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SHORT COMMUNICATION

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Quantitative ovary protein studies on *Dysdercus koenigii* F. after application of different insecticides

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ABSTRACT: Effect of different concentrations of insecticides viz, multineem, neemjeevan, imidacloprid, monocrotophos, quinolphos and oxydemeton-o-methyl on ovary of *Dysdercus koenigii* fouth instar female nymphs at different age interval i.e 1-day, 4-day and 7-day old after adult emergence was carried out . The nymphs when transformed into adults then females were dissected and ovary was taken out for the estimation of protein. The quantity of protein decreased in 1-day old adult female derived from IV instar treated with different insecticides and found to be insignificant when compared with control. In case of 4-day old female the amount of protein was also inhibited by the application of insecticides and more inhibition was found in higher concentration although oxydemeton-o-methyl found to be more effective on the 4-day old insects. However in 7-day old insects the inhibition was almost same as in 4-day old insects and significant in all most all concentrations of quinolphos and oxydemeton-o-methyl.

KEY WORDS: *Dysdercus koenigii*, protein, ovary, insecticides

INTRODUCTION

Protein metabolism plays an important role in reproductive development of insects. Proteins are the basic material used in formation of new cells in all living system thus they make up most of the dryness of a cells and provide the chief structural elements of muscles, glands and others tissues. Proteins determine the shape and structure of the cells and also serve as the main instrument of molecular recognition and catalysis. They are stored in fat bodies and much is deaminated or converted into carbohydrates or fat and thus used for energy production. During vitellogenesis remarkable amount of protein as well as lipids along with other substances is deposited as yolk in developing oocytes (Goltzene, 1977).

The protein component of yolk spheres is generally believed to be synthesized in the nurse cells (King *et al.,* 1956), but the fact is that it is synthesized in fat body and released into the haemolymph from where it is taken up by the growing oocytes (Price, 1973; Gelti-Douka *et al.,* 1974; Highnan and Hill, 1977; Chapman, 1985; Browns, 1986). A lot of work is on record regarding the effect of nutritional factors on ovarian development (Strangways, 1961; Dethier, 1962; Orr, 1964a; Engelmann, 1970; de-Wilde and de-Loof, 1973; Clift and Mc-Donald, 1976; Spradbery and Schweizer, 1979; Barton-Browne *et al.*, 1979; Vogt and Walker, 1987). Effect of neem oil has also been demonstrated to bring about significant reduction in protein level in gonads (Murugan *et al.,* 1993).

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MATERIALS AND METHODS

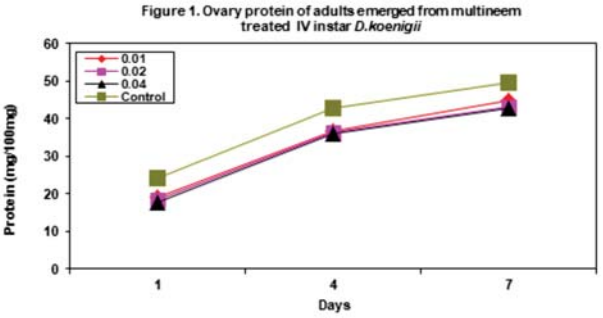
Newly moulted sixty nymphs of fourth instar (male and females) were sorted out from the mass culture and bioassay was carried out to assess the effect of insecticides on the total protein of ovaries of *D. koenigii.* Desired concentrations i.e 0.01, 0.02 and 0.04 percent of multineem (8 EC) and neemjeevan (0.3 EC) and 0.001, 0.002 and 0.004 percent of quinolphos (Byrusil, 25 EC), imidacloprid (Confidor, 200 SL), monocrotophos (Hilcron, 36 SL) and oxydemeton-O-methyl (Metasystox, 25 EC) were prepared for experimental purpose. They were applied topically @ 1µl/IV instar on the thoracic terga by means of a microapplicator and kept in a batch of 20 individuals in separate glass jars containing fresh food and sterilized sand at their bottom. The same numbers of nymphs were treated for each concentration of an insecticide. The food was changed at every 24 hours. The mortality also occurred during IV and V instars, which were discarded. They were sexed (females and males) after emergence. After pairing, each pair was kept in separate glass jars containing fresh food and sand at their bottom in order to obtain test insects of known age and a parallel untreated control was also run. After mating, the males were separated and didn't allow mate further. For each known age interval of females (1-day, 4-day and 7-day after emergence) a similar bioassay was carried out for each concentration of an insecticide. The adults of females of different age intervals were anaesthetized with chloroform and dissected in the insect saline (0.8 percent NaCl) under binocular microscope for the removal of ovary. Proteins were extracted according to the method describe by Searcy and Mac-Innis (1970). Known quantity of ovaries isolated from the test insect of different ages after emergence was homogenized in 5ml of 0.5N perchloric acid ($HClO_4$) and it was kept for precipitation in water bath at 100ºC for 20 minutes. The homogenate was cooled at room temperature and centrifuged at 3000rpm for 10 minutes. The supernatant containing RNA and DNA was taken in a volumetric flask. The residue was washed twice and centrifuged. Supernatant was taken in the same flask and made up to 5ml with 0.5N $HClO_4$. Residue was dissolved in distilled water and made up to 10ml. The solution was used for the estimation of protein. Estimation of protein was carried out according to Lowry *et al*. (1951).

