalis* infestation in all planting seasons of soybean crop that often occurred two weeks after the plant emergence. The leafhopper abundance (*E. terminalis*) has been reported as a soybean pest in India that was negatively correlated with rainfall (Parsai and Tiwari, 2002). It has been reported as minor pest on sesame, groundnut (Biswas and Das, 2011), mungbean (Chhabra *et al*. 1981) and green gram (Gatoria and Singh, 1984). Incidence of *E. terminalis* was observed throughout the year on different pulse crops. The incidence gradually increased from May to August

reaching a peak during October and declining subsequently at Bangalore, Karnataka (Ramakrishna, 1980). This species was also collected from Andhra Pradesh, India on geranium, sweet potato, frenchbean, greengram, redgram and rice by Ramu (2006).

2. *Empoasca* (*Empoasca*) *spirosa* Dworakowska and Viraktamath, 1979

Empoasca spinosa Dworakowska & Sohi, 1978b, Bull. Acad. Pol. Sci. Cl. II. Ser. Sci. BioI., 26 (7): 463-471.

Empoasca (Empoasca) spirosa Dworakowska & Viraktamath, 1979a, Bull. Acad. Pol. Sci. Cl. II.

Ser. Sci. BioI., 23 (8): 521-530.

The incidence of *E. spirosa* was observed on different crops (Okra, Bittergourd, Clusterbean, Cowpea, Pumpkin, Palak, Ridge gourd and Vegetable crops) by Bhandhavi (2010) and soybean, bottlegourd, bittergourd, cowpea and geranium (Ramu, 2006), groundnut, sunflower, castor, niger, mustard, greengram, blackgram and redgram (Ramasubharao *et al*., 2006) from Andhra Pradesh, India. Similarly, the incidence of *E spinosa* Dworakowska and Sohi was also reported on fenugreek from Junagadh, Gujarat, India (Joshi *et al*., 2009). Both the species of jassids were earlier reported on pulse crops in India by Ramakrishna (1980).

From the present observation it could be concluded that both the species of genus *Empoasca,* namely, *E. terminalis* and *E. spirosa* are major pests of soybean in Udaipur zone, Rajasthan. The maximum incidence was recorded during August and September in both years. The information regarding seasonal incidence of jassids and their identity will help the farmers to identify the pest and take up suitable management measures to reduce the losses caused by the pest.

Table: 1 Seasonal incidence of jassids in soybean during *kharif* season

2015					2016				
Date of observations	Mean		Total Rainfall (mm)	Mean Jassids/ 5Plants	Date of observations	Mean		Total Rainfall (mm)	Mean Jassids/ 5Plants
	Atm. Temp (°C)	RH (%)				Atm. Temp (°C)	RH (%)		
26/07/2015	27.84	80.50	58.40	16.25	24/07/2016	27.26	73.50	5.40	0.75
02/08/2015	24.26	86.86	233.80	1.50	31/07/2016	26.75	84.36	199.80	3.00
09/08/2015	27.11	71.07	0.00	27.75	07/08/2016	25.96	89.43	84..4	4.25
16/08/2015	27.26	83.07	98.60	13.25	14/08/2016	25.12	89.29	102.10	2.00
23/08/2015	26.93	75.43	6.80	16.25	21/08/2016	26.30	73.07	1.80	5.75
30/08/2015	27.10	69.79	0.00	43.50	28/08/2016	25.28	87.57	60.00	5.00
06/09/2015	26.98	67.57	0.00	37.25	04/09/2016	26.91	78.29	14.40	7.50
13/09/2015	26.74	61.14	0.00	29.00	11/09/2016	25.94	67.50	0.00	9.25
20/09/2015	29.76	60.79	24.60	35.00	18/09/2016	27.32	62.86	0.00	30.50
27/09/2015	24.84	76.86	17.00	12.75	25/09/2016	28.79	65.64	5.40	26.25
04/10/2015	25.94	48.36	0.00	9.25	02/10/2016	28.81	57.57	0.00	15.75
11/10/2015	26.91	44.79	0.00	7.75	09/10/2016	26.96	76.86	62.40	4.25
Coefficient of correlation (r) between population and Atm. Temp.				**0.58***					**0.62***
Coefficient of correlation (r) between population and RH				**-0.11**					**-0.71***
Coefficient of correlation (r) between population and Total Rainfall				**-0.53**					**-0.46**

*Significant at 5 per cent level of significance

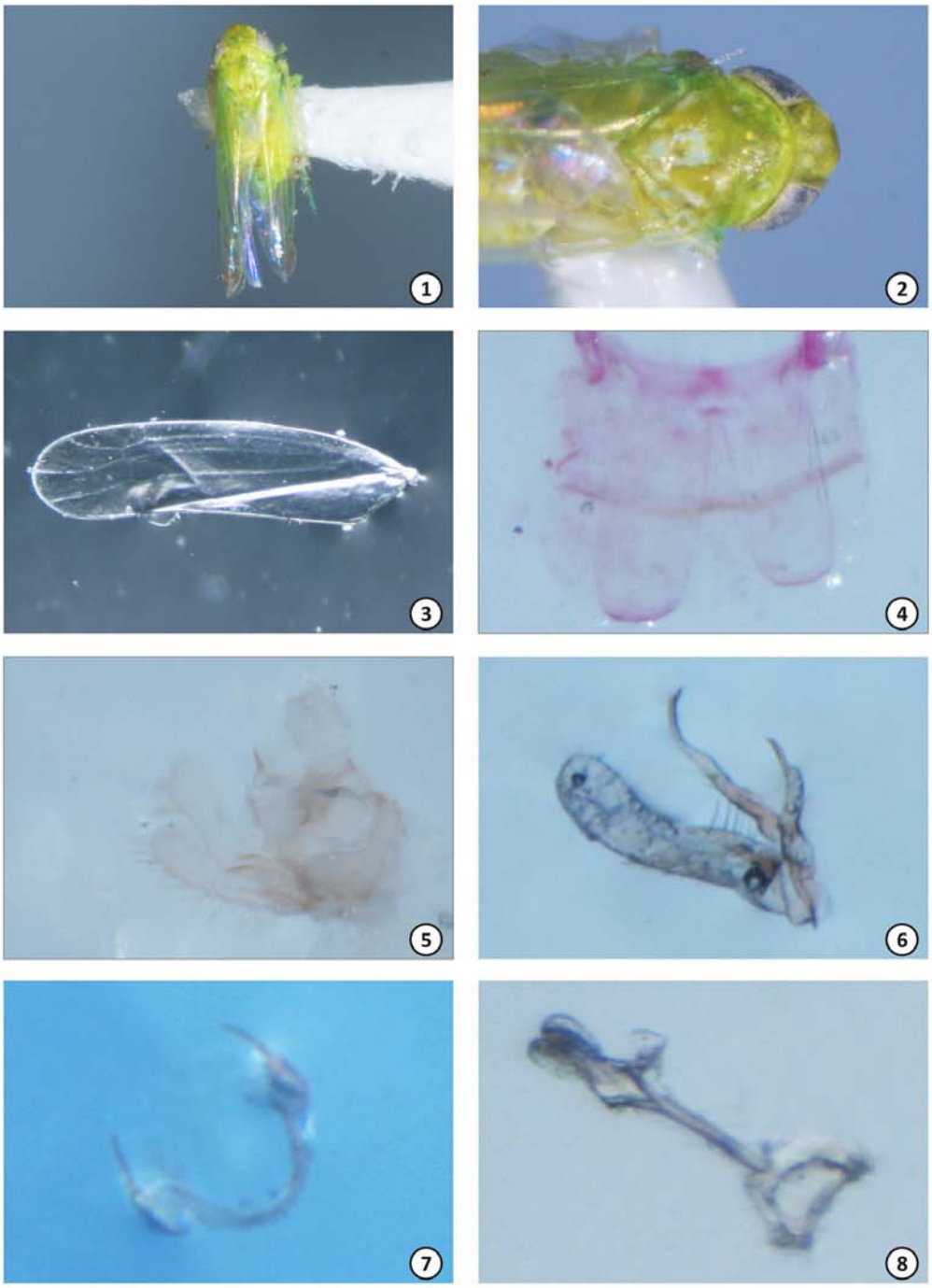

Plate I: Morphological characterization of *Empoasca* (*Empoasca*) *spirosa* Dworakowska & Viraktamath, 1979 (Male); 1-8: 1. Adult, dorsal view; 2. Head and thorax, dorsal view; 3. Forewing; 4. Abdominal apodemes; 5. Genitalia, right lateral view; 6. Subgenital plate with style and pygofer process; 7. Anal tube beak; 8. Aedeagus, dorsal view with Connective.

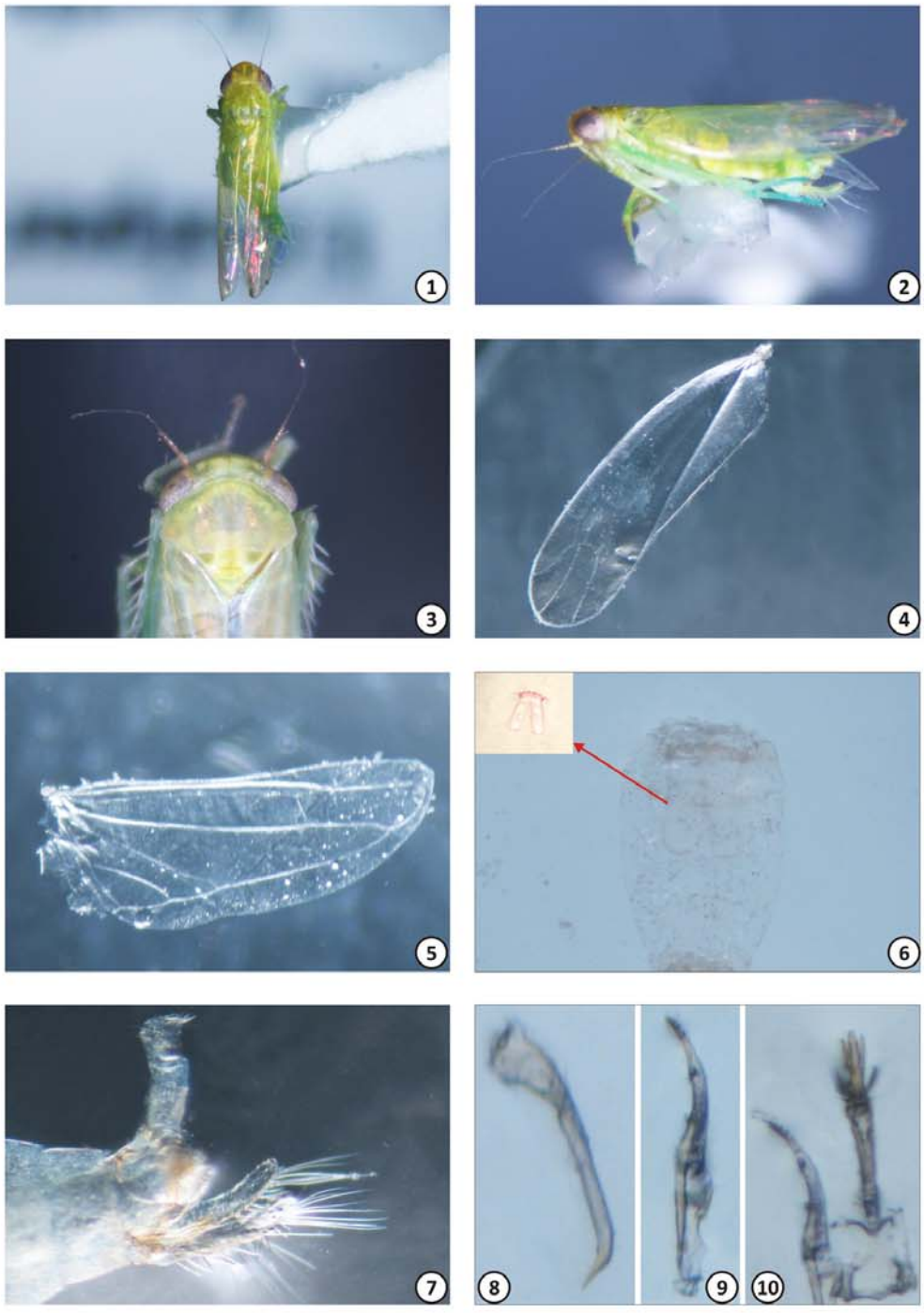

Plate II: Morphological characterization of *Empoasca* (*Distantasca*) *terminalis* Distant, 1918 (Male); 1-10: 1-2. Adult, dorsal and lateral view; 3. Head and thorax, dorsal view; 4. Forewing; 5. Hindwing; 6. Abdominal apodemes; 7. Genitalia, left lateral view with subgenital plate; 8. Style; 9. Pygofer process; 10. Aedeagus, dorsal view with connective.

Key to species of *Empoasca* Walsh, 1862

1. Subgenital plates elongated, with macro and micro setae present submarginally ; aedeagus without apical processes ..... *E. spirosa*

- Subgenital plates broad at the base, with numerous macro setae and hairs , aedeagus with two pairs of apical processes… …*E. terminalis*

Morphological characterization of the jassid species:

(1) *Empoasca (Empoasca) spirosa* Dworakowska and Viraktamath (Plate- I and Fig: 1-9)

Material Examined (30 ♂♂): India: Rajasthan, Udaipur; 10.IX.2015, Coll. A. K. Meena (RCA, Udaipur) (4); 25.IX.2015, Coll. A. K. Meena (RCA, Udaipur) (7); 15.VIII.2016, Coll. A. K. Meena (RCA, Udaipur) (1); 18.IX.2016, Coll. A. K. Meena (RCA, Udaipur) (14).

External morphology

Pale yellowish green in colour; head (0.79-0.82 mm) slightly wider than pronotum (0.74-0.78 mm); vertex subacute with distinct coronal suture. Ocelli are conspicuous and are close to the eyes. Pronotum wider than its length. Forewings light green colour with four apical cells, anteapical cells and appendix are absent. Hind wings hyaline. Abdominal apodemes well developed.

Male genitalia

Pygofer lobe longer with a few micro setae and its processes elongated, broad at base and narrowed towards apex, short tooth subapically and serrated at apex. Anal tube hook beak like apically. Subgenital plates elongated, with macro and micro setae submarginally and also hairs like setae basally. Genital styles broader basally tapering to pointed apex which is serrated apically. Connective trapezoidal, without arms, with a median notch at the apex. Aedeagal shaft tubular, broad in the middle and apex, with proximal end rod-like.

Measurements: The total length including fore wings 2.62-2.70 mm, width across the compound eyes 0.57-0.60 mm.

(2) *Empoasca* (*Distantasca*) *terminalis* Distant (Plate- II and Fig: 10-16)

Material Examined (84 ♂♂): India: Rajasthan, Udaipur; 27.VIII.2015, Coll. A. K. Meena (RCA, Udaipur) (18); 05.IX.2015, Coll. A. K. Meena (RCA, Udaipur) (29); 08.VIII.2016, Coll. A. K. Meena (RCA, Udaipur) (24); 15.VIII.2016, Coll. A. K. Meena (RCA, Udaipur) (15).

External Morphology

Yellowish green in colour; vertex shorter than broad between eyes, deeply sulcate in proximal region.

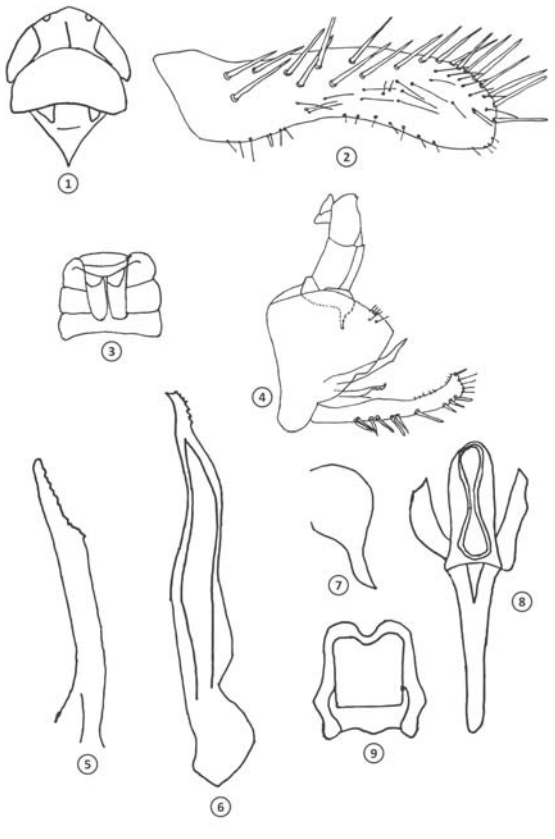

Figure: Line diagrams depicting morphological characters of *Empoasca (Empoasca) spirosa* Dworakowska and Viraktamath; Male 1-9: 1. Head and thorax, dorsal view; 2. Subgenital plate; 3. Abdominal apodemes; 4. Genitalia, right lateral view; 4. Style; 6. Pygofer process; 7. Anal tube beak; 8. Aedeagus, dorsal view; 9. Connective.

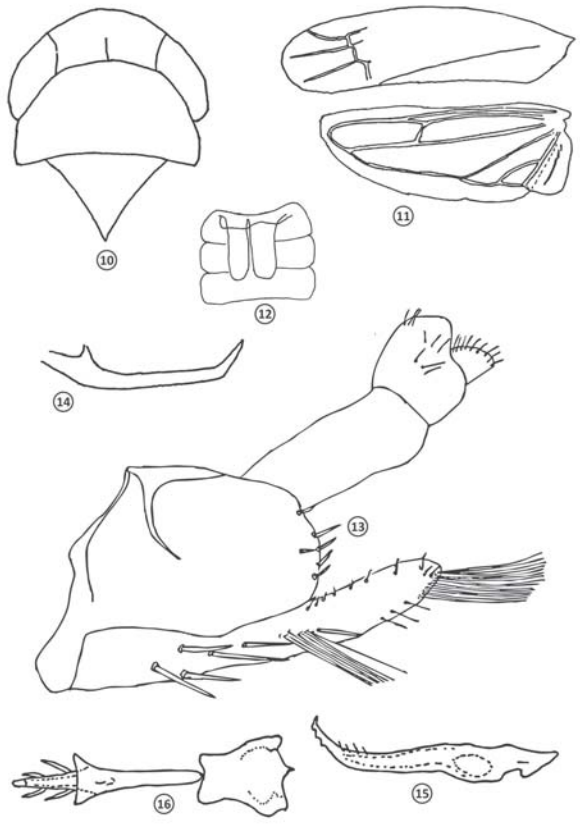

Figure: Line diagrams depicting morphological characters of *Empoasca (Distantasca) terminalis* Distant; Male; 10-16: 10. Head and thorax, dorsal view; 11. Forewing and hindwing; 12. Abdominal apodemes; 13. Genitalia, left laterals view with subgenital plate; 14. Style; 15. Pygofer process; 16. Aedeagus, dorsal view with connective.

Ocelli are large and distinct. Pronotum longer than vertex. Forewings are light pale green, shining and transparent. Abdominal apodemes well developed which are broad and elongated.

Male genitalia

Pygofer longer with a few micro setae and its process elongated slightly curved and pointed at apex. Subgenital plates are elongate, broad at the base, gradually narrowing and tapering at apex, numerous macro setae and hairs are present. Styles are slender, dentate and pointed at apex. Aedeagus narrower at base and broader at apex with two pairs of processes.

Measurements: The total length including fore wings 3.03-3.16 mm, width across the compound eyes 0.64-0.67 mm.

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Effect of change in mean monthly temperature and pH on the larvae of *Aedes triseriatus* Say, 1823 (Diptera: Culicidae) from North 24 Parganas of West Bengal

Rhitayu Chakraborti and Probir Kumar Bandyopadhyay*

Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, India.
Email: prabir0432@hotmail.com

ABSTRACT: *Aedes triseriatus* Say, 1823 commonly called the Eastern Tree Hole mosquito is the vector of La Crosse virus. Its larval density is highest in spring – early summer. Environmental parameters such as temperature and pH affect the life cycle of mosquitoes. Temperature affects every stage of the life cycle of *Aedes* sp. Effect of changes in the mean monthly temperature (MMT) and pH of the larval habitat on the larval count of *A. triseriatus* in North 24 Parganas district of West Bengal, India studies found that the larval count varied significantly with MMT (p value = 0.003) but not with pH (p value = 0.445). The maximal larval count was obtained in the temperature range of 27°C and 36°C with the highest at 33°C. The pH range of 6.65 to 7.05 supported a high larval count with the maximum count obtained at a pH of 7.05. © 2017 Association for Advancement of Entomology

KEY WORDS: *Aedes triseriatus*, life cycle, larval count, mean monthly temperature, pH

INTRODUCTION

Mosquitoes are one of the significant vectors of parasites and pathogens which have a devastating impact on human beings (Gajanana *et al*., 1997). A large portion of the world's population is greatly affected by mosquito borne diseases which are prevalent in more than hundred countries, being mostly prevalent in the tropical ones. Mosquitoes serve as vectors of malaria, yellow fever, dengue fever, chikungunya fever, filariasis and encephalitis. *Aedes triseriatus* or *Ochlerotatus triseriatus* Say, 1823 (Diptera: Culicidae) commonly called the Eastern Tree Hole mosquito or "Tris" is an invasive mosquito species which has been reported for the first time in India. The mosquitoes are terrestrial and are commonly found in forest regions where the canopies can be as high as 27m (Obenauer *et al*., 2009). The range of flight is limited to around 200m (Turell *et al*., 2005). Tree holes, tyres, artificial containers (Borucki *et al*., 2002) etc. serve as perfect breeding sites for its larvae in the urban areas. *A. triseriatus* is a known vector of La Crosse virus in North America which is the most common cause of paediatric arboviral encephalitis in U.S.A with 42 to 172 cases reported annually (Borucki *et al*., 2002). Apart from this, studies have shown *A. triseriatus* to be a competent vector of West Nile virus experimentally (Styer *et al*., 2007) and of Venezuelan equine encephalitis (Davis *et al*., 1966), Eastern equine encephalitis, Western equine encephalitis, Dengue (type I), St Louis encephalitis virus and Yellow Fever virus under laboratory conditions (Freier and Grimstad, 1983).