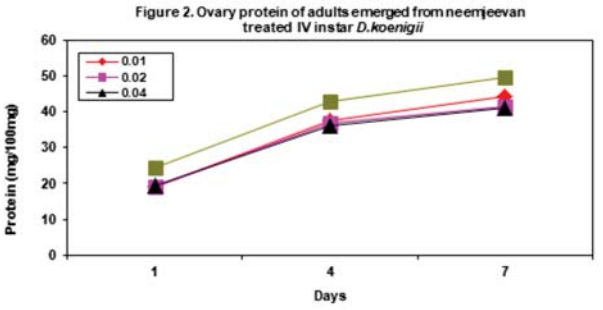
RESULTS

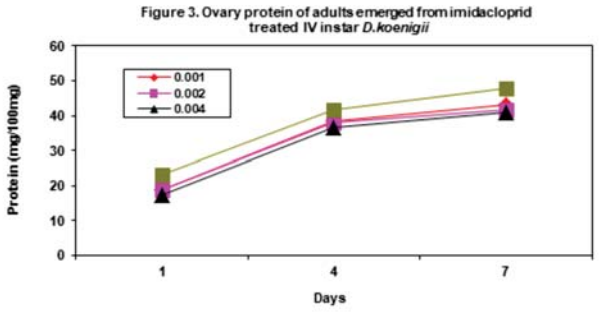
The quantity of protein was decreased in 1-day old female derived from IV instar treated with 0.01, 0.02 and 0.04 percent multineem but insignificant when compared with control. In 4-day old untreated female, total ovarian protein was increased to 42.855mg/100mg, while 4-day old females derived from treated IV instar, the magnitude of inhibition of total protein was also insignificant as compared to control and the obtained quantity was 36.743, 36.491 and 36.115 mg/100mg at three concentrations were used. However, in 7-day old females, the level of total protein was 49.670 mg/100mg and in those females derived from 0.01, 0.02 and 0.04 percent of multineen treated nymphs the level of total protein was 44.878, 43.073 and 42.775 mg/100mg but insignificant in compared to control.

The level of protein in 1-day old goes down after application of neemjeevan but not significant. It was found to be 19.264, 19.004 and 19.352 mg/100mg at three concentrations while 24.374 mg/100mg in control. Whereas in 4-day old untreated females, the level of total protein was increased from 24.374 to 42.855 mg/100mg, but in females derived from IV instar treated with 0.01, 0.02 and 0.04 percent neemjeevan, the level of protein was significantly inhibited at all concentrations. The amount of total ovary protein in untreated females continues to increase from 1-day to 7-day and found to be 49.670 mg/100mg. However, ovary obtained from 7-day old female derived from treated IV instar contains 44.302, 41.355 and 41.069 mg/100mg at 0.01, 0.02 and 0.04 percent concentrations respectively.