* Author for correspondence

Temperature affects the developmental stages of the life cycle of *Aedes* sp. The length of developmental stages has been found to be inversely proportional to enhancement of temperature with the ambient temperature for completion of the life cycle ranging between 20°C and 36°C (Marinho *et al*., 2016). Mortality is higher in environments with high nutrient concentration at 35°C (Farjana *et al*., 2012). Mosquito larvae can survive in a wide range of pH, much greater than those tolerated by other aquatic animals. There is no evidence that pH limits the survival of larvae in nature (Clark *et al*., 2004). The reported pH values for larval habitats range from 3.3 to 8.1 for *Ochlerotatus taeniorhynchus*, 4.4–9.3 for *A. geniculatus*, 3.3–9.2 for *Psorophora confinnis*, and 4.4–9.3 for *Anopheles plumbeus*. *A. flavopictus* has been reared in pH ranging from 2–9 and *Armigeres subalbatus* in the pH range of 2–10 in the laboratory (Clark *et al*., 2004). Thus a study on the effect of change in temperature and pH on the larval counts of important genera of mosquitoes is of great interest at the moment in India from the point of view of development of effective vector-control programme.

The work embodied in this paper probes the effect of changes in the mean monthly temperature (MMT) and pH of the larval habitat on the larval count of *A. triseriatus* in the North 24 Parganas district of West Bengal during the period June, 2015 and June, 2017.

MATERIALS AND METHODS

Mosquito larvae were collected from abandoned tyres, artificial containers and tree holes by immersing clean sampling bottles of 50ml capacity and brought to the Parasitology laboratory of the Department of Zoology, University of Kalyani, Kalyani,West Bengal, India for identification. A total of three samples were collected every month during the period June, 2015 and June, 2017.

Determination of mean monthly temperature (MMT) and pH: Temperatures were recorded using a thermometer on each day of the month. MMT (°C) was calculated as the average of the daily maximum temperatures of the month. Similarly, the average of the daily minimum temperatures of the month yielded the mean monthly minimum temperature. MMT was calculated as the average of the mean monthly maximum temperature and mean monthly minimum temperature. The pH of the water was measured during sample collection using a portable pH meter. The averages of the pH values measured each month during sampling have been used as the final pH values in this study.

Identification of larvae and determination of larval count: The mosquito larvae were identified by studying their body parts under the 10X objective of a phase contrast microscope (Olympus Corporation, Model : KH) following the work of Farajollahi and Price (Farajollahi and Price, 2013). The larval count per sample was ascertained.

Mean larval count (M), standard deviation (SD) and standard error of mean (SE) were calculated using the Graph Pad software (http://graphpad.com/quickcalcs/CImean1/).

Identification of adults: The larvae were reared at 26±2°C in a photoperiod of 12h light and 12h dark on a diet comprising of yeast extract and finely ground dog biscuits in the ratio 1:3 to obtain adults. The adults were identified following the adult pictorial key designed by the Crans and Reed of the Center for Vector Biology, School of Environmental and Biological Sciences, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901-8536 (http://vectorbio.rutgers.edu/Adult_Pictorial_Key.pdf).

Determination of optimal MMT and pH for maximal larval count: The data (Table 1) was organized into six continuous temperature classes and four continuous pH classes. For determining the optimal MMT and pH for maximal larval count, larval counts were plotted against the temperatures and pH values corresponding to the class marks (mean of the upper class limit and lower class limit) of the respective classes (Table 2 and Table 3). The larval counts were expressed as the sum of mean larval count (M) of the class and standard error of mean (SE) (M±SE). The data set corresponding to MMT

= 21°C was omitted from the plots as the mean larval count was zero in this case.

Statistical analysis: A one-way ANOVA was performed to determine whether larval count significantly varied with temperature and pH. Test of homogeneity of variances was performed using the Levene's test. Data analysis was performed using the SPSS software (version 19).

RESULTS AND DISCUSSION

Identification of larvae and adults: The features of the larval body parts were compared with the specimen studied by Farajollahi and Price in 2013 for identification. The specimen under study is a larva of *A. triseriatus* based on the larval body parts (Table 4, Fig. 1). The adults were identified using the adult pictorial key designed by Crans and Reed (http://vectorbio.rutgers.edu/Adult Pictorial Key.pdf). The current specimens are adults of *A. triseriatus* (Fig. 2).

Determination of optimal MMT and pH for maximal larval count: The data obtained during the sampling period (Table 1) was organized into six continuous temperature classes and four continuous pH classes for statistical analysis using SPSS. For determining

Table 1. Showing the Mean Monthly Temperature (MMT) (°C), pH, mean larval count (M), standard deviation (SD) and standard error of mean (SE) as obtained during the period of sampling

Period	MMT (°C)	pH	M	SD	SE
Jun'2015	33.5	6.9	76.33	6.03	3.48
Jul'2015	31.5	7.7	80	5	2.89
Aug'2015	31.5	6.7	79.67	2.52	1.45
Sep'2015	32	7.17	85.67	4.04	2.33
Oct'2015	30.5	8	72.33	8.74	5.04
Nov'2015	29	7.1	71	3.61	2.08
Dec'2015	26	7.4	68.33	3.51	2.03
Jan'2016	25	8	60	11.14	6.43
Feb'2016	29.5	6.7	77.33	6.81	3.93
Mar'2016	32.5	8	94	10.39	6
Apr'2016	36	8	79	5.29	3.06
May'2016	34.5	7.5	80.67	7.51	4.33
Jun'2016	34	7.3	81.67	3.51	2.03
Jul'2016	32	7.7	77.33	6.81	3.93
Aug'2016	31	7	86.67	7.64	4.41
Sep'2016	31	7.8	81.33	4.04	2.33
Oct'2016	29.5	7.62	76.33	5.13	2.96
Nov'2016	26.5	6.9	75.67	4.04	2.33
Dec'2016	25.5	7.37	73.33	12.22	7.06
Jan'2017	20	6.8	52.67	4.62	2.67
Feb'2017	24	7.3	55	10.44	6.03
Mar'2017	27	6.7	88.33	12.58	7.26
Apr'2017	31	7	88.33	2.89	1.67
May'2017	32	7.1	93.33	11.55	6.67
June'2017	31	7.5	87.33	2.52	1.45

Table 2. Showing the temperature classes, class mark, larval count, mean larval count (M), standard deviation (SD) and standard error of mean (SE)

Temperature classes (°C)	Class mark (°C)	Larval count	Mean larval count (M)	Standard Deviation (SD)	Standard Error of Mean (SE)
19.5 – 22.5	21	52.67±2.67	0	0	0
22.5 – 25.5	24	55±6.03 60±6.43	57.5	3.54	2.5
25.5 – 28.5	27	88.33±7.266 8.33±2.03 75.67±2.33 73.33±7.06	76.415	8.512	4.256
28.5 – 31.5	30	88.33±1.67 87.33±1.45 72.33±5.04 71±2.08 77.33±3.93 86.67±4.41 81.33±2.33 76.33±2.96	80.081	6.863	2.426
31.5 – 34.5	33	93.33±6.67 76.33±3.48 80±2.89 79.67±1.45 85.67±2.33 94±6 81.67±2.03 77.33±3.93	83.5	6.879	2.432
34.5 – 37.5	36	79±3.06 80.67±4.33	79.835	1.18	0.835

the optimal MMT and pH for maximal larval count, larval counts (mean larval count of the class (M) ± standard error of mean (SE)) were plotted against the temperatures and pH values corresponding to the class marks (mean of the upper class limit and lower class limit) of the respective classes (Table 2 and Table 3). MMT ranging between 27°C and 36°C showed a high larval count (M±SE) with the highest larval count (M±SE) at 33°C (Fig. 3). The larval count (M±SE) was high in a pH ranging between 6.65 and 7.05 with the maximum number of larvae surviving in the environment whose pH was neutral i.e. 7.05 (Fig. 3).

Levene's test of homogeneity of variances for both temperature and pH signified that the variances among the different classes of temperature and pH were homogeneous. The p values for the Levene's test for temperature and pH were 0.293 and 0.85 respectively. It became evident from one way ANOVA that the larval count varied significantly with the MMT (p value = 0.003) but, not with pH (p value = 0.445).

The study probed the effect of the two environmental parameters namely, mean monthly temperature and pH on the larval count of *A. triseriatus* collected from North 24 Parganas district of West Bengal, India. The district of North 24 Parganas in West Bengal, India covers an area of 4094 km^2 and spans between the coordinates; 22.6168°N and 88.4029°E and has a tropical wet and dry climate. The MMT (values corresponding to the class marks of temperature classes) in North

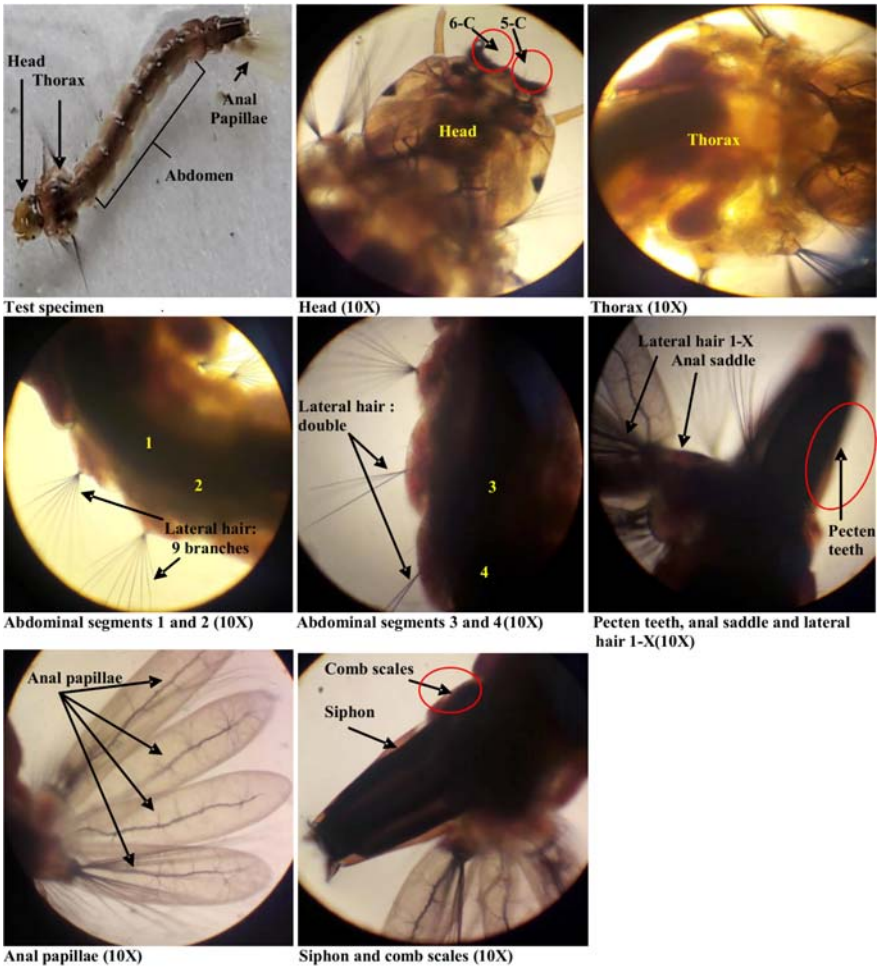

Fig. 1 Showing the body parts of the larval specimen under study

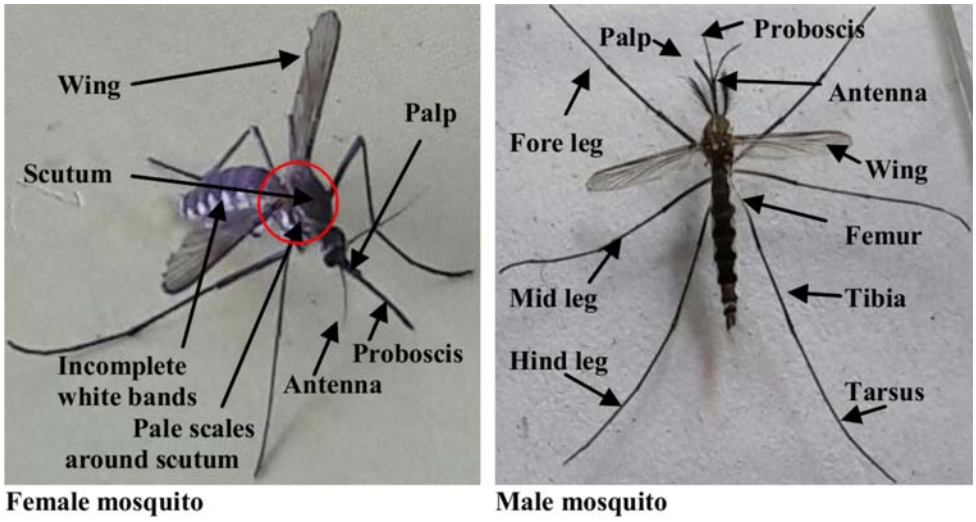

Fig. 2 Showing the body parts of the adult specimens under study

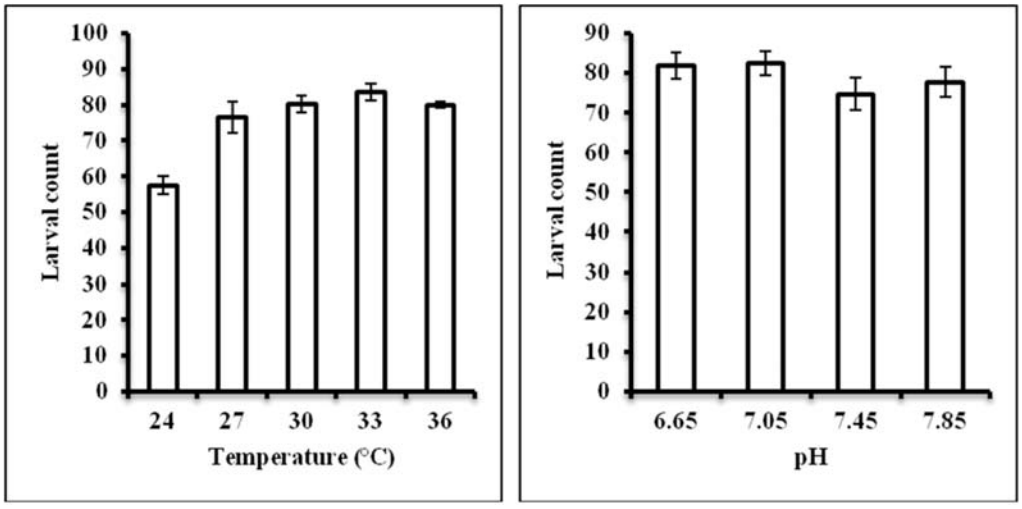

Fig. 3 Showing the plots of larval count versus MMT (°C) and pH

Table 3. Showing the pH classes, class mark, larval count, mean larval count (M), standard deviation (SD) and standard error of mean (SE)

pH classes	Class mark	Larval count	M	SD	SE
6.45 - 6.85	6.65	8.33±7.26 79.67±1.45 77.33±3.93	81.776	5.794	3.345
6.85 – 7.25	7.05	88.33±1.67 93.33±6.67 76.33±3.48 85.67±2.33 71±2.08 86.67±4.41 75.67±2.33	82.428	8.12	3.069
7.25 – 7.65	7.45	55±6.03 87.33±1.45 68.33±2.03 80.67±4.33 81.67±2.03 76.33±2.96 73.33±7.06	74.665	10.621	4.014
7.65 – 8.05	7.85	80±2.89 72.33±5.04 60±6.43 94±67 9±3.06 77.33±3.93 81.33±2.33	77.712	10.231	3.867

Table 4. Comparing the larval body parts of the specimen under study to the one studied by Farajollahi and Price, 2013

Larval body parts of *Aedes triseriatus*	Body parts as described by Farajollahi and Price	Remarks : Present or Undetected in the test specimen
Head hair	Has a box arrangement	Present
Upper head hair 5-C	Single	Present
Lower head hair 6-C	Double/triple	Present
Preantennal 7-C	Multiple	Undetected
Pecten teeth	Evenly placed	Present
Comb scales	Beyond pecten, partly double row.	Present
Anal saddle	Smooth	Present
Siphonal tuft 1-S	Double	Undetected
Lateral hair 1-X	On saddle, multiple	Present
Anal papillae	Unequal and tapering	Present

24 Parganas district of West Bengal, India ranges between 21°C and 36°C which is more or less similar to that of near by areas within the state where the average monthly temperature ranges between 19°C and 30°C (Khan *et al*., 2017). We found that the larval count varies significantly with the MMT (p value = 0.003) within temperature range of 24°C to 36°C with the maximum number of larvae surviving at 33°C. However, the larval count decreased above 33°C which may be due to suppressed embryonic development. The time taken for completing the life cycle and temperature are inversely related (Beserra *et al*., 2009). Above the optimal temperature, rate of development remains steady and may decrease slightly until the temperature reaches an upper limit of around 38°C to 42°C (Eisen *et al.,* 2014) which corroborates the results. Mosquito larvae can tolerate a wide range of pH from 2-10, much greater than those tolerated by other aquatic animals (Clark *et al*., 2004). However, there is no concrete evidence suggesting that pH limits the survival of mosquito larvae in nature. Tolerating sudden changes in pH suggests that major rearrangements pertaining to transporter expression are not required when faced with either a highly acidic or alkaline environment. The ability to withstand rapid changes in pH may be attributed to presence of separate mechanisms for acid and base secretion in larvae, rather than an adaptation providing the capacity to tolerate the sudden changes in pH. This is true in case of our findings wherein the larval count of *A. triseriatus* did not vary significantly with pH (p value = 0.445) although maximum larvae survived at a pH of 7.05.

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Secondary metabolites of *Musa* cultivars confer resistance against infestation by stem weevil, *Odoiporus longicollis* (Olivier) (Coleoptera: Dryophthoridae)

T. Ajitha, K. J. Kavitha, K. Shabith Raj, L. R. Bhavya, E. H. Vishnu Das and D. A. Evans*

Department of Zoology, University College, Thiruvananthapuram-695034, Kerala, India.
Email: drevansda@gmail.com

ABSTRACT: Banana, popularly known in Kerala as *Nendran* has diverse cultivars of indigenous and exotic or hybrid types. All *Nendran* cultivars are highly susceptible to infestation by *Odoiporus longicollis* Olivier (Coleoptera: Dryophthoridae), and they possess very low content of secondary metabolites (SM) such as total phenols (TP) and total flavonoids (TF). Activities of enzymes related to the synthesis of SM such as Phenylalanine Ammonia Lyase (PAL), Polyphenol Oxidase (PPO) and Peroxidase (PO) showed very low activity in *Nendran* cultivars and this may be one of the reasons for their susceptibility to infestation by *O. longicollis*. *Yangambi*, a *Musa* cultivar which is resistant to infestation by *O. longicollis* possessed very high content of TP, TF and elevated activity of PAL, PPO and PO. Under field condition, cultivar *Yangambi* did not show any symptoms of attack by this pest and rearing of larvae of *O. longicollis* in *Yangambi* resulted mortality within one week and wide spread cytopathological changes in the hemocytes and enzymatic changes in the hemolymph. Hemocytopaenia together with selective enhancement in the population of granulocytes and selective decrease in the population of plasmatocytes were observed in differential count. Cytopathological changes such as lack of cell membrane integrity, lack of nuclear membrane integrity and degeneration of cytoplasm was observed in hemocytes of larvae maintained in *Yangambi.* Intoxicated larvae showed sharp decrease in the contents of Trehalose through the elevated activity of Trehalase. Significant elevation of fat body glycogen and inhibition of glycogen phosphorylase was also observed in affected larvae. Sharp elevation of lactic acid through elevated activity of lactic acid dehydrogenase and inability to utilize glucose are other adverse effects caused by this pest resistant cultivar on the pest. Even though *Yangambi* is not a commercially viable *Musa* cultivar, the conservation of such cultivars is very much essential for knowing the molecular mechanism of pest resistance, which may help in the management of *O. longicollis* in an eco-friendly way.

KEY WORDS: *Odoiporus longicollis, Musa* cultivar *Yangambi*, resistance, secondary metabolites

INTRODUCTION

Banana is the major agriculture crop of Kerala state, India and globally India is the largest producer of this agricultural commodity. *Nendran* (AAB) cultivar of *Musa* is the most abundant and economically highly viable cultivar of Kerala (Kavitha *et al*., 2017) because of the desirable qualities such as short duration to set flower, large palatable ripe fruits, high commercial viability and comparatively good keeping quality. Field study conducted in various sites of Kerala proved that

* Author for correspondence

Nendran cultivars are the most pest susceptible *Musa* cultivars (Kavitha *et al*., 2015a), which was aggressively attacked by *Odoiporus longicollis* (Olivier) and if control measures are not properly applied, 70% crop loss will be certain (Padmanabhan and Sundararaju, 1999; Alagesan *et al*., 2016). Interaction made with traditional farmers of various districts in Kerala has resulted in the identification of many indigenous *Nendran* cultivars, and each of them is unique to a particular locality of Kerala. Recently, an indigenous *Nendran* cultivar from Guruvayoor of Thrissur District, Kerala has got GI tag and it was named as *Kazhchakkula*, a famous item in worship of Guruvayoor temple. In association with many indigenous *Nendran* cultivars, Agricultural Department of Kerala has introduced many exotic/ hybrid *Nendran* cultivars, which could not get wide appreciation from farmers of Kerala.