The amount of ovary protein of 1-day old untreated female was 23.307 mg/100mg of total protein, whereas the protein was partially affected in female derived from IV instar nymph treated with 0.001, 0.002 and 0.004 percent concentrations of imidacloprid. Almost insignificant inhibition was also







observed in of 4-day old female. However, the level of protein in the ovary of 7-day old untreated females was increased remarkably in comparison to ovary of 1-day old female. 7-day old female obtained from treated IV instar nymph and then the ovary protein was estimated to be 43.226, 41.794 and 41.117 mg/100mg at 0.001, 0.002 and 0.004 percent concentrations of imidacloprid respectively. 0.001 percent of insecticide caused significant inhibition of ovary protein but 0.002 and 0.004 percent did not cause significant effect.

The ovary protein of 1, 4 and 7-day old females derived from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations of monocrotophos was significantly and insignificantly inhibited. In 1-day old female obtained from treated IV instar the

Table 1. Ovary protein (mg/100mg) of emerged adults derived from multineem treated IV instar of *D.koenigii*.

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.01	18.965±0.419 *t*=2.382	36.743±1.325 *t*=2.032	44.878±1.397 *t*=2.152
.02	18.374±1.237 *t*=2.051	36.491±1.152 *t*=2.148	43.073±1.069 *t*=2.794
.04	17.896±0.980 *t*=2.252	36.115±0.638 *t*=2.496	42.775±1.152 *t*=2.778
Control	24.374±1.232	42.855±1.235	49.670±0.396

Table 2. Ovary protein (mg/100mg) of emerged adults derived from neemjeevan treated IV instar of *D.koenigii*

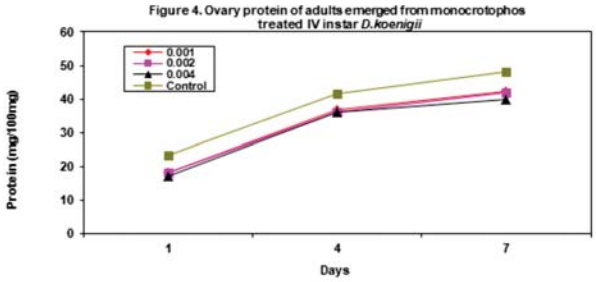
Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.01	19.264±1.043 *t*=1.972	37.532±1.741 *t*=2.028	44.302±0.887 *t*=2.693
.02	19.004±0.995 *t*=2.044	36.988±1.322 *t*=1.993	41.355±0.753 *t*=3.541
.04	19.352±0.339 *t*=2.366	36.025±0.637 *t*=2.513	41.069±0.496 *t*=4.090*
Control	24.374±1.232	42.855±1.235	49.670±0.396

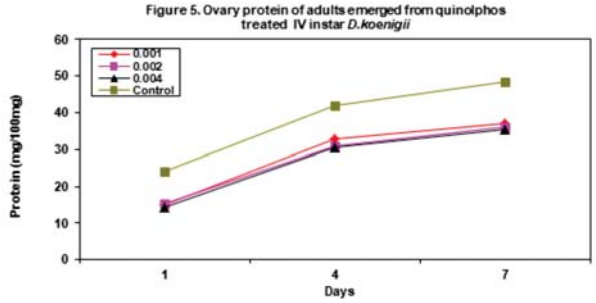
Table 3. Ovary protein (mg/100mg) of emerged adults derived from imidacloprid treated IV instar of *D.koenigii*

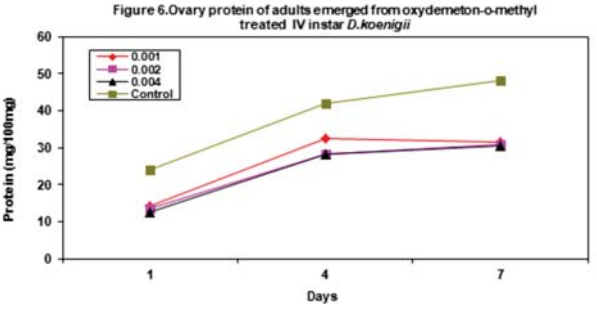
Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	18.841±1.287 *t*=1.950	38.417±1.676 *t*=1.624	43.226±0.542 *t*=3.451
.002	18.808±0.996 *t*=2.080	37.887±0.917 *t*=2.000	41.794±1.348 *t*=2.798
.004	17.380±1.195 *t*=2.567	36.752±1.020 *t*=2.352	41.117±1.138 *t*=3.108
Control	23.307±1.916	41.597±1.386	48.005±1.440