Presence of secondary metabolites in host plants is a major determining factor which influences herbivory (Harborne, 1982). All the *Nendran* cultivars, both indigenous and exotic are highly vulnerable to infestation by *O. longicollis* and since the destructive larvae are purely endophytic, farmers adopt systemic insecticides and also injection of Monocrotophos to control the pest. Interestingly, many indigenous *Musa* cultivars of Kerala are showing extreme degree of pest resistance through allelopathic interaction in the larvae of *O. longicollis* (Kavitha *et al*., 2015a, b). A comparative study on the content of total phenols, flavonoids and related enzymes in pest susceptible, indigenous or exotic/hybrid *Nendran* cultivars with a few pest resistant *Musa* cultivars and the mechanism of allelopathy induced by the pest resistant *Musa* cultivar on *O. longicollis* larvae form the subject matter of this communication.

MATERIALS AND METHODS

Indigenous *Nendran* cultivars were collected from Malappuram district of Kerala, India, where a traditional farmer is maintaining different indigenous *Nendran* cultivars in his sprawling fields. The exotic/hybrid cultivars of *Nendran* were collected from the Agriculture Department, Govt. of Kerala, at Kazhakuttom, Thiruvananthapuram district of Kerala, which supplies tissue cultured cultivars of *Nendran* types. *Ottamungili*, an indigenous *Nendran* cultivar was collected from Kottur, under Neyyar forest division Thiruvananthapuram district. *Yangambi* cultivar of *Musa* was collected from Agrifarm, a *Musa* diversity centre under the Agriculture Department, Peringammala, Thiruvananthapuram District, and Govt. of Kerala. The cultivars (suckers) brought from different sites were planted in the campus of University College and were provided with leaf litter as organic manure. *Changanassery Nendran*, *Chengazhikkodan, Manjeri Nendran, Mettupalayan, Swarnamukhi* and *Trichi manjeri* are the indigenous *Nendran* cultivars. *Ottamungili* is not cultivated in the any agroecosystems by farmers. All other cultivars are either exotic or hybrids.

Leaf sample collection: Tender cigar leaf, of 20 to 30 cm length was cut from the tip and kept in ice cold condition till weighing and processing.

Assay of enzymes, phenols and Flavonoids of host plants: All estimations were done as described in the standard techniques; total phenols (Mayr *et al.,* 1995), total flavonoids (Chang *et al.*, 2002) and assay of enzymes such as phenylalanine ammonia lyase (Whetten and Sederoff, 1992), polyphenol oxidase (Mayer *et al*., 1965), peroxidase (Hammerschmidth *et al*., 1982). Activity of enzymes was expressed as units/mg protein.

Rearing of *O. longicollis* larvae in *Musa* cultivars: *Yangambi*, a *Musa* cultivar which never showed infestation by *O. longicollis* under the field condition and which possessed very high activity of PAL, PO, PPO and bearing very high content of TP and TF was used for studying allelopathy in larvae. Those cultivars possessed very low contents of TP and TF, and very low activity of PAL, PO and PPO were used as control. Four month old cultivar with pseudostem of 25 to 30 cm circumference, whose crown was chopped down at a height of 100 cm above the ground. A small depression was made on the free cut end of live pseudostem and seven *O. longicollis* fourth instar larvae were released to it since they are voracious

feeders than younger instars, moderately large in size and easy to handle. The larvae were allowed to bore into the pseudostem and cut end was covered with a piece of mosquito net. In order to prevent the entry of rain water, the cut end was closed by a piece of plastic, if there was rain. On the seventh day, the live pseudostem (live stump) was cut 15 cm below the first cut and the larvae were carefully dissected out. Those cultivars which caused complete mortality of larvae within seven days were called Resistant (R) and those cultivars in which larvae showed no mortality were designated as Susceptible (S) (Kavitha *et al*., 2015a).

Study of haemocytes: The larvae were separated from pseudostem, washed in distilled water, blotted in filter paper, were used. Larvae were placed on a glass plate, kept on ice cubes and a sharp cut was given on the ventral side, without cutting the gut. The hemolymph was analysed for total count in standard counting chamber and differential count after staining by Giemsa stain.

Biochemical analysis of larval fat body and haemolymph: Larvae maintained in any of the susceptible *Nendran* cultivar (control) and *Yangambi* for four days were used for this experiment. Fat bodies of larvae were carefully separated. 100 mg fat body was weighed and homogenized in appropriate buffers under ice cold condition and used for estimating glycogen. 100 µl of hemolymph was centrifuged in a micro centrifuge and the supernatant was used for estimation of enzymes and biomolecules; glucose (Glucose Oxidase Peroxidase method, Trinder, 1969), trehalose (Roe, 1955), trehalase (Friedman, 1966), glycogen (Dubois *et al*., 1956), glycogen phosphorylase (Singh *et al*., 1961), lactic acid (Baker and Summerson, 1941) and lactic acid dehydrogenase (Queen, 1972).

The data collected from five leaf samples from each cultivar types was statistically analysed by one way analysis of variance (ANOVA) at pÃ0.05 level of significance.

RESULTS

The content of TP was very low in all the indigenous *Nendran* cultivars. Exotic/ hybrid cultivars possessed a slightly elevated TP and *Yangambi* possessed a very high content of TP compared to all other cultivars (Fig.1). *Ottamungili* is a commercially non viable cultivar which possessed only one or three fruits in the whole bunch, each fruit possessed a length of 35-40 cm. No flower bud could be located after one or two tier of fruits. It could not survive in the agroecosystem unless great care was provided. In Kottur forest, *Ottamungili* never showed symptoms of pest attack by *O. longicollis*. The content of TP in *Ottamungili* was low compared to that of exotic *Nendran* cultivars. The exotic cultivar, *Popaulu* showed slightly high content of TP than indigenous cultivars (Fig.1). Another group of secondary metabolites is flavonoids, the content of which was also very low in *Nendran* cultivars. Among the *Nendran* cultivars *Ottamungili*, *Changanassery Nendran*, and *Trichi Manjeri* possessed the lowest amount of TP and *Popaulu* the highest amount, which was almost of one third of the amount of TF in *Yangambi* (Fig.1)

Activity of PAL was very low in all the indigenous *Nendran* cultivars, (Fig.2). Some of the exotic cultivars such as *Popaulu* and *Mysore Ethan* showed a preferably good activity of PAL, almost one third of the activity of PAL in *Yangambi* cultivar. Another related enzyme PPO was also very low in all the different *Nendran* cultivars and it was least in *Changanassery Nendran* (Fig.2). All the exotic *Nendran* cultivars have maintained slightly elevated activity of PPO than to indigenous cultivars. Activity of PPO in *Yangambi* was several times higher than *Nendran* cultivars (Fig.2). Activity of PO was also low in all the *Nendran* cultivars, compared to *Yangambi*. Activity of PO in *Popaulu* and *Mysore Ethan* was almost one third to that of the activity of PO in *Yangambi* (Fig.2).

Rearing of 4th instar larvae of *O. longicollis* in either indigenous or exotic/hybrid cultivar of *Nendran* did not result any mortality or adverse effects in larvae. The larvae survived well in all the indigenous and exotic *Nendran* cultivars. All

the larvae of *O. longicollis* maintained in *Yangambi* died between 5th and 6th day of their maintenance. The hemolymph of the larvae on the third day of maintenance in *Yangambi* cultivar showed sharp hemocytopaenia (Table 1), together with significant change in the differential hemocyte count (Fig.3). Population of granulocytes have undergone a sharp increase, together with sharp decrease in the population of plasmatocytes. Wide spread cytopathological changes were observed in larvae maintained in *Yangambi* cultivar. Lack of cell membrane integrity, lack of nuclear membrane integrity and enucleation were observed in hemocytes (Fig.4 a&b).

The amount of glucose in the hemolymph of healthy larvae was very much lower than the fasting blood sugar of healthy human and it was 21.34±1.20, which became sharply decreased in larvae reared in *Yangambi* cultivar (Table 1). The amount of hemolymph trehalose was very much (15 times) higher than the amount of glucose and it became sharply reduced through the elevated activity of Trehalase under the influence of allelopathy by*Yangambi* cultivar (Table 1). The amount of fat body glycogen was significantly elevated in larvae reared in *Yangambi* and the enzyme glycogen phosphorylase was inhibited. Amount of lactic acid and its enzyme lactic acid dehydrogenase in the hemolymph was sharply elevated in larvae reared in *Yangambi* (Table 1).

DISCUSSION

TP and TF are phenolic compounds give bitter taste to the plants and host plants effectively used these compounds to get rid of the herbivorous insect pests (Georgima *et al*., 2015). In all the *Nendran* cultivars studied, the contents of TP and TF were low quantity when compared to *Yangambi*, a pest resistant *Musa* cultivar. The cultivar Yangambi was reported to be resistant to infestation by nematodes (Fogain, 1996; Valette *et al*., 1997). *Musa* cultivars exhibited high variation in the distribution of phenolic compounds in (Alfredo and Stalin, 2017) and phenolic compounds are acting as allelochemicals and have significant role in plant defense against herbivory (Usha Ravi and Ravibabu, 2011). Flavonoids are more bitter than phenols and also has significant role in pest defence (Joseph *et al*., 2004).

Activity of enzymes which are very much related to the formation of phenolic compounds such as PAL, PO and PPO were very low in all the *Nendran* cultivars when compared with *Yangambi*. It has been reported that PAL is a very important enzyme involved in the plant defence mechanism, which is evolved into phenyl propanoid pathway which imparts resistance against various types of pests (Ramesh kumar *et al*., 2012). Many investigators have reported the importance of PAL, PO and PPO in many crop plants including *Musa* cultivars and these enzymes showed elevated activity under the infestation of pests (Felipe Otalvaria *et al*., 2002; Valette *et al*., 1998). The mechanism of defence seen in plants against their insect enemies is through excessive synthesis of phenols and flavonoids and enzymes such as PAL, PO and PPO are key enzymes behind these secondary metabolites (Sung Kim and Hwang, 2014; Abdul *et al*., 2012).

Yangambi is not a CVC of *Musa* and farmers did not show interest in cultivating this cultivar because the ripe fruit bunch is small and attain weight of 8-10 Kg and in our experience, the ripe fruits are not so delicious and palatable and is slightl bitter , which may be due to the presence of excess of TP and TF. Under the field condition this cultivar was not attacked by *O. longicollis* but all the *Nendran* cultivars were attacked by this pest. *Yangambi* did not show any symptoms of attack by this pest such as small bore holes on the pseudostem with exudation of viscous fluid through the holes or breakage of pseudostem which are common symptoms of attack by *O. longicollis* (Kavitha *et.al*., 2015a,b). Rearing of this larva in *Yangambi* cultivars has resulted 100% mortality of 4th instar larvae in one week. Wide spread changes in the hemolymph which resulted hemocytopaenia together with cytopathological change in the hemocytes. Similar observations were reported in the hemolymph of *Dysdercus cingulatus* (Pandey and Tiwari, 2011) and *Papilio demoelus* (Pandey *et al.,* 2012) under toxicity by extracts of insecticidal

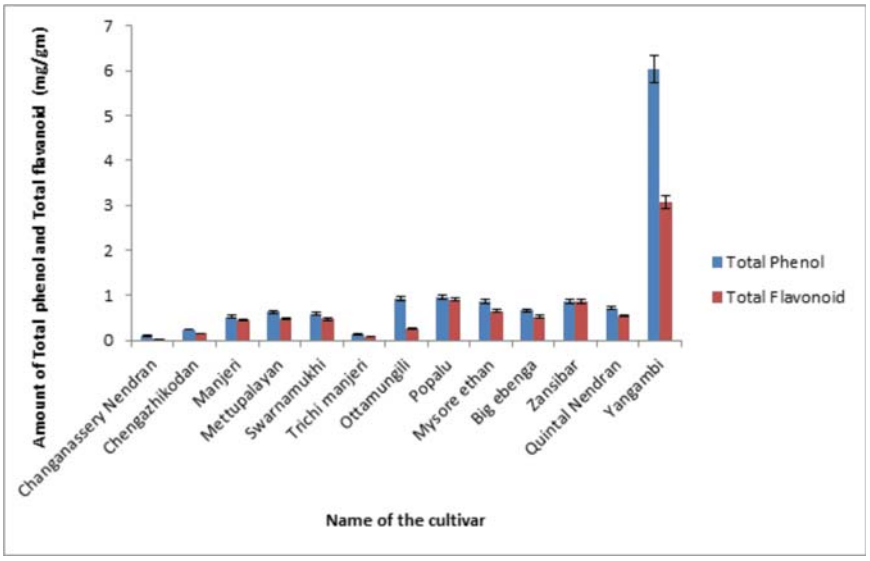

Fig.1. Amount of total phenols and flavonoids in different cultivars of *Musa*

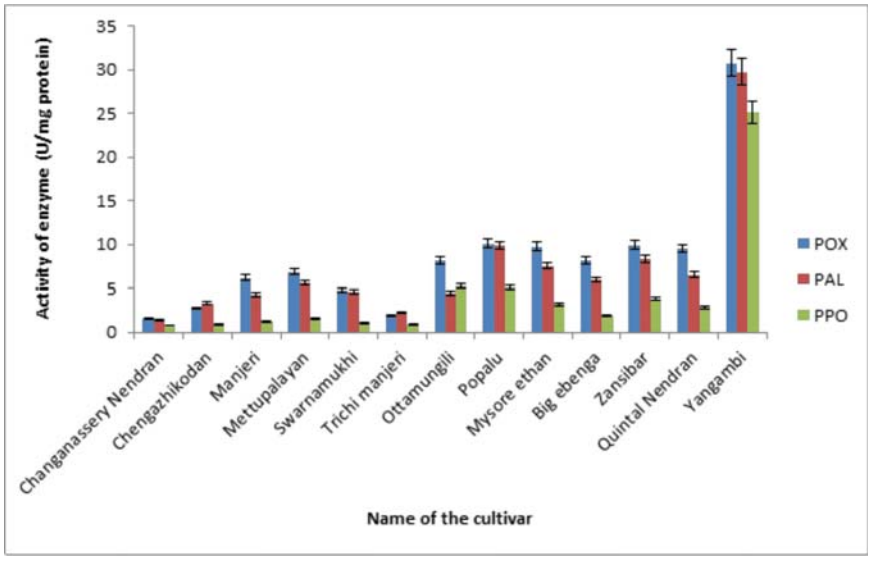

Fig. 2. Activities of three enzymes related to the production of secondary metabolites in different *Musa* cultivars

plants and in *Oryctes rhinoceros* larvae experimentally injected by *Bacillus thuringienesis* (Adhira *et al*., 2010, Adhira and Evans, 2011).

Cytopathological changes observed in *O. longicollis* larvae, reared in *Yangambi* cultivar indicated that the live pseudostem of this cultivar possessed toxic compounds. Differential hemocyte count of the larvae reared in *Yangambi* cultivar showed selective elevation of granulocytes and selective decrease in the population of plasmatocytes. Similar type of observation was also observed in *O. rhinoceros* larvae infected by *B.thuringienesis* (Adhira *et al*., 2010). Lack of membrane integrity of hemocytes of the *O. longicollis* larvae reared in *Yangambi* is indicated that this cultivar has cytotoxic molecule in the pseudostem. Cytopathological changes were observed mostly in plasmatocytes and granulocytes are are the main hemocytes concerned with the immunity of insects and the cells are phagocytic in function and act against pathogens entering in to

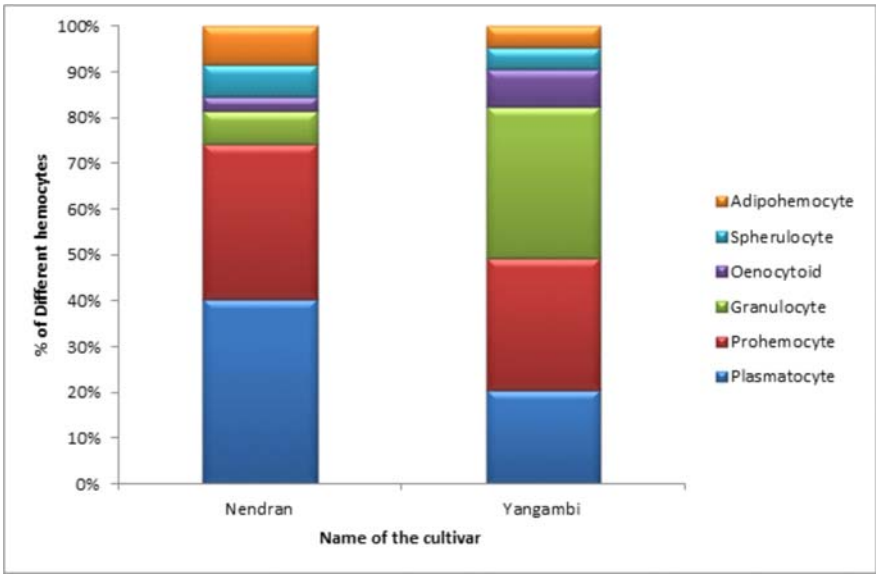

Fig.3. Allelopathic reactions of *Yangambi* cultivar on differential hemocyte count

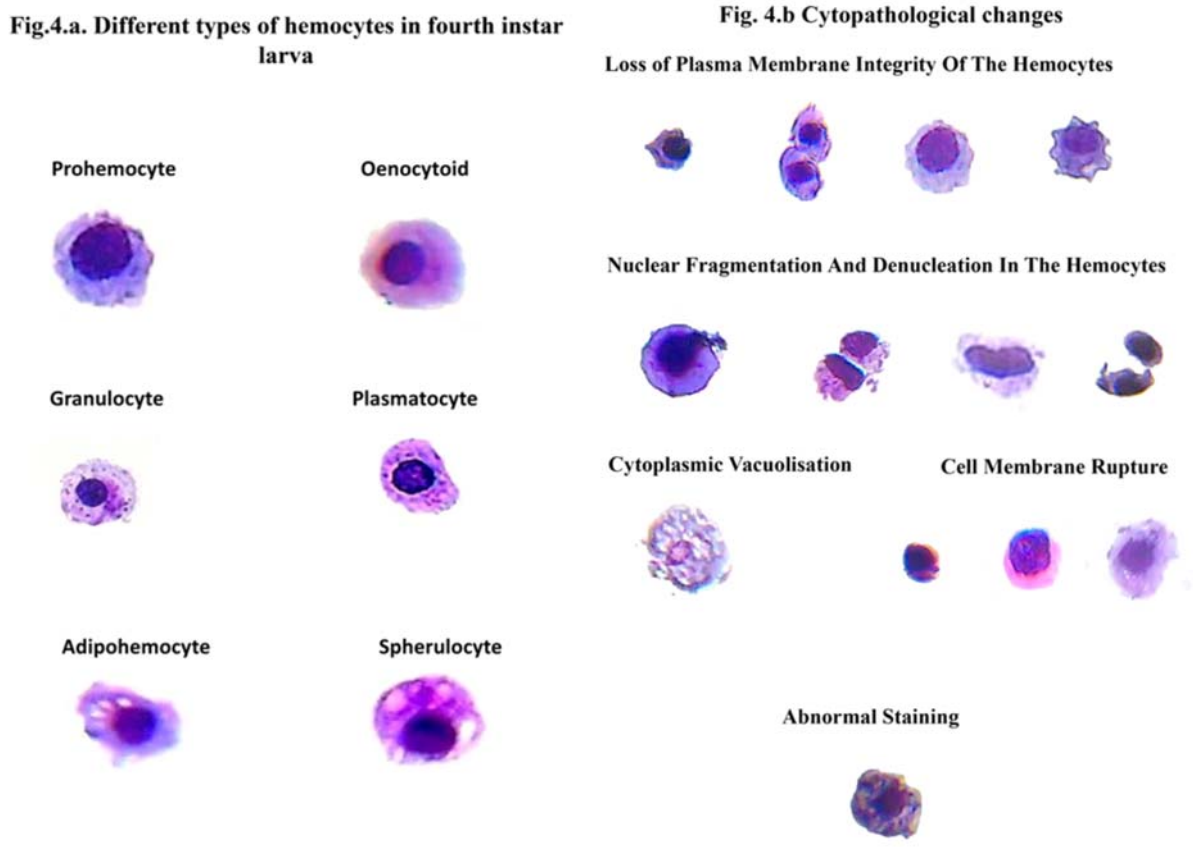

Fig.4. Normal hemocytes (4a) and Cytopathological changes (4b) induced by *Yangambi* cultivar in the hemocytes of *O. longicollis*

Table. 1. Allelopathic reactions induced in larvae by *Yangambi* cultivar during the third day of existence in the pseudostem

Sl. No.	Biochemical/Cellular parameters	Control (*Nendran* cultivar)	Test (*Yangambi*)
1	Glucose	21.34±1.20	14.42±0.96
2	Trehalose	318.36±18.50	248.56±12.8
3	Trehalase	42.43±2.52	58.35±3.15
4	Glycogen	354.76±20.16	404.12±19.54
5	Glycogen phosphorylase	365.52±18.50	274.92±16.52
6	Lactic acid	249.17±11.80	439.12±22.52
7	Lactic acid dehydrogenase	106.44±8.96	297.04± 20.7
8	Total hemocyte count	4438± 202	2328±102

All values are mean I SD, n=6, p≥0.05 with respect to corresponding control values.

1. Amount of glucose is expressed as mg/100ml hemolymph.
2. Trehalose is expressed as glucose units in mg/100ml hemolymph.
3. Activity of trehalase is expressed as amount of glucose in micromoles liberated/minutes/mg protein.
4. Glycogen content of fat body is expressed as microgram of glucose equivalent/100mg tissue.
5. Glycogen phosphorylase activity is given as micromoles of organic phosphate liberated/minutes/mg protein.
6. Lactic acid in microgram/ml of hemolymph.
7. Activity of lactic acid dehydrogenase is expressed as micromoles of lactic acid liberated/minutes/mg protein.

the body of the insect. Plasmatocytes are more involved in phagocytosis of non self cells whereas granulocytes are apparently the only hemocytes that engulf the dead cells (Ling and Yu, 2006; Amral *et al*., 2009).

The content of glucose in the hemolymph of the healthy larvae of *O. longicollis*, very much agreed with the observation in *O. rhinoceros* larvae and in larvae of *Oecophylla smaragdina*. The glucose level of larval hemolymph of these insects was very low when compared to that of fasting blood of healthy humans (Adhira, 2015; Vidhu, 2015). In *O. longicollis*, the content of Trehalose was very much higher than that of the amount in glucose which is also agreeable with the observation in *O. rhinoceros* (Adhira, 2015). Rearing the *O. longicollis* has resulted sharp decrease in the content of trehalose which may be due to elevated activity of trehalase, under the influence of *Yangambi* cultivar. The storage polysaccharide glycogen became significantly elevated in larvae reared in *Yangambi* and such elevation may be through the inhibition of glycogen phosphorylase. In *O. rhinoceros*, experimental infection of *Bacillus thuringiensis* and exposure to cold shock also resulted similar changes in glycogen phosphorylase and glycogen content (Adhira and Evans, 2014). The content of lactic acid became greatly increased in larvae maintained in *Yangambi* cultivar which very well attested the weak, lethargic appearance of larvae which were reared in *Yangambi* cultivar and the elevated activity of lactic acid dehydrogenase substantiated the increase in lactic acid content.

Development of resistance against a serious pest of *Musa* by a natural *Musa* cultivar may be through years of evolution. Modern agricultural practices aimed only on commercial gains are not interested in commercially non-viable and pest resistant cultivars. So these types of *Musa* cultivars require

special conservation efforts to keep their germplasm healthy and viable for studying the molecular mechanism of pest resistance.

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An updated distributional checklist of Bees of the subfamily Nomiinae (Hymenoptera: Apoidea: Halictidae) with new records from south India

Arati Pannure and Vasuki V. Belavadi*

Department of Entomology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bengaluru 560 065, India.
E mail: vvbelvadi@gmail.com

ABSTRACT: The first comprehensive distributional checklist of the species belonging to Nomiinae of south India is presented. Totally, 48 species under 13 genera are listed, of which *Curvinomia strigata* (Fabricius, 1793), *Macronomia antennata* (Smith, 1875) and *Steganomus lieftincki* Pauly, 2009 are newly recorded from south India. Sixteen of these species are reported for the first time for Karnataka, three species for Andhra Pradesh, two species for Tamil Nadu and one species each for Kerala and Telangana. Distribution of species of Nomiinae in south India is provided.

KEYWORDS: Apiformes, distribution, fauna, Halictidae, diversity

INTRODUCTION

Bees belonging to the subfamily Nomiinae are distinctive members of the family Halictidae. The subfamily includes an incredibly diverse group of metallic and non-metallic species, which are largely solitary, except for a few communal nesting species (Batra, 1966, 1977; Michener, 2007). Though majority of these bees are generalist foragers, they are very important pollinators for many agricultural crops and wild plant species (Cane, 2002, 2008; Isaacs and Kirk 2010). The great majority of Nomiinae occurs in Afrotropical, Oriental, Australian and Palaearctic regions, but poorly distributed in Nearctic region and totally absent in Neotropical region (Astafurova, 2013). This subfamily is comprised of approximately 600 spe-cies worldwide and the Oriental fauna includes 154 valid species (Pauly, 2009; Astafurova, 2013). According to Michener (2007), the subfamily includes 11 genera worldwide, which were considered to be belonging to a single genus, *Nomia* Latreille for a long time. Generic level classification of Nomiinae of Afrotropical and Oriental species has undergone several changes owing to tremendous species diversity and heterogeneity. Taxonomy of Afrotropical and Oriental species was restructured and many changes in nomenclature have been made subsequently by the revisionary works of fauna of these regions undertaken by Pauly (1980, 1984, 1990, 1991, 2000, 2005, 2008, 2009, 2014) and Karunaratne *et al*. (2005) resulting in subdivision of Nomiinae genera into a number of separate genera including raising several subgenera to generic level. In the present study, recent classification of Oriental fauna by Pauly (2009) is followed.

* Author for correspondence

The Halictidae fauna is rich and diverse, yet it is poorly studied from India. Most species of Nomiinae were studied and described in the 19th century. Smith (1853, 1875ab, 1879), Westwood (1875), Cameron (1897, 1898, 1902, 1904, 1907ab, 1908), Nurse (1902, 1904), Cockerell (1911, 1917, 1919, 1920), Meade-Waldo (1916) and Baker (2002) have made valuable contributions to Indian Halictidae. Bingham (1897) in his Fauna of British India (Hymenoptera Vol. I.) reported 26 species under 2 genera belonging to Nomiinae from Indian subcontinent (with no information on south Indian species). Online version of the checklist published by Gupta (2010) unfortunately is a mere checklist without any updated locality data or changes in classification. However, classification of Nomiinae from Oriental region, New Guinea and Islands of the Pacific Ocean by Pauly (2009), a web based Atlas Hymenoptera (Pauly 2015/2016) and Discover Life's bee species guide and world checklist (Ascher and Pickering 2016) provide a better understanding of the diversity and distribution of these bees from India. Saini and Rathor in 2012 listed 72 species in 15 genera from India mainly based on published data, of which only 17 species are reported from south India. Considering the scattered and incomplete knowledge about south Indian fauna, the current study was attempted to provide a complete checklist of south Indian Nomiinae bees with updated distributional records. South India is the area encompassing India's states of Andhra Pradesh, Karnataka, Kerala, Telangana, Tamil Nadu, as well as the union territories of Lakshadweep and Puducherry, occupying 19.31% of the geographical area of the country. We list a total of 48 species under 13 genera from south India. Distribution ranges of 27 species are appreciably extended owing to the new specimens examined. Floral records are also provided wherever available.

MATERIALS AND METHODS

The present checklist is based on the examination of a total of 1062 specimens deposited in the Department of Entomology, University of Agricultural Sciences, Gandhi Krishi Vignan Kendra, Bengaluru, collected from various localities in Karnataka and other south Indian states. Material from College of Horticulture (Mudigere) was also examined. External morphological examinations of specimens were made using a Nikon SMZ 1000 (Lens – WD 70 (model- C-FIR) -1007956) microscope. Specimens were identified using keys in Bingham (1897); Pauly (2009, 2014) and Atlas Hymenoptera (Pauly, 2015/2016). Both adult females and males (when available) were examined. Digital colour images of species were made using a Leica M205A stereomicroscope with DFC 420 camera attachment. All images were edited with Adobe Photoshop CS2 (Version 9.0).

The genera and species of Nomiinae are listed alphabetically together with specimens examined. The checklist also contains the general distribution of the listed species. New distribution records are marked with an asterisk (*). The distribution of species is given in this order: (1) the states of south India arranged in alphabetical order along with the localities within it, (2) the other Indian states (Elsewhere in India), (3) the other counties (outside India) in alphabetical order. Attempts have been made to incorporate the current names of the localities and present geographical distribution. The list of new names include Bengaluru (Bangalore), Chennai (Madras), Firozpur (Ferozepore), Jeypore (Tey pore), Kodagu (Coorg), Kolkata (Calcutta), Mangaluru (Mangalore), Mumbai (Bombay), Mysuru (Mysore), Puducherry (Pondicherry), Pune (Poona), Shivamogga (Shimoga), Tumakuru (Tumkur) etc. To produce the distribution map, all localities from south India from where Nomiinae bees have been collected were used (only records with detailed locality indication or complete coordinates were included). Mapinfo Professional 7.5SCP was used for generating the distribution map.

RESULTS

The subfamily Nomiinae is differentiated from other subfamilies of Halictidae by the following characters: marginal cell usually rounded at apex, third submarginal cell in forewing usually about as long as first, and distinctly longer than second

(*Steganomus* has only two submarginal cells), basal vein of the forewing varies from slightly and uniformly curved to strongly curved, prepygidial fimbria not divided medially, antenna arising near midlength of eyes, episternal groove present up to scrobe but sometimes as a weak depression below scrobal groove. Male: S7 a transverse plate with short apodemal arms and no midapical projection, highly variable in leg modifications (adapted from Michener, 2007).

Genus *Austronomia* Michener, 1965

Austronomia arcuata Pauly, 2009

No specimens examined.

Distribution:

South India	Karnataka (Bengaluru-Bannerghatta National Park), Kerala (Walayar forest), Puducherry (Karaikal)
Elsewhere in India	Madhya Pradesh (Satpura Hills, Pachmarhi), Maharashtra (Mumbai)
Outside India	-

Austronomia capitata (Smith, 1875)

(Fig. 1 & 2)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 12°58' N 77°35' E, 1♀, 12.ix.2011, coll. Latha, C; 1♂, 28.i.2012, coll. Shrikant; 1♀, 31.vii.2013, coll. Girish, R.; 1♀, 10.i.2014, coll. Arati Pannure; 1♀, 29.x.2013, coll. Najeer; Hebbal, 900 m, 13°02' N 77° 35' E, 1♂, 19.x.2014, 1♀, 9.iii.2015, coll. Zameeroddin; Sadahalli, 906 m, 13°12'N 77°38' E, 1♀, 30.x.2014, coll. Zameeroddin. Haveri, Ranebennur, 957 m, 14°36' N 75°37' E, 1♀, 27.x.2010, coll. Sudha, M. Telangana: Medak, Narayankhed, 479 m, 18°04' N 77°50' E, 1♂, 12.ix.2012, coll. Yeshwanth, H.M.

Distribution:

South India	Karnataka (Bengaluru, *Haveri), *Telangana
Elsewhere in India	Maharashtra, Punjab (Ludhiana)
Outside India	Sri Lanka

Floral records: *Phaseolus vulgaris*, *Calotropis* sp., *Tectona grandis*.

Austronomia notiomorpha (Hirashima, 1978)

No specimens examined.

Distribution:

South India	Karnataka (Bengaluru), Kerala (Wayalar Forests)
Elsewhere in India	Maharashtra
Outside India	Sri Lanka

Austronomia pseudoscutellata Pauly, 2009

No specimens examined.

Distribution:

South India	Kerala (Walayar forest), Tamil Nadu (Nilgiri Hills, Devala).
Elsewhere in India	-
Outside India	-

Austronomia ustula (Cockerell, 1911)

(Fig. 3 & 4)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♂, 1.xi.1988; 1♂, 11-20.ix.1989, coll. Ghorpade, K; GKVK, 876 m, 13°5' N 77°34' E, 1♀, 1.xii.2011, 927 m, 13°5' N 77°34' E, 2♀, 28.ii.2012, 1♀, 30.iv.2012, coll. Arun, B.C; 1♀, 12.x.2012, coll. Girish, R; 1♂, 23.x.2014, 1♀, 5.xi.2014, coll. Zameeroddin; 1♂, 8.iv.2015, coll. Sunil M.T; Hebbal, 900 m, 13°02' N 77° 35' E, 1♀, 11.xi.2014, 1♀, 1.4.xii.2014, 1♀, 30.i.2015, 1♀, 5.ii.2015, coll. Zameeroddin. Sadahalli, 906 m, 13°12'N 77°38' E, 1♀, 4.xi.2014, 1♂, 6.ii.2015, coll. Zameeroddin; Chikkamagaluru, Mudigere, 979 m, 13°06' N 75°37' E, 1♀, 10.xi.2014, coll. Prashantha, C. Kolar, Horticulture college, 830 m, 13°07' N 78°10' E, 1♂, 16.xii.2014, coll. Pradeep. Mysore, Chinnamballi, 716 m, 12°05' N 76°49' E, 1♂, 30.vii.2014, coll. Prashantha, C.

Distribution:

South India	Karnataka (*Bengaluru, Chamarajanagara-Bandipur National Park, *Chikkamagaluru, *Kolar, *Mysuru)
Elsewhere in India	Maharashtra
Outside India	Sri Lanka

Genus *Curvinomia* Michener, 1944

***Curvinomia fulvata* (Fabricius, 1804)**

No specimens examined.

Distribution:

South India	Tamil Nadu (Nilgiri Hills, Gudalur)
Elsewhere in India	-
Outside India	Cambodia, China, Germany, Indonesia, Malaysia, Nepal

***Curvinomia iridescens* (Smith, 1857)**
(Fig. 5 & 6)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♀, 19.v.1980, 1♀, 11.viii.1980, 1♀, 14.vii.1980, coll. Veena; GKVK, 2♀, 13.v.2006, coll. Suma, S; 1♀, 30.iv.2009, 1♂, 19.v.2009, 1♂, 21.iv.2009, coll. Latha, H.C; 1♂, 7.iv.2009, coll. Geeta, R.N; 1♀, 16.iv.2009, coll. Mutthuraju, G.P; 1♀, 18.x.2011, coll. Dhanyavathi, P.N; 1♂, 1.vii.2014, coll. Sunil Rathod; Sadahalli, 906 m, 13°12' N 77°38' E, 1♂, 30.x.2014, coll. Zameeroddin. Chikkamagaluru, Mudigere, Malaymarutha, 980 m, 13°07' N 75°30' E, 1♀, 28.iv.2013, coll. Girish; Thogarihankal, 1023 m, 13°28' N 75°48' E, 1♀, 21.ix.201, coll. Prashantha, C; Sringeri, 672 m, 1♂, 20.vi.2015, coll. Sachin Hegde; 672 m, 1♀, 19.vi.2015, coll. Abhishek. Coorg, 1♂, 4.iii.2007, coll. Chengappa. Mandya, VC farm, 727 m, 12°48' N 76°74' E, 6♀ & 1♂, 10.viii.1982, coll. B. Mallik. Mangalore, 45 m, 12°92' N 74°85' E, 1♂, 9.v.1983, coll. B. Mallik. Mysore, Hunsur, 1♀, 10.iii.2009, coll. Dhanyavathi, P.N.

Distribution:

South India	Karnataka (*Bengaluru, *Chikkamagaluru, Kodagu-Madikeri, *Mandya, *Mangalore, *Mysuru), Tamil Nadu (Anaimalai Hills, Coimbatore, Nilgiri hills)
Elsewhere in India	Maharashtra, West Bengal (Barrackpore)
Outside India	It is distributed from India to Indonesia

Floral records: *Cajanus cajan*, *Anacardium occidentale*

***Curvinomia strigata* (Fabricius, 1793)** (Fig. 7)

Specimens examined. INDIA: Andhra Pradesh: Visakhapatnum, Araku valley, 934 m, 18° 33' N 82° 87' E, 1♀, 22.ix.2013, coll. Vinayak, T. Karnataka: Bangalore, 916 m, 1♀, 19.v.1980, 1♀, 11.viii.1980, 1♀, 14.vii.1980, coll. Veena. Chikkamagaluru, Mudigere, 1♀, 6.vi.2002, coll. Anas; Jannapura, 931 m, 29°10' N 75°46' E, 1♀, 3.iv.2012, coll. Y. Diwakar. Dakshina Kannada, Puttur (DCR), 1♀, 9.ii.2015, coll. K. Vanitha. Coorg, 1♀, 4.iii.2007, 1♂, 21.iv.2009, coll. Chengappa; Ponnampet, 858 m, 12°08' N 76°56' E, 1♀, 21.vii.2015, coll. Prashantha, C. Uttara Kannada, Badagunda, Ganeshgudi, 570 m, 15°16' N 074° 33' E, 1♀, 17.x.2015, coll. Prashantha C. Tamil Nadu: Coimbatore, 3♀, 13.x.2000, coll. C.A.Viraktamath.

Distribution:

South India	*Andhra Pradesh, *Karnataka, *Tamil Nadu
Elsewhere in India	-
Outside India	India to Indochina, Indonesia to Java and Borneo

Floral records: *Mimosa pudica, Oryza sativa*

Genus *Gnathonomia* Pauly, 2005

***Gnathonomia aurata* (Bingham, 1897)** (Fig. 8)

Specimens examined. INDIA: Karnataka: Bidar, Ghorwadi, 628 m, 1♂, 15.x.2015, coll. Zameer. Mandya, VC farm, 727 m, 12° 48' N 76° 74' E, 1♂, 10.viii.1982, coll. B. Mallik. BM, 2♂, date and

location unknown, coll. B. Mallik. Tumkur, Yarabahalli, 861 m, 1♂, 29.vii.2014, coll. Revansidda.

Distribution:

South India	*Karnataka, Tamil Nadu (Coimbatore -Kovai)
Elsewhere in India	Punjab (Chohla Sahib), Uttarakhand
Outside India	Malaysia, Myanmar, Laos, Sri Lanka, Thailand

***Gnathonomia radiata* Pauly, 2009** (Fig. 9 & 10)

Specimens examined. INDIA: Karnataka: Chamarajanagar, BRT wildlife sanctuary, Khaggali, 2♂, 16.v.2011, coll. Suman. Chikkamagaluru, Mudigere, 981 m, 13°07' N 75°37' E, 1♀, 3.vi.201, coll. Ashwath, P.C. Coorg, 2♀, 22.x.2008, coll. Chengappa; Ponnampet, 858 m, 12°08' N 76°56' E, 1♀, 21.vii.2015, coll. Prashantha, C. Dakshina Kannada, Puttur (DCR), 1♀, 9.ii.2015, coll. K. Vanitha. BM 123, BM 129, BM 130, BM 132, date and locality unknown, coll. B. Mallik.

Distribution:

South India	*Karnataka, Tamil Nadu (5 km S. Theppakadu Mudumalai National Park, 30 km NW Ooty)
Elsewhere in India	-
Outside India	Malaysia, Thailand

***Gnathonomia thoracica* (Smith, 1875)** (Fig. 11 & 12)

Specimens examined. INDIA: Karnataka: Coorg, 1♀, 17.ix.2008, coll. Chengappa. Mangalore, Ullal (ARS), 6 m, 12°81' N 74° 84' E, 2♂, 18.viii.1983, ♀ & 1♂, 25.vii.1985, coll. B. Mallik.

Distribution:

South India	*Karnataka, Kerala, Tamil Nadu
Elsewhere in India	Sikkim, West Bengal (Kolkata)
Outside India	It is distributed from India to Indonesia (Java, Philippines), China

Genus *Hoplonomia* Ashmead, 1904

***Hoplonomia elliotii* (Smith, 1875)** (Fig. 13 & 14)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 13°04' N 77°34' E, 1♀, 21.iii.2015, coll. Sunil, M.T; 1♀, 18.vi.2009, coll. Kalleshwaraswamy; 1♀, 28.x.2009, coll. Suma, S. Chikkamagaluru, Mudigere, 980 m, 13°06' N 75°37' E, 3♀ & 2♂, 1.vii.2014, coll. Prashantha, C; Kodagu, Ponnampet, 858 m, 12°08' N 76°56' E, 2♀ & 3♂, 21.vii.2015, coll. Prashantha, C. Mandya (Sasalu), 841 m, 12° 47' N 76° 26' E, 2♀, 6.x.2014, coll. Pradeep. Mangalore, Ullal (ARS), 6 m, 12° 81' N 76° 26' E, 1♀, 30.ix.2011, coll. B, Mallik. Mysore, Hunsur, 1♀, 10.iii.2009, coll. Dhanyavathi, P.N. Kerala: Kannur, Madayipara, 38 m, 12°01' N 75°15' E, 1♂, 13.viii.2015, coll. Prashantha, C; 1♀, 13.viii.2015, coll. Pradeepa, S.D.

Distribution:

South India	*Karnataka, Kerala (Ponmudi Range, Trivandrum, *Kannur), Tamil Nadu
Elsewhere in India	Maharashtra, West Bengal (Barrackpore)
Outside India	It is distributed from India to Indochina and southern China

Floral records: *Cajanus cajan*, *Portulaca* sp., *Glycine max*.

***Hoplonomia westwoodi* (Gribodo, 1894)** (Fig. 15 & 16)

Specimens examined. INDIA: Andhra Pradesh: Bapatla, 1♂, 12.xii.2006, coll. David, K.J. Karnataka: Bangalore, GKVK, 930 m, 12° 58' N 77°35' E, 1♀, 5.vi.2013, coll. Girish; 1♀, 23.iv.2012, coll. Arun B.C; 1♀, 7.i.2014, 1♀, 20.i.2014, coll. Arati Pannure; 1♂, 10.ii.2008, coll. Nayana, E.D; 4♀ & 1♂, 4,5,6,ix.2014, coll. Pradeep. GKVK, 934 m, 13° 08' N 77° 58' E, 1♀ & 3♂, 31.i.1982, coll. B. Mallik; Hebbal, 900 m, 13° 02' N 77° 35' E, 2♀, 31.x.2014, 1♀, 11.xi.2014, 1♀, 16.xi.2014, 1♀, 21.ii.2015, 1♂, 6.i.2015, 1♂, 20.xi.2015, coll.

Zameeroddin; Sadahalli, 906 m, 13°12'N 77°38' E, 1♀, 6.ii.2015, 1♀, 26.ii.2015, coll. Zameeroddin. Belgaum, Arabhavi, 582 m, 16°13' N 74°49' E, 2♀, 20.ix.2014, coll. Revansidda. Bellary, 1♀, 21.ix.2011, coll. M. Srinivasa. Chikkamagaluru, Kadur, 758 m, 13°33' N 76°49' E, 1♀, 24.xi.2014, coll. Prashantha, C; Mudigere (20 km SW), 2♂, 15.iii.2008, coll. Nayana, E. Hassan, karekere, 934 m, 13°06' N 76°10' E, 2♀, 23.vi.2014, coll. Zameeroddin. Dakshina Kannada, Kankandi, 20 m, 12°81' N 74°88' E, 1♀, 5.iii.2015, coll. Prashantha, C. Kodagu, Ponnampet, 858 m, 12°08' N 76°56' E, 1♀, 21.vii.2015, coll. Prashantha, C. Kolar, Horticulture college, 830 m, 13°07' N 78°10' E, 1♀, 16.xii.2014, coll. Zameeroddin. Koppal, Munirabad, 466 m, 15°33' N 76°33' E, 1♀, 5.xii.2012, coll. Najeer. Mandya (Sasalu), 841 m, 12° 47' N 76° 26' E, 2♀, 6.x.2014, coll. Pradeep; VC farm, 727 m, 12° 48' N 76° 74' E, 2♂, 10.viii.1982, coll. B. Mallik. Mysore, Chinnamballi, 716 m, 12°05' N 76°49' E, 2♀, 19.vii.2015, coll. Prashantha, C; COH, 824 m, 12° 22' N 76°31' E, 1♂, 20.vii.2015, coll. Prashantha, C; Hunsur, 1♀, 18.iv.2009, Dhanyavathi, P.N; Nanjangud, 1♂, 24.i.2009, coll. Dhanyavathi, P.N; Banur, 1♀, 23.iv.2009, Dhanyavathi, P.N. Udupi, Brahmavar, 1♂, 12.iv.1985, coll. A.R.V Kumar.