*Significant at 0.05; SE = Standard Error

ovary protein was not significantly inhibited at different concentrations of monocrotophos as compared to control. While in 4-day old female the quantity of ovary protein was 36.752, 36.147 and 36.188 mg/100mg was found at 0.001, 0.002 and 0.004 percent respectively. Whereas in control the total ovary protein was considerably higher than that of 1-day old females. Further it was observed that a two fold increase in the amount of ovary protein in 7-day old females in comparison to that







of 1-day old. The protein was significantly inhibited in 7-day old females derived from 0.002 and 0.004 percent concentrations of monocrotophos treated IV instar nymphs and insignificant at 0.001 percent concentration. The quantity of protein was 42.267, 42.039 and 40.037 mg/100mg at different concentrations of monocrotophos while 48.005 mg/100mg in untreated control.

The result obtained after analysis in case of quinolphos showed that 0.001 and 0.002 percent of quinolphos did not cause significant inhibition of ovary protein in 1 and 4-day old females derived from IV instar while in the control ovary the protein was 24.041 and 41.910 mg/100mg, in 1 and 4-day old females respectively. In 7-day old female, the amount was significantly inhibited at all concentrations, i.e 0.001, 0.002 and 0.004 percent which was found to be 36.962, 36.007 and 35.440 mg/100mg respectively in comparison to 48.336 mg/100mg in the control. 0.004 percent of insecticide was proved to inhibit the protein of ovary more significantly than other concentrations tested. 1-day old female obtained from IV instar treated with 0.001, 0.002 and 0.004 percent concentrations of oxydemeton-o-methyl the amount of ovary protein

Table 4. Ovary protein (mg/100mg) of emerged adults derived from monocrotphos treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	18.332±1.032 *t*=2.149	36.752±1.020 *t*=2.164	42.267±1.4454 *t*=2.634
.002	18.231±0.620 *t*=2.397	36.147±0.911 *t*=2.326	42.039±0.892 *t*=3.204*
.004	17.031±0.342 *t*=2.840	36.188±1.161 *t*=2.195	40.037±0.787 *t*=3.758*
Control	23.307±1.232	41.597±1.386	48.005±1.440

Table 5. Ovary protein (mg/100mg) of emerged adults derived from quinolphos treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	14.950±1.517 *t*=2.436	32.954±1.114 *t*=2.702	36.962±1.545 *t*=3.368*
.002	15.287±1.261 *t*=2.512	30.966±1.117 *t*=2.958	36.007±1.287 *t*=3.751*
.004	14.212±1.211 *t*=2.684	30.584±1.036 *t*=3.059	35.440±0.613 *t*=4.944*
Control	24.041±1.273	41.910±1.403	48.336±2.058

Table 6. Ovary protein (mg/100mg) of emerged adults derived from oxydemeton-o-methyl treated IV instar of *D.koenigii*

Conc.(%)	1-day old (Mean±SE)	4-day old (Mean±SE)	7-day old (Mean±SE)
.001	14.271±1.261 *t*=2.649	32.618±1.746 *t*=2.438	31.572±1.849 *t*=3.738*
.002	13.605±0.971 *t*=2.910	28.402±1.598 *t*=2.972	30.962±0.920 *t*=4.964*
.004	12.766±0.706 *t*=3.220*	28.356±1.075 *t*=3.297*	30.487±0.585 *t*=5.826*
Control	24.041±1.273	41.910±1.403	48.336±2.058

* Significant at 0.05; SE = Standard Error

was found to be 14.271, 13.605 and 12.766 mg/100mg respectively while 24.041 mg/100mg in control. Inhibition of ovary protein was also significant at in 4-day old females derived from 0.004 percent-treated IV instar and the amount was 32.618, 28.402 and 28.356 mg/100mg at different concentrations respectively. A significant decrease in the quantity of ovary protein was found in 7-day old females obtained from IV instar treated with 0.001, 0.002 and 0.004 percent of oxymeton-o-methyl while the quantity was 31.572, and 30.962 and 30.487 mg/100mg respectively in comparison to 48.336 mg/100mg in untreated control.