Distribution:

South India	*Andhra Pradesh, *Karnataka, Puducherry (Karaikal), Tamil Nadu (Coimbatore-Kovai)
Elsewhere in India	Maharashtra (Pune), Rajasthan (Udaipur), West Bengal (Barrackpore)
Outside India	Afghanistan, Pakistan, Sri Lanka

Floral records: *Leucas aspera*, *Cajanus cajan*, *Ocimum* sp., *Grewia* sp., *Gossypium hirsutum*, *Duranta erecta*, *Euphorbia pulcherrima*.

Genus *Leuconomia* Pauly, 1980

***Leuconomia interstitialis* (Cameron, 1898)** (Fig. 17 & 18)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 12° 58' N 77°35' E, 1♂, 21.i.2013, coll. Girish; Sadahalli, 906 m, 13°12'N 77°38' E, 1♂, 30.x.2014, 4♀, 4.xi.2014, 1♀, 17.xi.2014, 1♂, 18.i.2015, 3♀, 29.i.2015, 2♀, 15.ii.2015, coll. Zameeroddin. Bidar, Markal, 584 m, 17°59' N 75°28' E, 1♀, 7.i.2011, coll. A.N.Reddy. Bijapur, 598 m, 16°46'N 75°44' E, 1♂, 16.viii.2011, coll. A.N.Reddy. Mysore, 1♀, 18.iii.2009, coll. Dhanyavathi, P.N.

Distribution:

South India	*Karnataka, Kerala (Walayar Forests)
Elsewhere in India	Punjab (Chohla Sahib), Uttar Pradesh (Allahabad), Uttarakhand (Mussoorie)
Outside India	-

Floral records: Grasses, *Cuphea hyssopifolia, Triticum aestivum*

***Leuconomia rufitarsis* (Smith, 1875)**

No specimens examined.

Distribution:

South India	Kerala (Walayar Forests)
Elsewhere in India	-
Outside India	Africa

Genus *Lipotriches* Gerstaecker, 1858

***Lipotriches* (*Rhopalomelissa*) *bombayensis* (Cameron, 1908)**

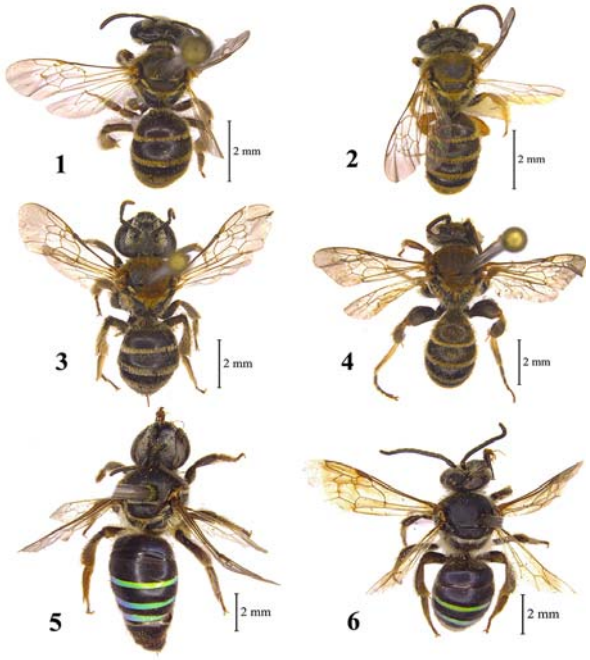
No specimens examined.

Distribution:

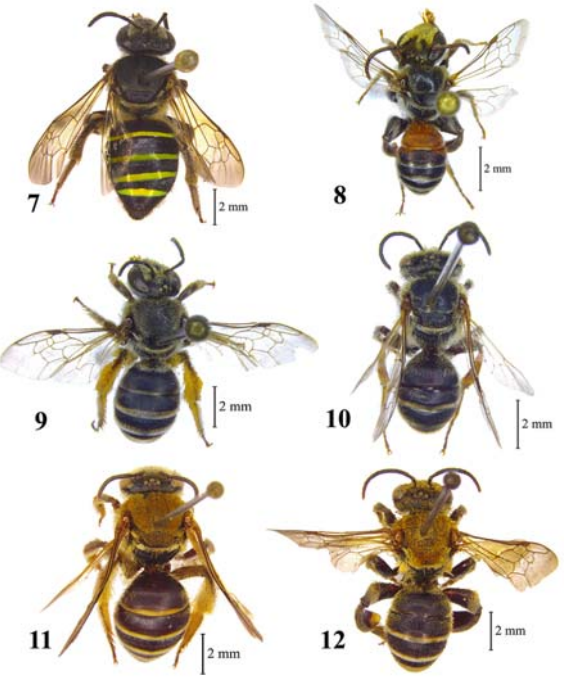
South India	Tamil Nadu (Coimbatore)
Elsewhere in India	Goa (Mormugao), Gujarat (Deesa), Maharashtra (Bombay)
Outside India	Sri Lanka

***Lipotriches* (*Lipotriches*) *bouceki* Pauly, 2014** (Fig. 19)

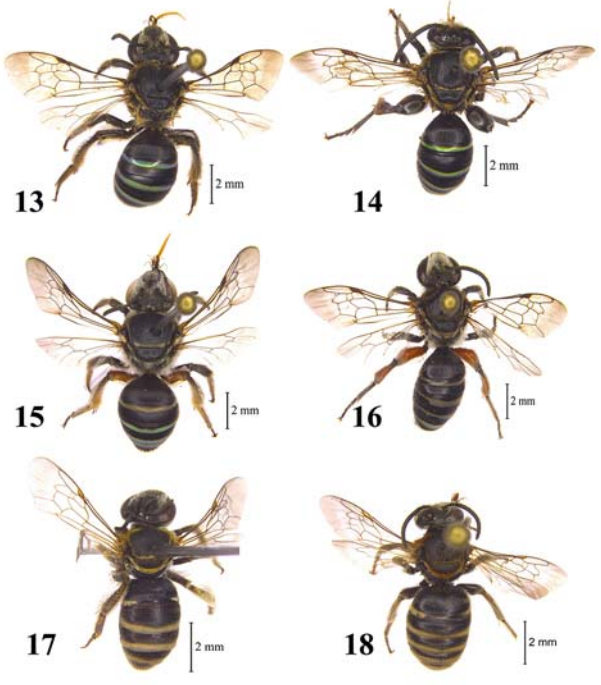
Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 939 m, 12° 58' N 77° 35' E, 1♀, 8.vi.2012, coll. G. Keshavareddy; Hebbal, 1♀, 25.x.1976, coll. Students.



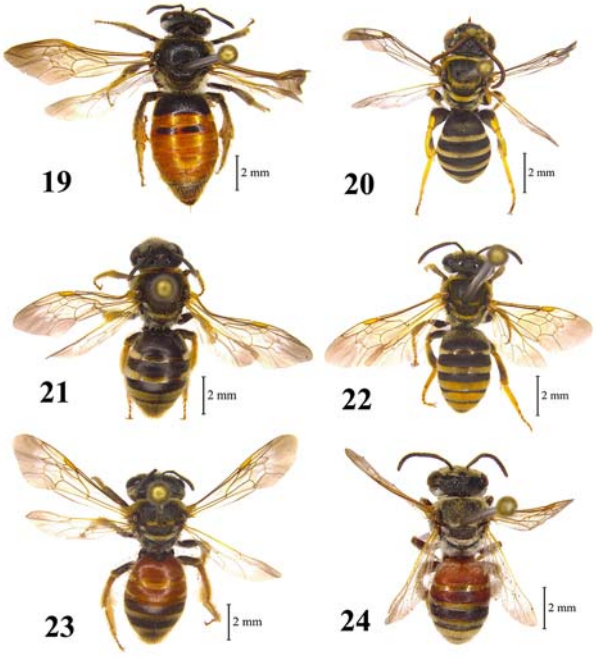
Figures 1–6. 1. ***Austronomia capitata*****, female; 2.** ***Austronomia capitata*****, male; 3.** ***Austronomia ustula*****, female; 4.** ***Austronomia ustula*****, male; 5,** ***Curvinomia iridescens*****, female; 6.** ***Curvinomia iridescens*****, male.**



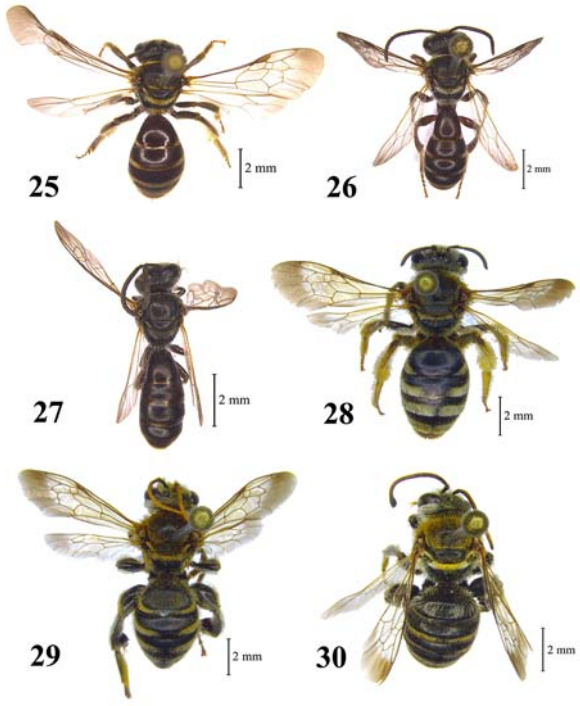
Figures 7–12. 7. ***Curvinomia strigata*****, female; 8.** ***Gnathonomia aurata*****, male; 9.** ***Gnathonomia radiata,*** **female; 10.** ***Gnathonomia radiata,*** **male; 11.** ***Gnathonomia thoracica*****, female; 12.** ***Gnathonomia thoracica*****, male.**



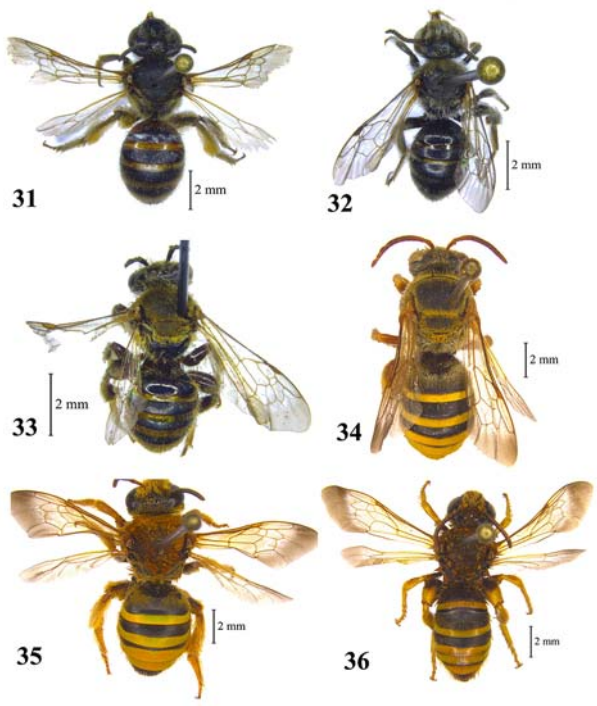
Figures 13–18. 13. ***Hoplonomia elliotii*****, female; 14.** ***Hoplonomia elliotii*****, male; 15.** ***Hoplonomia westwoodi*****, female; 16.** ***Hoplonomia westwoodi*****, male; 17.** ***Leuconomia interstitialis*****, female; 18.** ***Leuconomia interstitialis*****, male.**



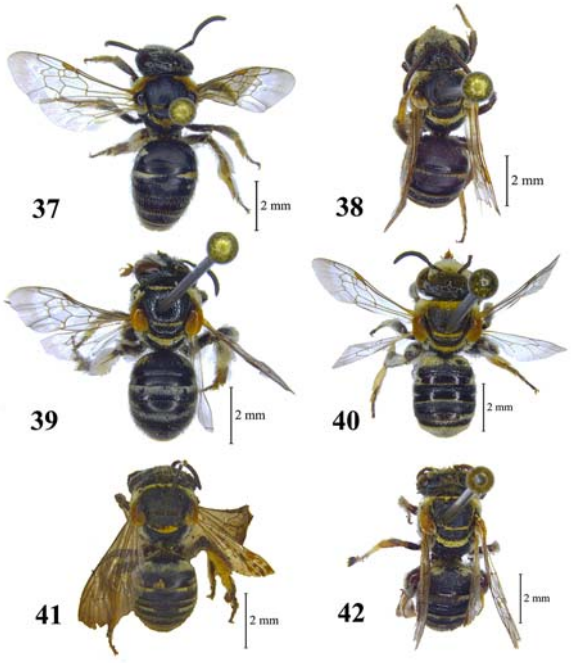
Figures 19–24. 19. ***Lipotriches*** **(*Lipotriches*)** ***bouceki*****, female; 20.** ***Lipotriches*** **(*Armatriches*)** ***fervida,*** **male; 21.** ***Lipotriches*** **(*Lipotriches*)** ***fulvinerva*****, female; 22,** ***Lipotriches*** **(*Lipotriches*)** ***fulvinerva*****, male; 23.** ***Lipotriches*** **(*Lipotriches*)** ***phenacura*****, female; 24,** ***Lipotriches*** **(*Lipotriches*)** ***phenacura*** **male.**



Figures 25–30. 25. ***Lipotriches*** **(*Rhopalomelissa*)** ***pulchriventris*****, female; 26,** ***Lipotriches*** **(*Rhopalomelissa*)** ***pulchriventris*****, male; 27.** ***Lipotriches*** **(*Rhopalomelissa*)** ***tubulisetae*****, male; 28.** ***Macronomia antennata*****, female; 29.** ***Macronomia antennata*****, male; 30.** ***Macronomia dilatata*****, male.**



Figures 31–36. 31. ***Macronomia karnatakaensis*** **female, 32.** ***Macronomia karnatakaensis*****, male; 33.** ***Macronomia walayarensis*****, male; 34.** ***Nomia curvipes*****, female; 35.** ***Nomia crassipes,*** **female; 36.** ***Nomia crassipes,*** **male.**



Figures 37–42. 37. ***Pachynomia aliena*****, female; 38.** ***Pachynomia aliena*****, male; 39.** ***Pseudapis oxybeloides*** **female; 40,** ***Pseudapis oxybeloides*****, male; 41.** ***Pseudapis patellata,*** **female; 42,** ***Pseudapis patellata*****, male.**

Figures 43–46. 43. ***Steganomus bipunctatus*****, female; 44.** ***Steganomus bipunctatus*****, male; 45.** ***Steganomus lieftincki,*** **female; 46.** ***Steganomus lieftincki,*** **male.**

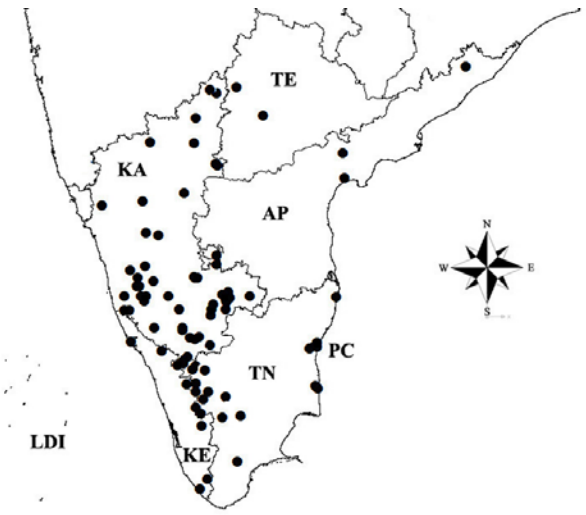


Figure 47. Summarized distributional map of bees belonging to Nomiinae recorded so far from south India. Current states and union territories of south India abbreviated as follows, AP: Andhra Pradesh, KA: Karnataka, KE: Kerala, TE: Telangana, TN: Tamil Nadu, LDI: Lakshadweep Islands and PC: Puducherry.

Distribution:

South India	Karnataka (Bengaluru)
Elsewhere in India	-
Outside India	-

***Lipotriches* (*Rhopalomelissa*) *ceratina* (Smith, 1857)**

No specimens examined.

Distribution:

South India	Puducherry (Karaikal)
Elsewhere in India	Orissa (Jeypore)
Outside India	China, Indonesia (Borneo, Sumatra, Sulawezi), Japan, Korea, Laos, Malaysia, Myanmar, Philippines, Taiwan, Thailand, Vietnam

***Lipotriches* (*Rhopalomelissa*) *exagens* (Walker, 1860)**

No specimens examined.

Distribution:

South India	Tamil Nadu (Coimbatore-Marudamalai Hills)
Elsewhere in India	Goa (Mormugao), Orissa (Jeypore), Punjab (Ludhiana), Rajasthan, Thar Desert
Outside India	Sri Lanka

***Lipotriches* (*Armatriches*) *fervida* (Smith, 1875)** (Fig. 20)

Specimens examined. INDIA: Karnataka: Raichur, 939 m, 16°15' N 77°20' E, 2♂, 25.viii.2013, coll. Veereshkumar.

Distribution:

South India	Andhra Pradesh, *Karnataka, Puducherry (Karaikal, Nettapakam), Tamil Nadu (Coimbatore), Telangana (Hyderabad)
Elsewhere in India	Gujarat (Deesa), Haryana, New Delhi (Sakeet), Punjab (Firozpur), Rajasthan (Mount Abu), Thar Desert, Uttar Pradesh (Agra, Allahabad)
Outside India	Pakistan, Sri Lanka

Floral records: *Tribulus terrestris*.

***Lipotriches* (*Lipotriches*) *fulvinerva* (Cameron, 1907)** (Fig. 21 & 22)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 12° 58' N 77° 35' E, 1♂, 11.ii.2014, coll. Unknown; Hebbal, 900 m, 13° 02' N 77° 35' E, 1♀, 20.xi.2014, 1♀, 4.xii.2014, 1♂, 31.x.2014, coll. Zameeroddin.

Distribution:

South India	*Karnataka, Puducherry (Karaikal), Tamil Nadu (Coimbatore)
Elsewhere in India	Assam (10 min N. of Tinsukia), Bihar, Gujarat (Deesa), Maharashtra, West Bengal (Barrackpore, Kanchrapara, Kolkata)
Outside India	Bangladesh, Myanmar, Pakistan, Sri Lanka

***Lipotriches* (*Rhopalomelissa*) *minutula* (Friese, 1909)**

No specimens examined.

Distribution:

South India	Tamil Nadu (Anamalai Hills, Cinchona)
Elsewhere in India	Maharashtra
Outside India	China, Indonesia (Borneo, Sumatra, Java, Sulawezi), Laos, Malaysia, Philippines, Thailand, Vietnam

***Lipotriches (Lipotriches) phenacura* (Cockerell, 1911)** (Fig. 23 & 24)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♂, 3.xi.1987, coll. K. Ghorpade; GKVK, 930 m, 12°58' N 77°35' E, 1♀, 8.vi.2012, coll. Veereshkumar; Hebbal, 900 m, 13° 02' N 77° 35' E, 2♀, 4.xii.2014, coll. Zameeroddin.

Distribution:

South India	*Karnataka, Kerala (Walayar Forest), Puducherry (Karikal), Tamil Nadu (Coimbatore)
Elsewhere in India	Maharashtra (Pune-Yerandavna, Nasik)
Outside India	Sri Lanka

***Lipotriches* (*Rhopalomelissa*) *pulchriventris* (Cameron, 1897)** (Fig. 25 & 26)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 12° 58' N 77° 35' E, 1♀, 21.vi.2011, 1♂, 28.vi.2011, coll. Girish; 1♂, 21.vi.2011, 1♀, 1.viii.2011, coll. E.D. nayana; 1♂, 24.xi.2011, coll. P. Nirmala; 1♂, i.vi.2014, coll. Students; Hebbal, 900 m, 13° 02' N 77° 35' E, 1♀, 20.xi.2014, 2♀, 16.xi.2014, 1♀, 5.ii.2015, coll. Zameeroddin; Lalbagh, 1♂, 9.vi.1976, coll. Students; Cubbon Park, 916 m, 1♂, 4.xi.1976, coll. A.R.V. Kumar. Hassan, karekere, 934 m, 13°06' N 76°10' E, 5♀, 23.vi.2014, coll. Prashantha, C; 3♀, 23.vi.2014, coll. Zameer.

Distribution:

South India	Karnataka (Bengaluru, *Hassan, Kodagu - Madikeri), Tamil Nadu (Nilgiri -Devala, Singara)
Elsewhere in India	Assam (Chabua, 6mi NW Digboi), Maharashtra (Nasik), Uttarakhand (Mussoorie, Kumaon), West Bengal (Kanchrapara)
Outside India	Australia (Queensland), China (Hainan, Hunan), Indonesia (Borneo, Sumatra, Java, Timor, Sulawezi, Moluccas), Laos, Malaysia, Nepal, New Guinea, Philippines, Solomon Islands, Sri Lanka, Thailand, Vietnam

***Lipotriches* (*Rhopalomelissa*) *taprobanae* (Cameron, 1897)**

No specimens examined.

Distribution:

South India	Puducherry (Auroville), Tamil Nadu
Elsewhere in India	-
Outside India	Sri Lanka (Colombo)

***Lipotriches* (*Rhopalomelissa*) *tubulisetae* Pauly, 2009** (Fig. 27)

Specimens examined. INDIA: Karnataka: Kodagu, Chettalli, 1001 m, 12°22' N 75°49' E, 1♂, 9.xi.2012, coll S. Ramani. Uttara Kannada, Sirsi, Unchalli falls, 510 m, 14°82' N 74°92' E, 1♂, 20.xi.2012, coll. Vinayaka, T; 1♂, 20.xi.2012, coll. R. Girish.

Distribution:

South India	Karnataka (Kodagu, *Uttara Kannada), Tamil Nadu (Coimbatore, Nilgiri Hills)
Elsewhere in India	-
Outside India	-

Genus *Macronomia* Cockerell, 1917

***Macronomia anamalaiensis* Pauly, 2009**

No specimens examined.

Distribution:

South India	Tamil Nadu (Chennai, Anamalai Hills-Kadamparai, Cinchona)
Elsewhere in India	-
Outside India	-

***Macronomia antennata* (Smith, 1875)**
(Fig. 28 & 29)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 12°58' N 77°35' E, 1♀, 26.ii.2015, Sunitha, N.D; 930 m, 13°04' N 77°34' E, 1♂, 23.x.2014, coll. Zameeroddin; Sadahalli, 906 m, 13°12'N 77°38' E, 1♀ & 1♂, 12.x.2014, 1♀ & 3♂, 30.x.2014, 1♀ & 1♂, 29.x.2014, 2♀ & 1♂, 04.xi.2014, 2♀ & 1♂, 17.xi.2014, 1♀, 5.xii.2014, 1♀ &1♂, 6.ii.2015, 3♂, 26.ii.2015, Zameeroddin. Raichur, 939 m, 16° 15' N 77° 20' E, 1♀, 27.ix.2014, coll. Veereshkumar. Kerala: Kannur, Madayipara, 38 m, 12°01' N 75°15' E, 1♂, 13.viii.2015, coll. Prashantha, C.

Distribution:

South India	*Karnataka, *Kerala
Elsewhere in India	Madhya Pradesh (Jabalpur), Maharashtra (Mumbai, Pune), Uttar Pradesh
Outside India	-

Floral record: *Tephrosia* sp.

***Macronomia dilatata* Pauly, 2009** (Fig. 30)

Specimens examined. Tamil Nadu: Tarapura, 277 m, 10°49' N 77°27' E, 1♂, 10.xi.2010, coll. A. N. Reddy.

Distribution:

South India	Kerala (Walayar Forest, Malabar), Tamil Nadu (Nilgiri Hills- Moyar Camp, Coimbatore-Marudamalai Hills)
Elsewhere in India	-
Outside India	-

***Macronomia karnatakaensis* Pauly, 2009**
(Fig. 31 & 32)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 13°04' N 77°34' E, 1♂, 5.xi.2014, coll. Zameeroddin. Chikkamagaluru, Mudigere, Ettina Bhuja, 1005 m, 12°59' N 75°35' E, 1♀, 14.xii.2013, coll. Najeer. Kolar, Horticulture College, 830 m, 13°07' N 78°10' E, 2♂, 16.xii.2014, coll. Pradeep.

Distribution:

South India	Karnataka (Bengaluru-Doddagubbi, Bannerghatta National Park, *Chikkamagaluru, *Kolar), Kerala (Walayar Forest), Tamil Nadu (Coimbatore)
Elsewhere in India	-
Outside India	Sri Lanka

***Macronomia madrasensis* Pauly, 2009**

No specimens examined.

Distribution:

South India	Tamil Nadu (Chennai)
Elsewhere in India	-
Outside India	-

***Macronomia nilgiriensis* Pauly, 2009**

No specimens examined.

Distribution:

South India	Tamil Nadu (Nilgiri Hills-Moyar Camp, Singara)
Elsewhere in India	-
Outside India	-

***Macronomia savannakheti* Pauly, 2009**

No specimens examined.

Distribution:

South India	Tamil Nadu (Nilgiri Hills-Singara, Gudabu)
Elsewhere in India	-
Outside India	Laos

***Macronomia walayarensis* Pauly, 2009** (Fig. 33)

Specimen examined. INDIA: Tamil Nadu: Burliar, 860 m, 1♂, 19.xi.2000, coll. K. Ghorpade.

Distribution:

South India	Kerala (Walayar Forest), *Tamil Nadu
Elsewhere in India	-
Outside India	-

Genus *Maynenomia* Pauly, 1984

***Maynenomia chalcea* (Cockerell, 1920)**

No specimens examined.

Distribution:

South India	Kerala (Manantoddy, Wynad)
Elsewhere in India	-
Outside India	-

***Maynenomia keralaensis* Pauly, 2009**

No specimens examined.

Distribution:

South India	Kerala (Walayar Forest), Puducherry (Karaikal), Tamil Nadu (Coimbatore, Marudamalai hills).
Elsewhere in India	-
Outside India	-

***Maynenomia lonavlaensis* Pauly, 2009**

No specimens examined

Distribution:

South India	Puducherry, Tamil Nadu
Elsewhere in India	Maharashtra (Lonavla)
Outside India	-

***Maynenomia nathani* Pauly, 2009**

No specimens examined

Distribution:

South India	Kerala (Walayar Forest), Puducherry (Indra Nagar), Tamil Nadu (Anamalai Hills, Cinchona)
Elsewhere in India	-
Outside India	Sri Lanka

Genus *Nomia* Latreille, 1804

***Nomia curvipes* (Fabricius, 1793)** (Fig. 34)

Specimen examined. INDIA: Gujarat: 19 km S. Baroda, 1♂, 18.ix.1990, coll. K. Ghorpade.

Distribution:

South India	Kerala (Walayar Forests), Puducherry (Karaikal), Tamil Nadu (Coimbatore, Kovilpatti, Nagapattinam)
Elsewhere in India	Gujarat (Baroda, Deesa), Madhya Pradesh, Maharashtra (Mumbai), Punjab (Ludhiana), Uttar Pradesh, West Bengal
Outside India	Pakistan (Karachi), Myanmar, Nepal

***Nomia crassipes* (Fabricius, 1798)**
(Fig. 35 & 36)

Specimens examined. INDIA: Karnataka: Bangalore, GKVK, 930 m, 13°04' N 77°34' E, 1♂, 22.x.2014, 1♀, 5.xi.2014, coll. Zameeroddin; 2♀, 10, 25.ix.2014, coll. Pradeep; 1♀, 22.x.2013, coll. Srinivas; 3♀, 10,12,19.x.2012, coll. Girish, R; 927 m, 13°5' N 77°34' E, 1♂, 7.v.2012, 1♂, 16.vi.2012, coll. Arun, B.C; Hessaraghatta, 1♀, 1.x.2005, coll. Suma, S; 1♀, 25.vii.2009, 1♂, 26.vii.2009, coll. Yeshwanth, H.M. Sadahalli, 906 m, 13°12'N 77°38' E, 1♀, 12.x.2014, 2♀ & 1♂, 30.x.2014, 1♂, 4.xi.2014, 1♀ & 1♂, 6.ii.2015, coll. Zameeroddin. Belgaum, Arabhavi, 582 m, 16°13' N 74°49' E, 1♀, 20.ix.2014, coll. Revansidda. Chikkamagaluru, Mudigere (Jannapura, YFC), 931 m, 29°10' N 75°46' E, 1♀, 3.iv.2012, coll. Y. Diwakar. Dakshina Kannada, Vittal, 1♂, 30.ix.2011, coll. Aswathy, T.V. Gulberga, 1♂, viii.1981, coll. A.R.V Kumar. Kerala: Kasaragod, Padannakkad, 14 m, 12°15' N 75°07' E, 2♀, 14.viii.2015, coll. Arati Pannure.

Distribution:

South India	*Karnataka, Kerala (*Kasaragod, Walayar

	Forest), Tamil Nadu (Coimbatore)
Elsewhere in India	Odisha (Jeypore), New Delhi (nr Sakeet), Maharashtra
Outside India	Bhutan, China, Pakistan, Sri Lanka, Taiwan, Thailand

Floral records: *Croton bonplandianum, Coffea arabica*

Genus *Nomiapis* Cockerell, 1919

***Nomiapis carcharodonta* (Baker, 2002)**

No specimens examined.

Distribution:

South India	Kerala (Walayar Forest)
Elsewhere in India	-
Outside India	-

Genus *Pachynomia* Pauly, 1980

***Pachynomia aliena* (Cameron, 1898)**
(Fig. 37 & 38)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♂, 24.vii.1984, coll. B. Mallik; 3♀, 18.ii.1978, fields, coll. k. Ghorpade; 934 m, 13°08' N 77°58' E, 3♂, 14-16.xi.1981, coll. B. Mallik; 1♂, 4.ix.1984, coll. Prasad; 930 m, 12°58' N 77°35'E, 5♀, 17-20.i.2014, coll. Arati Pannure; 2♀, 24.xi.2011, coll. Nayana, E.D; 3♀, 15.i.2013, coll. Girish, R; 2♀ & 1♂, 18.xi.2014, 1♂, 23.x.2014, 1♂, 5.i.2015, coll. Zameeroddin. Hebbal, 900 m, 13°02' N 77° 35' E, 1♀, 14.x.2014, 2♀, 31.x.2014, 2♀, 20.xi.2014, 1♀, 11.xi.2014, 1♀, 21.ii.2015, 1♀, 30.i.2015, 1♀, 9.iii.2015, coll. Zameeroddin; Sadahalli, 906 m, 13°12' N 77°38' E, 1♀, 12.x.2014, 1♀, 5.xii.2014, 3♀, 29.i.2015, coll. Zameeroddin. Gulberga, Bheemarayan Gudi, 454 m, 1♀, 16.viii.1981, coll. A.R.V Kumar. Mysore, COH, 824 m, 12°22' N 76°31' E, 1♀, 20.vii.2015, coll. Prashantha, C. Raichur, 421 m, 16° 12' N 77°22' E, 2♂, 19.ix.2014, coll. Veereshkumar. Kerala: Walayar, 2♀, 25.v.1982, coll. V.V. Belavadi.

Distribution:

South India	*Karnataka, Kerala (Walayar)
Elsewhere in India	Maharashtra (Nasik, Pune), Uttarakhand (Mussoorie)
Outside India	Sri Lanka

Floral records: Grasses, *Helianthus annuus*, *Solanum* sp.

***Pachynomia nathani* Pauly, 2009**

No specimens examined.

Distribution:

South India	Kerala (Walayar Forest), Tamil Nadu (Nilgiri Hills-Kallar)
Elsewhere in India	-
Outside India	Sri Lanka

Genus *Pseudapis* Kirby, 1900

***Pseudapis oxybeloides* (Smith, 1875)**
(Fig. 39 & 40)

Specimens examined. INDIA: Andrha Pradesh: Guntur, Ananthavaram, 105 m, 16°30' N 80°26' E, coll. Girish, R. Karnataka: Bangalore, GKVK), 930 m, 12°58' N 77°35' E, 2♂, 24.xi.2011, coll. Nayana, E.D; 1♀, 21.v.2012, coll. Arun, B.C; 1♀, 20.i.2014, coll. Arati Pannure; 1♀, 5.i.2015, coll. Prashanth, C. Belgaum, Arabhavi, 595 m, 16°13' N 74°50' E 17.viii.2011, coll. H.M. Yeshawant. Chikkamagaluru, Mudigere, ARS, 930 m, 13°13' N 75°65' E , 1♀, 12.xi.1985, coll. T. Shivashankar. Dakshina Kannada, Puttur (DCR), 2♀ & 1♂, 25.ii.2015, coll. K. Vanitha; 90 m, 12°45' N 75°01' E, 3♀ & 3♂, 3.iii.2015, coll. Prashanth, C; Moodabidri, 80 m, 13°06' N 75°06' E, 2♀, 25.vii.2015, coll. Prashantha, C. Koppal, Munirabad, 466 m, 15°33' N 76°33' E, 1♀, 5.xii.2012, coll. Najeer. Mangalore, Ullal (ARS), 6 m, 12°81' N 74°84' E, 2♀, 16.xi.1983, 1♀, 28.xii.1985, coll. B, Mallik. Mysore, Hunsur, 3♂, 17.i.2009, coll. Dhanyavathi, P.N. Shimoga (40 km SW), 1♀ & 1♂, 21.v.2008, coll. Nayana, E.D; Navile, 640 m,

2♀ & 1♂, 24.xii.2014, coll. Sunil Rathod. Tumkur, Pavagada, 876 m, 14°5' N 77°21' E, 1♀, 17.i.2012, coll. Arun, B.C. Kerala: Kannur, Madayipara, 38 m, 12°01' N 75°15' E, 1♀, 13.viii.2015, coll. Prashantha, C. Pondicherry, 1♀, 19.ix.1979, coll. ARV Kumar. Tamil Nadu: Dindigul (Gandhi gram), 331 m, 10°16' N 77°56' E, 1♂, 17.x.2010, coll. A.N. Reddy.

Distribution:

South India	*Andhra Pradesh (Guntur), Karnataka, Kerala (Kannur), Puducherry (Karaikal), Tamil Nadu (Annamalai hills, Chennai, Coimbatore, *Dindigul, Thanjavur)
Elsewhere in India	Gujarat (Banaskantha, Deesa), Maharashtra (Bombay, Lonavla, Khandala, Nasik, Pune), Madhya Pradesh (Jabalpur), Punjab (Ludhiana), Rajasthan (Mount Abu, Udaipur), Uttar Pradesh (Allahabad), West bengal (Kolkata)
Outside India	Pakistan to Bangladesh, Sri Lanka

Floral records: *Anacardium occidentale, Sorghum bicolor,* Grasses, *Tridex procumbens, Aegle marmelos, Callistemon citrinus.*

***Pseudapis patellata* (Magretti, 1884)**
(Fig. 41 & 42)

Specimens examined. INDIA: Karnataka: Mandya, VC farm, 727 m, 12°48' N 76°74' E, 1♀, 10.viii.1982, coll. B. Mallik. Mysore, Megalapura, 605 m, 12°39' N 77°13' E, 1♂, 8.viii.1982, coll. B. Mallik. Tamil Nadu: Kodaikanal (Bryant Park), 1♀, 15.xii.1985, coll. T. Shivashankar.

Distribution:

South India	*Karnataka, Puducherry (Karaikal), Tamil Nadu (Coimbatore, *Kodaikanal, Thanjavur- Kurumbagarm)
Elsewhere in India	-
Outside India	Africa, Oman, Saudi Arabia, Sudan, UAE, Yemen

Genus *Steganomus* Ritsema, 1873

***Steganomus bipunctatus* (Fabricius, 1804)**
(Fig. 43 & 44)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♀, 25.xi.1989, coll. K. Ghorpade; GKVK, 921 m, 13°04' N 77°34' E, 1♀, 12.x.2012, 1♀, 28.xi.2012, 1♂, 4.iii.2013, coll. Girish, R; 2♀, 10.ix.2014, coll. Pradeep; 3♀, 17.ix.203, coll. Najeer; Sadahalli, 906 m, 13°12'N 77°38' E, 1♀, 15.ii.2015, coll. Zameeroddin. Chikkamagaluru, Kadur, 758 m, 13°33' N 76°49' E, 1♀, 24.xi.2014, coll. Prashantha, C; Mudigere, Jannapura, 931 m, 29°10'N 75°46' E, 1♀, 3.iv.2012, coll. Y. Diwakar. Hassan, karekere, 934 m, 13°06' N 76°10' E, 1♂, 24.vi.2014, coll. Prashantha, C. Shimoga, Navile, 640 m, 13°93' N 75°56' E, 6♀, 24.xii.2014, coll. Prashantha, C. Ramanagara, Magadi, Savandurga, 12°55' N 77°16' E, 1♂, 15.x.2009, coll. Yeshwanth, H.M. Mysore, COH, 824 m, 12°22' N 76°31' E, 1♀ & 1♂, 21.viii.2015, coll. Prashantha, C.

Distribution:

South India	*Karnataka, Tamil Nadu
Elsewhere in India	Punjab (Ludhiana), Uttar Pradesh (near Lucknow), West Bengal (Barrackpore)
Outside India	Pakistan, Sri Lanka.

Floral records: *Phaseolus vulgaris*

***Steganomus gracilis* Cameron, 1898**

No specimens examined.

Distribution

South India	Puducherry (Karaikal)
Elsewhere in India	Uttarakhand
Outside India	Sri Lanka

***Steganomus lieftincki* Pauly, 2009** (Fig. 45 & 46)

Specimens examined. INDIA: Karnataka: Bangalore, 916 m, 1♀, 10.x.1988, coll. K. Ghorpade; GKVK, 930 m, 13°04' N 77°34' E, 1♂, 12.xi.2014,

coll. Zameeroddin; 1♀, 11.x.2012, coll. Prashantha, C; 900 m, 13°04' N 77°34' E, 1♀, 29.xi.2014, 1♀, 11.x.2012, coll. Prashantha, C; 876 m, 13°04' N 77°34' E, 1♀, 8.xi.2011, coll. Arun, B. C; Sadahalli, 906 m, 13°12'N 77°38' E, 1♂, 30.x.2014, 3♀, 4.xi.2014, coll. Zameeroddin. Belgaum, Arabhavi, 582 m, 16°13' N 74°49' E, 2♀ 20.ix.2014, coll. Revansidda. Hassan, karekere, 934 m, 13°06' N 76°10' E, 1♂, 23.vi.2014, coll. Prashantha, C.; 1♀, 23.vi.2014, coll. Zameeroddin. Kolar, Horticulture College, 830 m, 13°07' N 78°10' E, 1♂, 16.xii.2014, coll. Pradeep; 3♀ & 1♂, 16.xii.2014, coll. Arati Pannure. Tumkur, Ankasandra, 1086 m, 13°32' N 76°53' E, 1♀, 26.xii.2012, coll. Prashantha, C; Yarabahalli, 816 m, 2♀ & 2♂, 29.vii.2004, coll. Veereshkumar.

Distribution:

South India	*Karnataka
Elsewhere in India	New Delhi, Ranchi (Namkum), Eastern Rajasthan, West Bengal (Barrackpore)
Outside India	-

Floral records: *Grewia hirsuta*

DISCUSSION

Bees, like most other insects in India, have been largely ignored, except for a few noteworthy literature resources, proper taxonomic and distributional studies on this economically imperative insect group are lacking. Bingham's (1897) work had almost exclusively been limited to the northern and eastern parts of India. Online checklist by Gupta (2010) and checklist of halictid bees by Saini and Rathor (2012) are without complete species list, localities or details of distri-bution. Since no comprehensive list of these bees from south India has been ever published, the present list has been compiled with updated information from different south Indian states keeping in mind the recent nomenclatural changes by Pauly (2009). Altogether, 48 species belonging to 13 genera of Nomiinae bees are reported, among them sixteen species are reported for the first time for Karnataka, three species for Andhra Pradesh, two species for Tamil Nadu and one species each for Kerala and Telangana. *Curvinomia strigata* (Fabricius, 1793), *Macronomia antennata* (Smith, 1875) and *Steganomus lieftincki* Pauly, 2009 are newly recorded from south India. The current nomiinae bee diversity in south India represents around 8.2 % and 31.8 % of world and Oriental fauna respectively. Two species of *Macronomia,* one species each of *Lipotriches, Maynenomia* and *Nomia* remain unidentified.

The study also indicates that most areas of the south Indian states like Andhra Pradesh, Telangana, Tamil Nadu, Northern parts of Karnataka and Kerala are unexplored for bees (Fig. 47). Considering the Western Ghats and Eastern Ghats are unknown for bees, the true number of species in the region could be undeniably larger than reported here. There is further scope of discovery of many more bees from the study area, if more extensive and intensive surveys of the unexplored areas are undertaken. The updated checklist of the Nomiinae bees of south India presented here therefore can facilitate future research of this area.

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Seasonal incidence of sucking insect pests and their association with predatory coccinellid beetles on bitter gourd

Sunil*, M. Thippaiah, K. S. Jagadish and A. K. Chakravarthy#

Department of Agricultural Entomology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru 560065, India; #Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru 560090, India. Email: rathodsunil915@gmail.com

ABSTRACT: The seasonal incidence of sucking insect pests (aphid, leafhopper, thrips and whitefly) on bitter gourd and their association with predatory coccinellid beetles was studied during *kharif* and *rabi* seasons, 2014-15. The mean population of aphid, leafhopper, thrips and whitefly varied from 0.40, 0.65, 0.30 and 0.60 in *kharif*, 3.86, 1.66, 1.50 and 0.11 in *rabi*, respectively. Similarly the numbers of predatory coccinellid beetles varied from 0.15 in *kharif* and 0.48 in *rabi*. The incidence of aphids, leafhopper and predatory coccinellids were positively correlated (r = 0.85, 0.62, 0.86) respectively, with maximum temperature. The association of sucking pests and predatory coccinellids revealed a positive correlation. A significant positive correlation existed between aphid and predatory coccinellid beetles (r = 0.69 and r = 0.94 per cent) during *kharif* and *rabi* season, respectively. These results showed that increase in the incidence of sucking insect pests led to increased population of predatory coccinellid beetles on bitter gourd. Numbers of predatory beetles and other natural enemies should maintain populations of sucking pests below economic injury level on bitter gourd.

KEYWORDS: Bitter gourd, seasonal incidence, sucking insect pests, predatory coccinellids

INTRODUCTION

Bitter gourd, like other cucurbits, is attacked by a wide array of insect and non-insect pests, the major being fruit fly, red pumpkin beetle, *Epilachna* beetle, whitefly, aphids and thrips. Infestation by these pests is an important limiting factor in the commercial cultivation of the crop. Attack of these pests begin at very early stage of crop growth and continues till harvest and degree of infestation depends upon prevailing agronomic conditions (Vandana *et al*., 2001). Sucking insect pests like aphids, whitefly, thrips and leafhoppers attack the crop throughout the growth period resulting in the reduction of yields. So management interventions are required to save the yield loss. Coccinellids are used as an effective predator for sucking insect pest management (Elliott and Kieckhefer, 1990). The beetles prey on a number of species of aphids on different host plants (Sakuratani, 1977; Winder *et al*., 1994). The lady beetles are predacious both at larval and adult stages and feed on pests such as aphids, brown plant hopper and thrips (Rawat and Modi, 1969; Sumalde *et al*., 1993). This paper deals with the seasonal incidence of sucking insect pests on bitter gourd and determines the role of predatory coccinellid beetles in suppressing sucking pest populations. The present hypothesis of the investigation was that predatory beetles do effectively suppress sucking pests on bitter gourd.

* Author for correspondence

MATERIALS AND METHODS

The present investigation was conducted during *kharif* and again during *rabi* season of 2014-15 at the Department of Horticulture , UAS, GKVK, Bengaluru (12° 58' N lati and 77° 35' E long, at an alti of 930 m AMSL) and at IIHR, Hesaraghatta, Bengaluru (13° 13' N lati and 77° 48' E long, at an alti of 890 m AMSL), respectively. For this study, seeds of the variety 'Arka Harit' were sown during second week of August (*kharif)* and during second week of November (*rabi*). The experiment was laid out in randomised block design with three replications with a plot size 8 m X 11 m. To record observations on sucking insect pests populations, ten plants per plot (total n = 30) were randomly selected, labelled and on each selected plant, three leaves each from top, middle and lower parts were observed. The observations on pest activity and predatory coccinellid beetles were recorded at weekly intervals.

Standardised sampling procedures were adopted while counting the insects on bitter gourd. For sucking pests [aphids (*Aphis gossypii* Glover), leafhoppers (*Empoasca motti* Pruthi, *Amrasca biguttula biguttula* (Ishida)), white flies (*Bemicia tabaci* Gennadius) and thrips (*Thrips palmi* Karny)], observations were recorded by counting the number of nymphs and adults on three leaves i.e., one each from top, middle and bottom canopy of the selected and labelled plants (Barma and Jha, 2013; Mari and Bugti, 2016; Singh *et al*., 2013).

The adult and grub stages of two predatory coccinellid beetles (*Cheilomenes sexmaculata* Fab. and *Coccinella transversalis* Fab.) were counted on thirty randomly selected whole plants at weekly intervals during *kharif* and *rabi* season on bitter gourd plants (Vennila *et al*., 2007; Patel and Purohit, 2014).

The data were statistically ananlysed by correlation analysis between sucking insect pests, predatory coccinellid with weather parameter and also between sucking insect pest with predatory coccinellid beetle. The data on sucking insect pest and predatory coccinellid were subjected to multiple regression analysis to know their association (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

During *kharif* season, aphid numbers on bitter gourd varied from 0.00 to 1.73 with a mean of 0.40 aphids per three leaves per plant. However, higher aphid numbers were recorded during first week of November (45th SW). Similarly, during *rabi* season, the aphids numbers varied from 0.00 to 13.33 with a mean of 3.86 aphids per three leaves per plant of bitter gourd. However, the maximum numbers of aphids were observed during first week of March (9th SW) (Tables 1 and 2). The correlation studies revealed that, during *kharif*, weak positive correlation existed between incidence of aphids and maximum temperature (r = 0.08), whereas, negative correlation was observed with minimum temperature (r = -0.49), maximum RH (r = -0.005) and minimum RH (r = -0.40). However, during the *rabi* season, significant positive correlation existed between aphids and maximum temperature (r = 0.85), whereas, significant negative correlation existed between minimum temperature (r = -0.77), maximum RH (r = -0.86) and minimum RH (r = -0.79) (Tables 3 and 4). These observations are in conformity with the observations made earlier by Chakraborthy (2011) who reported that abiotic factors such as temperature and relative humidity significantly influenced *A. gossypii* population on tomato crop.

During *kharif*, leafhopper numbers varied from 0.13 to 2.60, with a mean of 0.65 per three leaves per plant. Similarly, during *rabi* season, leafhopper numbers varied from 0.50 to 3.26, with a mean of 1.66 leafhoppers per three leaves per plant (Tables 1 and 2). During *kharif*, a weak positive correlation existed between the infestation of leafhoppers and minimum temperature (r = 0.24) and minimum relative humidity (r = 0.09). However, non-significant negative correlation was observed between the infestation of leafhoppers with maximum temperature (r = 0.10), maximum relative humidity (r = -0.04) and rainfall (r = -0.29). Moreover, during the *rabi* season, a significant positive correlation existed between leafhoppers and

Table 1. Seasonal incidence of sucking pests and predatory coccinellids on bitter gourd at GKVK during *kharif*, 2014

Month	Standard week	Aphids*	Leaf hoppers*	Thrips*	Whitefly*	Coccinellids**
August	35	0.00	0.16	0.00	0.00	0.00
	36	0.00	1.70	0.40	0.33	0.00
September	37	0.00	2.60	0.76	0.50	0.00
	38	0.00	1.40	0.40	0.30	0.06
	39	0.00	0.56	0.50	0.56	0.16
	40	0.00	0.13	0.10	1.16	0.00
October	41	0.43	0.16	0.26	1.33	0.16
	42	1.40	0.13	0.43	1.03	0.26
	43	0.16	0.26	0.00	0.63	0.00
	44	0.00	0.13	0.00	0.00	0.16
November	45	1.73	0.13	0.26	0.93	0.30
	46	1.10	0.50	0.50	0.50	0.73
	Mean	0.40	0.65	0.30	0.60	0.15
	Max	1.73	2.60	0.76	1.33	0.73
	Min	0.00	0.13	0.00	0.00	0.00
	SD	0.63	0.80	0.24	0.43	0.21

*Mean no./ 3leaves/plant **mean no./plant

Table 2. Seasonal incidence of sucking pests and predatory coccinellids on bitter gourd at Hessaraghatta during *rabi*, 2014 - 15

Month	Standard week	Aphids*	Leaf hoppers*	Thrips*	Whitefly*	Predatory Coccinellids**
November	47	0.00	0.50	0.20	0.00	0.00
	48	0.00	0.66	0.60	0.00	0.00
December	49	0.60	0.83	0.66	0.00	0.13
	50	0.50	0.83	2.50	0.00	0.20
	51	1.16	0.83	0.96	0.13	0.13
	52	1.33	1.46	1.03	0.60	0.26
January	01	1.50	1.86	1.83	0.33	0.26
	02	1.46	0.83	1.63	0.30	0.36
	03	1.83	1.93	3.40	0.26	0.43
	04	4.13	2.33	2.53	0.00	0.50
February	05	6.33	2.43	1.60	0.00	0.60
	06	7.66	2.83	1.57	0.06	0.83
	07	6.50	2.30	1.30	0.00	1.10
	08	11.66	2.10	1.36	0.00	1.30
March	09	13.33	3.26	1.40	0.00	1.16
Mean		3.86	1.66	1.50	0.11	0.48
Max		13.33	3.26	3.40	0.60	1.16
Min		0.00	0.50	0.20	0.00	0.00
SD		4.30	0.87	0.82	0.18	0.42

*Mean no./ 3leaves/plant **mean no./plant

maximum temperature (r = 0.62), whereas, significant negative correlation existed between leafhopper and minimum temperature (r = -0.83), maximum RH (r = -0.57) and minimum RH (r = -0.78) (Tables 3 and 4). These observations are in agreement with the observations of Deepika *et al*. (2013) who observed that leafhoppers population was significantly and positively correlated with maximum temperature. The infestation of leafhoppers was negatively correlated with rainfall. During *kharif*, thrips numbers varied from 0.00 to 0.76, with a mean of 0.30 thrips per three leaves per plant. Similarly, during *rabi*, thrips numbers varied from 0.20 to 1.50, with a mean of 1.50 thrips per three leaves per plant (Tables 1 and 2). During *kharif*, a non-significant negative correlation existed between the thrips incidence and maximum temperature (r = -0.22), minimum temperature (r = -0.01), maximum relative humidity (r = -0.04), minimum relative humidity (r = -0.12) and rainfall (r = -0.03). Similarly, during *rabi*, non-significant positive correlation was observed between thrips population and maximum temperature (r = 0.08) and non-significant negative correlation existed between thrips and minimum temperature (r = -0.25), maximum RH (r = -0.03) and minimum RH (r = -0.25) (Tables 2 and 4). This observation is in agreement with observations of Krishna Kumar *et al*. (2006) who reported the population of thrips increased from three to six weeks after sowing of watermelon. During *kharif*, whitefly numbers varied from 0.00 to 1.33 with a mean of 0.60 whitefly per three leaves per plant. Similarly, during *rabi*, whitefly numbers varied from 0.00 to 0.60, with a mean of 0.11 whitefly per three leaves per plant (Tables 1 and 2). During *kharif*, a non-significant positive correlation existed between incidence of whitefly and maximum temperature (r = 0.39), minimum temperature (r = 0.05) and rainfall (r = 0.23). While, non-significant negative correlation was observed with maximum RH (r = -0.35) and minimum RH (r = -0.12). Similarly, during *rabi*, positive correlation existed between whitefly population and minimum temperature (r = 0.34), maximum RH (r = 0.29) and minimum RH (r = 0.36) (Tables 3 and 4). This observation was similar to that of Lekshmi *et al*. (2014), who reported that the maximum and minimum temperatures were significantly and negatively correlated with the population build-up of whitefly.

The number of predatory coccinelids during *kharif* season ranged from 0.00 to 0.73, with a mean of 0.15 per plant. During *rabi* season their numbers ranged from 0.00 to 1.16 with a mean of 0.48 beetles per plant (Tables 1 and 2). During *kharif,* predatory coccinellid beetle population was non-significantly and positively correlated with rainfall (r = 0.61) and non-significant and negatively correlated with maximum temperature (r = -0.22) and maximum RH (r = -0.30). The relationship was significantly and negatively correlated with minimum temperature (r = -0.71) and minimum RH (r = -0.61). In the *rabi* season, significant positive correlation existed between coccinellid population and maximum temperature (r = 0.86). Significant negative correlation existed between coccinellids and minimum temperature (r = -0.80), maximum RH (r = -0.77) and minimum RH (r = -0.74) (Tables 3 and 4). These results are in conformity with Singh *et al*. (2013) who reported that coccinellid beetle population had a negative correlation with minimum and mean temperature, rainfall and maximum and minimum RH. Khuhro *et al*. (2014) revealed that temperature had overall positive impact on all the insect pests and their predators on tomato crop.

During *kharif* season the aphid population was significantly and positively correlated with the predatory coccinellid population (r = 0.69). Similarly, during *rabi* season the aphid population was significantly positively correlated with predatory coccinellid population (r = 0.94) (Tables 5 and 6). These results were in agreement with the findings of Patel and Purohit (2014) where predatory coccinellid beetles had significant positive correlation with aphids during *kharif* and *rabi* season in sorghum crop. Similarly, Singh *et al*. (2013) reported that the predatory coccinellid beetles showed positive correlation with aphid population and maximum temperature in okra ecosystems. The multiple linear regression equation suggests that the predatory coccinellid population on bitter gourd crop was influenced to an extent of 47 per cent due to aphid population during *kharif* and 89 per cent during *rabi* (Figures 1 and 2). Similarly, leafhopper population

Table 3. Correlation between sucking pests and predatory coccinellids in bitter gourd with weather parameters during *kharif,* 2014

Weather parameters	Aphids	Thrips	Leaf hoppers	Whitefly	Predatory coccinellids
Maximum tem. (°C)	0.085	-0.22	-0.10	0.39	-0.22
Minimum tem. (°C)	-0.49	-0.01	0.24	0.05	-0.73**
Maximum RH (%)	-0.05	-0.08	-0.04	-0.35	-0.30
Minimum RH (%)	-0.40	-0.39	0.09	-0.12	-0.61*
Rainfall (mm)	-0.05	-0.03	-0.29	0.23	0.61

**Correlation is significant at P ≤ 0.01 level (2-tailed); *.Correlation is significant at the P ≤ 0.05 level (2-tailed)

Table 4. Correlation between sucking pests and predatory coccinellids in bitter gourd with weather parameters during *rabi*, 2014 - 15

Weather parameters	Aphids	Thrips	Leaf hoppers	Whitefly	Predatory coccinellids
Maximum tem. (°C)	0.85**	0.089	0.62**	-0.20	0.86**
Minimum tem. (°C)	-0.77**	-0.25	-0.83**	0.34	-0.80**
Maximum RH (%)	-0.86**	-0.03	-0.57*	0.29	-0.77**
Minimum RH (%)	-0.79**	-0.25	-0.78**	0.36	-0.74**
Rainfall (mm)	-	-	-	-	-

**Correlation is significant at P ≤ 0.01 level (2-tailed); *.Correlation is significant at the P ≤ 0.05 level (2-tailed)

Table 5. Correlation between aphids, leafhoppers, thrips and whitefly numbers with predatory coccinellids during *kharif,* 2014

	Correlation	Regression equation	R^2 value
Aphids Vs. Predatory coccinellids	0.69*	Y = 0.06 + 0.22 Aphids	0.47
Leafhoppers Vs Predatory coccinellids	-0.28	Y = 0.20 - 0.07 Leafhoppers	0.08
Whitefly Vs Predatory coccinellids	0.26	Y = 0.08 + 0.23 Thrips	0.07
Thrips Vs Predatory coccinellids	0.12	Y = 0.11 + 0.06 Whitefly	0.015

**Correlation is significant at P ≤ 0.01 level (2-tailed); sucking pests numbers/3 leaves/plant with predatory coccinellids per plant

Table 6. Correlation between aphids, leafhoppers, thrips and whitefly with predatory coccinellids during *rabi,* 2014-15

	Correlation	Regression equation	R^2 value
Aphids Vs. Predatory coccinellids	0.94*	Y = 0.12 + 0.09 Aphids	0.89
Leafhoppers Vs Predatory coccinellids	0.81*	Y = -0.17 + 0.39 Leafhoppers	0.66
Whitefly Vs Predatory coccinellids	0.18	Y = 0.33 + 0.09 Thrips	0.03
Thrips Vs Predatory coccinellids	-0.26	Y = 0.55 - 0.62 Whitefly	0.06

**Correlation is significant at P ≤ 0.01 level (2-tailed); sucking pests numbers/3 leaves/plant with predatory coccinellids per plant

during *kharif* season was negatively correlated with the predatory coccinellids (r = -0.28).

Similarly, during *rabi* season the pest population was significantly and positively correlated with predatory coccinellids population (r = 0.81) (Tables 5 and 6). The multiple linear regression equation suggests that the incidence of predatory coccinellid population on bitter gourd crop was influenced by 8 per cent due to leafhopper during *kharif* and 66 per cent during *rabi* (Figs. 1 and 2). During *kharif* season the thrips population was positively

correlated with the predatory coccinellids population (r = 0.12). Similarly, during *rabi* season the pest population was negatively correlated with predatory coccinellids population (r = -0.26) (Tables 5 and 6). The multiple linear regression equation suggests that the incidence of predatory coccinellids population on bitter gourd crop was influenced by thrips to the extent of 1.5 per cent during *kharif* and 6 per cent during *rabi*, by thrips population (Figs. 1 and 2). During *kharif* season the thrips population was positively correlated with the predatory coccinellids population (r = 0.12). During *rabi* season the pest population was negatively correlated with predatory coccinellids population (r = -0.26) (Tables 5 and 6). The multiple linear regression equation suggests that the numbers of predatory coccinellid beetles on bitter gourd crop was influenced by thrips to an extent of 1.5 per cent during *kharif* and 6 per cent during *rabi* (Figs. 1 and 2). From the above results, it is confirmed that the numbers of predatory coccinellids was influenced by increasing population of sucking insect pests especially aphids and leafhoppers. The above results are similar with the findings of Solangi *et al*. (2008).

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First report of tomato pinworm, *Tuta absoluta* (Meyrick) on egg plant *Solanam melongena* L. from Kerala, India

T. Sivakumar*, A. Josephrajkumar and N. Anitha*****

** ICAR-KVK- Alappuzha, ICAR-CPCRI (RS), Kayamkulam 690 533, Kerala; **Division of Crop Protection, ICAR-CPCRI(RS), Krishnapuram, Kayamkulam 690 533, Kerala; *** Department of Agrl. Entomology, College of Agriculture, Vellayani 695522 Thiruvananthapuram, Kerala*
Email: sivanagri@yahoo.co.in

ABSTRACT: Invasive insect pests are threat to native flora as well as cultivated crops all around the globe. *Tuta absoluta* is a recent invasion to India and caused economic damage to tomato in South-Central India. The pest was noticed on *Solanum* melongena L. in southern most part of the Country and caused heavy defoliation of crops. A promising native ant species, *Diacamma rugosum* was found feeding on pupae from the ventral surface of the leaf. The ant predator could check the pest infestation in one plot. This the first report of the pest on eggplant and its ant predator from this part of the world. © 2017 Association for Advancement of Entomology

KEY WORDS: *Tuta absoluta*, invasive pest, brinjal, predator, *Diacamma rugosum*

IUCN defines an invasive alien species as a species that is established outside of its natural past or present distribution, whose introduction and/or spread threaten biological diversity. Even though these invasive species include all categories of living organisms, plants and mammals, insects comprise the most common invasive alien species (Reghubanshi *et al*., 2005). The tomato pin worm or leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a serious invasive pest of tomato, *Lycopersicum esculentum* Mill. (Pereyra and Sánchez, 2006). Identified first in South America in 1917, *T. absoluta* has spread to Europe, North Africa (Desneux *et al*., 2010) and Indian sub-continent (Shashank *et al*., 2015). Active as well as passive means of dispersal, apart from human assisted transfer were reported by Desneux *et al*. (2011).

Apart from tomato, host range of *T. absoluta* include other Solanaceous crops such as brinjal or eggplant *Solanum melongena*; potato, *Solanum tuberosum* (L.), sweet pepper, *Solanum muricatum* L. and tobacco *Nicotiana tabacum* L. (Vargas 1970; Campos 1976), non-cultivated Solanaceae (*S. nigrum* L., *S. eleagnifolium* L., *S. bonariense* L., *S. sisymbriifolium* Lam., *S. saponaceum*, *Lycopersicum puberulum* Ph. etc.) and other plants such as *Datura ferox* L., *D. stramonium* L. and *Nicotiana glauca* Graham (Garcia and Espul 1982; Larraý´n 1986). *T. absoluta* has been reported on bean *Phaseolus vulgaris* in Italy (EPPO 2009). *Chenopodium album* L. (Fa. Convolvulaceae) and *Capsicum annuum* L. were also recorded as a host of *T. absoluta* from Turkey (Portakaldali *et al*., 2013).

This micro-lepidopteran has caused immense damage to tomato crop in all invaded regions incurring a crop loss up to 80-100 per cent in tomato (Desneux *et al*., 2010; Shashank *et al*., 2015). Coupled with indiscriminate use of insecticide and environmental hazard, excess interventions by insecticide usage accounted for huge expenses in

* Author for correspondence

pest management in tomato crop (Desneux *et al*., 2011).

In India, the pest was reported from Karnataka (Sridhar *et al*., 2014), Maharashtra (Shashank *et al*., 2015), Andra Pradesh (Kalleshwaraswamy *et al*., 2015), Telangana (Kumari *et al*., 2015) and of late, from Tamil Nadu (Shanmugam *et al*., 2016). These studies revealed the attack of *T. absoluta* on tomato plants under open conditions. But from across the globe pest infestation was observed both in green house and open conditions. (Desneux *et al*., 2010).

Field observations in farmers' plot during 2015 and 2016 in Southern Kerala, India revealed the presence of some leaf miners on egg plant, *S. melongena*. Further microscopic observations confirmed it as a lepidopteran pest. The larvae were reared out and morphometric observations were made. The identity of the pest was confirmed as *T. absoluta* with the taxonomic experts and using the keys explained by Roditakis *et al*. (2010).

Apical, tender leaves of egg plants were seen affected by the pest. Symptoms appeared as irregular blisters on dorsal leaf surface (Plate 1). Larvae were found feeding on the internal mesophyll tissues by remaining within the galleries formed. As many as twenty six blisters harbouring larvae of different life stages were noticed on a single leaf of *S. melongena*. Complete destruction of severely affected plants was also noticed. No fruit infestation was observed as reported in tomato (Ballal *et al*., 2016). Low to severe infestation by *T. absoluta* was observed from different locations in Thiruvananthapuram, Kollam, Pathanamthitta and Alappuzha districts in Southern Kerala. Lack of knowledge and skill in identifying the symptoms of the pest by the growers and field extension staff made the pest infestation went unnoticed. The tomato plants adjacent to the brinjal plants were not affected by the pest.

Pupation of *T. absoluta* was reported in soil, leaf surface or within mines (EPPO, 2009). But we observed pupation on the ventral surface of the leaves, near to main vein of the leaf in brinjal (Plate 2). Under laboratory rearing conditions also the same behavior was observed. The morphometrices of larvae, pupae and adult are presented in table.1. Full grown larvae at prepupal stage had a mean length of 5.52±0.787 mm. The pupae had 4.0±0.432 mm length and 1.34±0.075 mm breadth (Plate 3). Adults were dark straw coloured swift flying tiny moths with wings folded parallel to their body while resting (Plate 4). Adult moths had long sharply bicoloured (with dark and light colouration) filiform antennae and fringed wings with a body length of 3.96±0.233mm from head to wing tip. Pupal period lasted 5 to 7 days.

Infestation by *T. absoluta* resulted in seared appearance of leaves which finally dried and fell off immaturely. Nevertheless eggplant was reported as a host plant, this observation proves the potential of causing regional wise crop specific damage by the pest. The pest has developed resistance against a number of conventional insecticides as well as new generation pesticides (Lietti *et al.,* 2005; Silva *et al.,* 2011) which forms a major impediment in the control strategies. The multivoltine nature, high dispersal rate and r-related species status (Pereyra and Sánchez, 2006) coupled with insecticide resistance make the management strategies against the pest more expensive (Desneux *et al*., 2011). Several natural enemies had been reported on *T. absoluta* from different regions of the World (Desneux *et al*., 2010). Spiders (*Argiope* sp), mirid bug *Nesidiocoris tenuis* (Reuter), parasitoids such as *Trichogramma achaeae* Nagaraja and Nagarkatti, *Neochrysocharis formosa* (Westwood), *Habrobracon* sp. and *Goniozus* sp were also reported as natural enemies of *T. absoluta* from India (Sridhar *et al*., 2014; Kumari *et al*., 2015; Ballal *et al*., 2016). In our field observations, the pupal cases were found bitten along with an incision on the leaf blade around the cocoon. Further investigations revealed the presence of an ant predator, *Diacamma rugosum* Le Guillou (Hymenoptera: Formicidae) (Plate 5). The robust black ant was found predating on cocoon of *T. absoluta* by biting open the cocoon with its mouth parts and also found carrying the pupae on their mandibles. The ants attacked the pupae not in groups, but singly. One ant was found consuming as many as three cocoons in 45 minutes. All

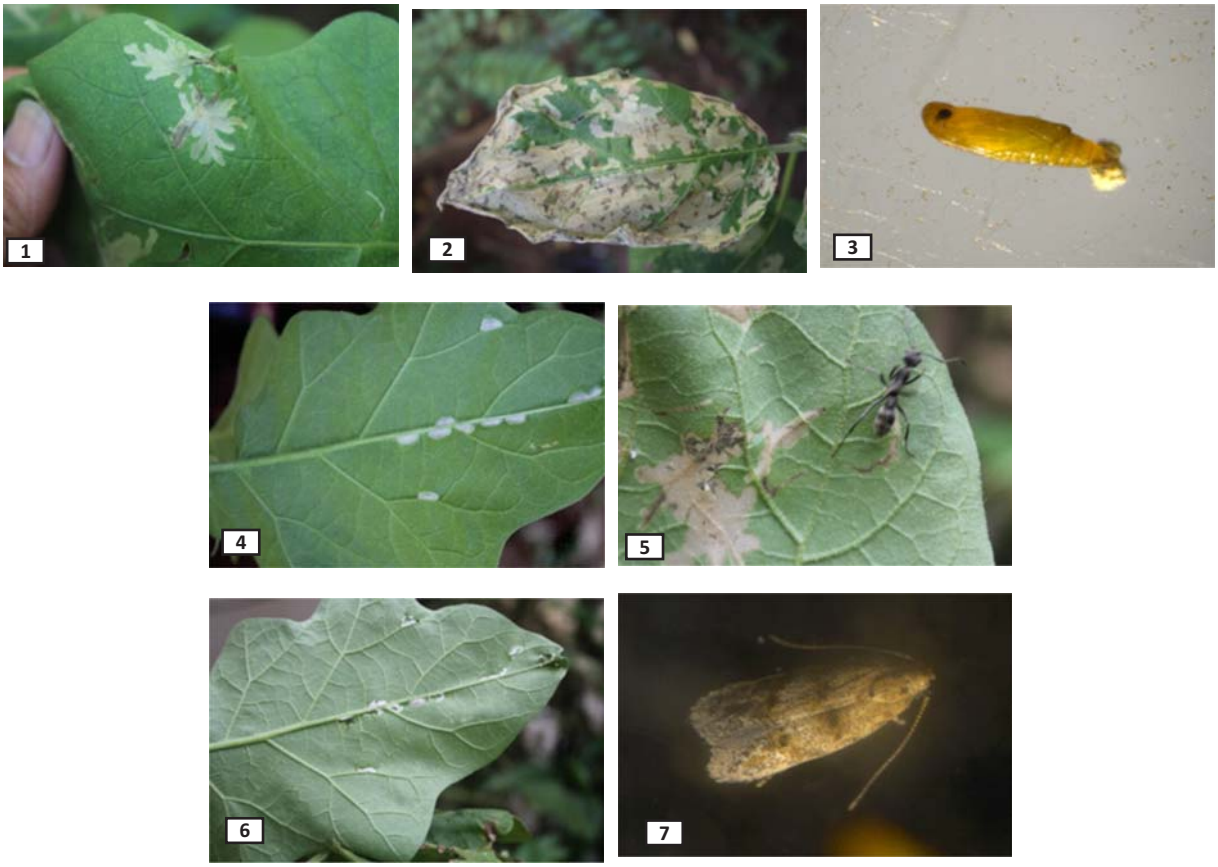

Plate. *T. absoluta* infestation on egg plant
1. Early symptom; 2. Severely damaged leaf; 3. Pupa; 4. Cocoons on ventral surface of leaf
5. Ant predator *D. rugosum*; 6. Predated cocoons; 7. Adult moth

fourteen cocoons found on a single leaf were devoured by the predatory ants in 24 hour. A typical incision was made on the leaf surface while the ants cut the cocoons with their sharp mandibles (Plate 6). Ants were found wandering on the leaf surface in search of cocoons, but attack on larvae was not noticed. The other formicid ant predators, *Pheidole* sp., *Solenopsis saevissima* and *Solenopsis geminata* were reported from Brazil and Ecuador (Desneux *et al*., 2010).

This is the first report on incidence of *T. absoluta* as a major pest in brinjal from India and first report of this pest from Kerala on any reported host. The predation of *T. absoluta* by formicid, *D. rugosum* was not reported earlier from any part of the World. Large scale transport of tomato and brinjal from Tamil Nadu/Karnataka could be some of the initial source of spread in Kerala. As suggested by Garzia *et al.* (2012), the ecological and biological strategies of the pest might have caused the rapid adaptation to its new environment. A sustainable management strategy comprising of correct blending of biological, chemical, behavioral and cultural methods has to be developed for the control of this noxious pest. Even though the native ant species, *D. rugosum* promises natural control of the pest in this region, other predators and parasites should be explored for their efficacy on *T. absoluta.*

Table 1. Morphometric of different growth stages of *T. absoluta*

T. absoluta		Mean (mm)*	SD
Full grown larva	- Length	5.52	0.787
Pupa	- Length	4.0	0.433
	- Breadth	1.34	0.075
Adult	- Length	3.96	0.233
	- Breadth	1.45	0.131

*Mean of 10 observations; SD=standard deviation

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Teak defoliator: changing host preference - may be climatic effect in Madhya Pradesh, India

Salman Khan*, Nikita Soni[#] and Mohd. Yousuf

Forest Entomology Division, Forest Research Institute, P.O. New Forest, Dehradun, Uttarakhand-248006, India; [#]Tropical Forest Research Institute, Jabalpur, Madhya Pradesh-482021, India.
Email: salman1315@gmail.com; Tel: +91-135-222-4269

ABSTRACT: *Hyblaea puera* is a key defoliator pest of *Tectona grandis* commonly known as teak. Preliminary examination of teak shows that there is no attack or negligible attack on *H. puera* in Madhya Pradesh, India. But *Vitex negundo* which is a medicinal plant growing in the region which is moist enough have been attacked by these larvae. The larvae were collected from 150 plants and categorized in accordance with the no. of larvae collected. The change in host plant from Teak to *Vitex negundo* is a phenomenon which involve climatic parameters and biological parameters.

KEYWORDS: *Hyblaea puera*, *Tectona grandis*, *Vitex negundo,* host preference, climatic effect

Teak defoliator, *Hyblaea puera* Cramer native to South-East and belonging to the order Lepidoptera and family Hyblaeidae is a serious pest of Teak (*Tectona grandis*) (Arun and Mahajan, 2012). Teak is multipurpose tree species being used in building boats, deck houses, doors, furniture, etc., because it produces very good quality timber. Many insect pests attack this beautiful tree and *Hyblaea puera* is one of them. The life cycle of *H. puera*is generally completed within a month and around twelve generations are possible every year. The eggs are laid on the leaves of the food plants. Typically, the larvae turn over the leaf marginand attach it to the rest of the leaf with a silken thread. The larvae are red headed and have orange colour margin or wholly black body.

The population of insect are dependent on two factor: density dependent factors (i.e., the direct or indirect negative feedback exerted by the increasing population) and density independent factor (abiotic, like weather factors) (Turchin, 1995). In a two-year light trap study at Jabalpur in 1978 and 1979 (Vaisharnpayan *et al.*,1987), collection of teak: defoliator moths were restricted to July, August and September. Saur *et al.* (1999) has recorded the defoliation of *Avicennia germinans* by *H. puera.* Similarly, the infestation of Asian *Avicennia* species by *H. puera* has been observed in Thailand (Murphy 1990) and India (Mehlig and Menezes, 2005). Javaregowda and Naik (2007) reported the incidence of *Hyblaea puera* in Karnataka, India. Peak population of *H. puera* in Madhya Pradesh is available in June and July and least was reported at September onwards (Khan *et al.,*1988). Nair *et al.* (1985) reported that the moths migrates up to 10 km in search of suitable host trees. Similarly, Nair and Sudheendrakumar (1986) reported the migration of adult *H. puera* from one locality to others.

Vitex negundo also called Chinese chaste tree is

* Author for correspondence

a bushy shrub or small tree growing from 2 to 8 m. They are mostly found near water bodies. Both Teak and Chinese chaste tree belongs to the family Lamiaceae. Leaves are palmately compound with 3-5 foliate; and distributed in Andaman & Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Delhi, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Punjab, Sikkim, Rajasthan, Tamil Nadu, Tripura, Uttar Pradesh and West Bengal (Western Ghats website).

The larvae of *Hyblaea puera* were collected from Bhedaghat area of Jabalpur (Madhya Pradesh) India. The geographical coordinates of the Bhedaghat is 23.132° N 79.801° E. The observation was taken in the month of August and September, 2016. The moisture content of the area was too high. Also because of the rainy season, the water flow of Narmada river was also on its peak. Almost 150 small shrubs of *Vitex negundo* were identified infected with these larvae. The average height of the shrubs was approximately 5 feet (Figure-1). The *H. puera* has been collected mostly from the apex leaves of each branch of *V. negundo*. The apex of each branch was seen folded and when it was opened, there was *H. puera* eating the leaves from inside and preparing itself for turning into the pupae.

The defoliator *H. puera* mostly feed on *Tectona grandis*. The *H. puera* is Oligophagus in nature and feeds and breeds on many plants belonging to the family Verbenaceae, Bignoniaceae, Araliaceae, Juglandaceae and Oleaceae (Beeson, 1941; Mathur, 1960 and Mohandas, 1986).

During the survey in Jabalpur during August and September for the collection of *H. puera*, a large area dominated with *Tectona grandis* was found free from *H. puera*. There was no serious attack of this larvae. The Teak Skeletonizer *Eutectona machaeralis* was somewhere available but still no significant sign of large attack was observed on teak. The larvae of *H. puera* needs good moisture content and relative humidity. Though the Jabalpur area is good for the development of the *Hyblaea* larvae but still negligible amount of larvae was collected from teak.

Bhedaghat is large area of which river Narmada flows and has water falls. The large area of Bhedaghat has *V. negundo* shrubs growing over the marble rocks. *V. negundo* shubs is dominating the area as there was sufficient amount of moisture content and relative humidity over the area. As *V. negundo* also require such climates to grow so it is being dominating and growing in some space to each other. But wherever it grows 2-5 stems are originating from a single place means they are growing in cluster or bunches of stems at one point of emergence of the shrubs of *V. negundo*.

H. puera was feeding on the leaves of *V. negundo* by folding it. The apical part of almost every branch was folded and *H. puera* was collected from it (Figure-1). Observation regarding no. of larvae collected from each plant has been taken and average height of the shrubs has also been estimated by ocular method. Different category has been formed according to the larvae collected from *V. negundo viz.* number of larvae collected, 1-5 larvae collected, 6-10, 11-15, 16-20, 21-25, 26-30 and 31-35 larvae collected. No. of plants in each category has been identified summed up to understand the no. of plants of *V. negundo* attack

Table1. Number of *Hyblaea puera* collected in *Vitex negundo* shrubs

S.No.	*H. puera* larvae	Shrubs *V. negundo*
1	0	11
2	1-5	23
3	6-10	29
4	11-15	22
5	16-20	26
6	21-25	15
7	26-30	8
8	31-35	16

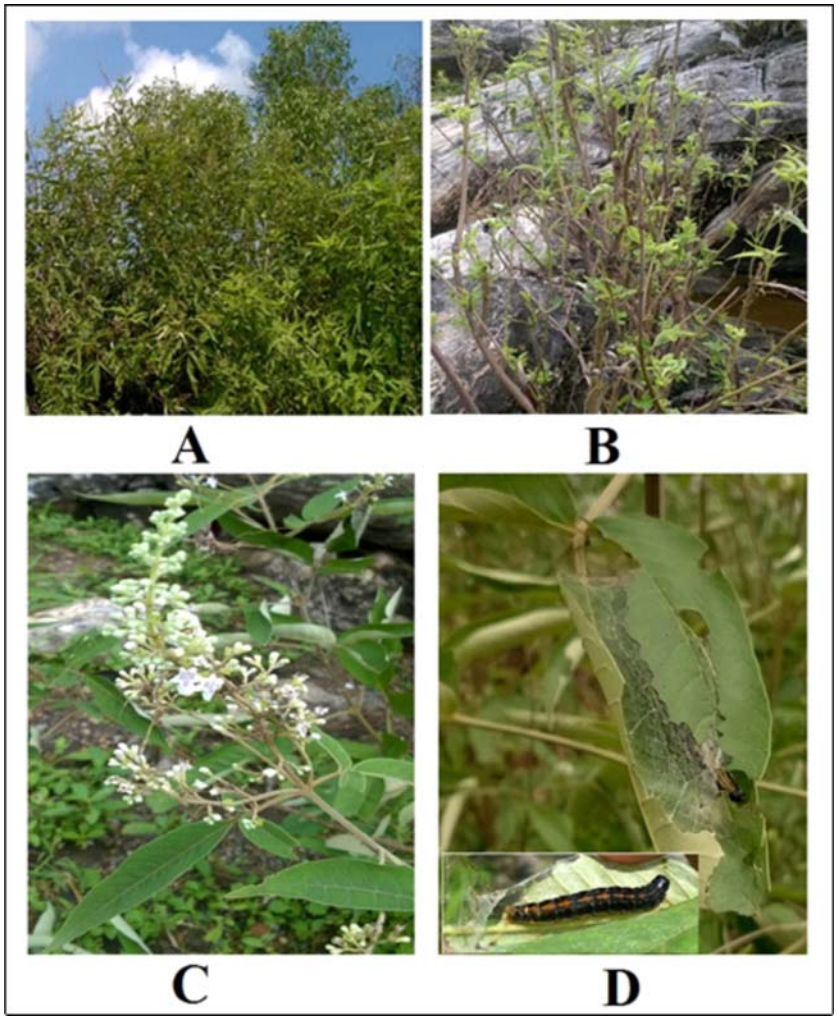

Figure 1. [A-D]: A-Healthy *Vitex negundo* plant, B-Infected *Vitex negundo,* C-Flowering of *Vitex negundo* plant showing 3-5 foliage pattern and D- Larvae of *Hyblaea puera*Inside the leaves folding and inset of D showing stretched leaves with *H. puera* larvae.

by *H. puera* (Table 1). It has been concluded that the *V. negundo* has been attacked by *H. puera* on functional basis. 31-35 larvae of *H. puera* has been collected from 16 plants out of 150 plants of *V. negundo*. Plants of *V. negundo* with more than 5 larvae collected were identified as 116 which is 77.3% of total plant studied (Figure 2). This shows that the *V. negundo* has been severely attacked by *H. puera* in the region and at the same time it has been found that *Tectona grandis* does not have more than 5 larvae on any single tree. This may be because of the climatic effect as the teak growing in the region have changes in the moisture or temperature regime. The area inhabitant with *V. negundo* has good climate required for the *H. puera* and because of the same family of *V. negundo* and *T. grandis* which is Lamiaceae the chemical constituents of both the species could be same up to some extent.

But Insects are insect and they destroy the food crops some are beneficial but in the case of *H. puera* it is not. It destroys the teak area in all over world which indirectly give less returns to the farmers who is growing the teak plantation and that leads to the gap in demand and supply to the wood-market. However, in Java the *H. puera* is being collected for edible purpose (Lukiwati, 2010) but in

Indiasuch activity is not being reported. As *V. negundo* is a medicinal plant which is being used in treating many disorders it is very important plant as per medicinal point of view. It is being used in analgesic, anti-inflammatory, anticonvulsant, antioxidant and insecticidal and pesticidal activities (Tandon, 2005). If the *H. puera* is been shifted towards *V. negundo* off course, there would be some positive in context of teak but from medicinal part it is would highly impact the *Vitex* plant and there would be negative impact.

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Abraham Verghese, GPS Institute of Agricultural Management, Peenya, Bengaluru, India

Abrol, D.P., Sher-e-Kashmir University of Agricultural Sciences & Technology, Chatha, Jammu, Jammu & Kashmir, India

Alain Pauly, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

Amrita, V.S., Kerala Agricultural University, Vellayani, Thiruvananthapuram, Kerala, India

Avtar Kaur Sidhu, High Altitude Regional Centre, Zoological Survey of India, Saproon, Solan, Himachal Pradesh, India

Balakrishan, N., National Centre for Disease Control - Plague Surveillance Unit, Bengaluru, Karnataka, India

Bhat, N.S., University of Agricultural Sciences, Bengaluru, Karnataka, India

Bindu Lakshmanan, College of Veterinary and Animal Sciences, Thrissur, Kerala, India

Biplob Kumar Modok, Sidho Kanho Birsa University, Purulia, West Bengal, India

David, B.V., International Institute of Bio-tech and Toxicology, Padappai, Chennai, Tamil Nadu, India

Devasahayam, S., ICAR - Indian Institute of Spices Research, Calicut, Kerala, India

Dharma Rajan Priyadarsanan, Ashoka Trust for Research in Ecology and the Environment, Srirampura, Bengaluru, Karnataka, India

Evans, D.A., University of Kerala, Thiruvananthapuram, Kerala, India

Francesco Vitali, Cerambycoidea Specialist, Luxembourg city, Luxembourg

Gayathri Elayidam, NSS College, Ottapalam, Kerala, India

Harish Kumar Sharma, Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Harry Brailovsky, Instituto de Biologia UNAM, Depto. de Zoologia, Apod, Ciudad de Mexico, Mexico

Haseena Bhaskar, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Hebsi Bhai, Thiruvananthapuram, Kerala, India

Hemant V. Ghate, Department of Zoology, Modern College, Shivajinagar, Pune, India

Ian J. Kitching, Natural History Museum, Cromwell Road, London, U.K

Inoka P. Karunaratne, W. A., Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

Jagbir Singh Kirti, Punjabi University, Patiala, Punjab, India

Jorge Ari Noriega, Dept. of Biogeography and Global change, Madrid, Spain

Josephrajkumar, A., ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam, Kerala, India

Kolla Sreedevi, ICAR-Indian Agricultural Research Institute, New Delhi, India

Maicykutty P. Mathew, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, India

Mallik, B., University of Agricultural Sciences, Bengaluru, Karnataka, India

Mani, M., Bengaluru, Karnataka, India

Mohankumar, S., Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Mohd Yousuf, Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India

Padmanaban, B., ICAR - National Research Centre for Banana, Tiruchirapalli, Tamil Nadu, India

Pandey, J. P., Central Tasar Research & Training Institute (Central Silk Board), Piska Nagari Ranchi, Jharkhand, India

Panicker, K.N., Amrita Institute of Medical Sciences, Kochi, Kerala, India

Panicker, K.N., Amrita Institute of Medical Sciences, Kochi,Kerala, India

Philip Samuel, P., Centre for Research in Medical Entomology (ICMR), Madurai, Tamil Nadu, India

Pushpalatha, E., University of Calicut, Malappuram, Kerala, India

Rabindra, R.J., Coimbatore, Tamil Nadu, India

Raman, A., Charles Sturt University & Graham Centre for Agricultural Innovation, Orange, NSW, Australia

Ramani, S., Bengaluru, Karnataka, India

Ramaraju, K., Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Ramasubramanian, ICAR - Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India

Ramesha, B., Kerala Agricultural University, Padannakkad, Kasaragod, Kerala, India

Ravi Kumar, G., Seri-Biotech Research Laboratory, Central Silk Board, Kodathi, Carmelram, Bengaluru, India

Richard Mally, University Museum of Bergen, Natural History Collections, Bealfagbygget, Bergen, Norway

Sabu K Thomas, St. Joseph's College, Devagiri, Calicut, Kerala, India

Selvanarayanan, V., Faculty of Agriculture, Annamalai University, Cuddalore, Tamil Nadu, India

Srinivasa Murthy, K., ICAR – National Bureau of Agricultural Insect Resources, Bengaluru, India

Subaharan, K., ICAR - National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

Subramanian,K. A., Southern Regional Centre, Zoological Survey of India, Chennai, India

Sudhir Singh, Forest Research Institute, Dehradun, Uttarakhand, India

Sundararaj, R., Institute of wood science and Technology, Bengaluru, India

Suresh, S., Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Sureshan, P. M., Zoological Survey of India, Western Ghat Regional Centre, Kozhikode, Kerala, India

Sushama Bharadwaj, RHR&TS, Mashobra, Shimla, Himachal Pradesh, India

Swaminathan, R., Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan, India

INFORMATION TO CONTRIBUTORS

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Department of Entomology, Kerala Agricultural University,
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