DISCUSSION

Ovary exhibited an increase in total protein in untreated *D. koenigii* from 1-day and reaching maximum in 7-day old females. Present findings are in conformity with Clarke and Smith (1966), who observed higher rate of protein synthesis in aged *Drosophila subobscura* than in younger ones. Whereas, females undergoing normal cycles of oogenesis, cyclic changes in protein synthesis would be anticipated (Ring, 1973). A slow but steady decline followed during adult life to senescence. While, Lang *et al.* (1965) suggested that such decreasing curves are due to the fact that protein is increasing more rapidly than nucleic acids. No sexual differences were found in either sex. Mills *et al.* (1966) reported in American cockroach that a rapid increase occurs reaching its peak on day two followed by a decline and another rapid increase begins, reaching a second peak on day five when the oocyte is taking up the greatest amount of protein. Thomas and Nation (1966) reported decrease in level of protein, which may be consequence of a decrease in RNA synthesis. The possible correlation of these changes in protein and RNA metabolism with that of gonadal cycle in female of *Periplaneta americana*. It was also revealed by Venugopal and Kumar (1997) that ovary and fat body showed a sudden increase in number of proteins after eclosion in *D. koenigii*, while trophocytes showed the presence of nucleic acids, proteins and glycogen but poor in lipids and the oocytes at maturity were packed with protein and lipid yolk granules (Kaur *et al.*, 1991) in *D. koenigii.* Increase in protein level during period of attaining maturity is related with their demands for the synthesis of vitellogenic proteins to be incorporated in the developing oocytes. In 4-day old total protein content is greater than 1-day because of vitellogenesis started on somewhere in 2- and 3-day after emergence of *D. koenigii*. This is also supported by Sharma and Sharma (1979) in *Z. subfasciatus* and Zaidi and Khan (1979) in *D. cingulatus*. These authors reported that increase in protein level was due to increase demand of oocyte protein during early period of vitellogenesis. Adults of 7-day old revealed maximum proteins contents as the ovaries were in 2nd reproductive cycle and the vitellogenesis is almost completed as found in present study.

Handels(1976) work on *Aedes atropalpus* shows that gradual accumulation of protein in the maturing eggs and increase with their size upto the time of chorion formation (Oliviera *et al.*, 1986). Sifat and Khan (1974) concluded in *D. cingulatus* that total protein concentration of ovaries varies in relation to maturation and oviposition of eggs.

Survivors from insecticide treated IV instar nymph exhibited reduction in the level of ovarian protein as compared to that of control. However, the decline was more with increasing concentrations of insecticides. Once the inhibition in the ovarian protein takes place, the level was almost same in 4-day and 7-day old females with a slight fluctuation. Multineem and neemjeevan found to be almost equitoxic but 0.04 percent neemjeevan caused a significant inhibition in 7-day old female. It may be due to some unknown reason because neem formulations did not show any residual effect within an organism. Srivastava and Krishna (1992) also observed fall in protein content after treatment with eucalyptus oil in adult virgin female of *D. koenigii*. This is further supported by Murugan *et al.* (1993) in *H. armigera*. Moth obtained from treated larvae with neem kernel extract showed suppression in protein level as well as fat bodies that would severely impaired reproductive system. Similarly Toja *et al.* (1985) observed reduction in protein concentration of larval fat body of *S. litura* when single dose of azadirachtin (1µg/gm of body

weight) applied. Padamaja and Rao (1999) found that the protein content in all treatments was significantly lower in *H. armigera* treated with oil of *Artemesia annula*, *Ageratum conyzoids* and *A. indica*. Vijayaraghavan and Chitra (2002) revealed reduction of total protein content when all the stages from second instar to adult of *S. litura* treated with neem and annona seed extract. The protein content was dose dependent and lowest in 200µg treated precocene I topically applied on *D. koenigii* (Ramalakhshami *et al.*, 1985).

Females derived from imidacloprid treated IV instar showed a similar nature of reduction in the ovarian proteins at different age intervals as compared to that of neem formulations but in 7-day old females, the inhibition of protein was more than 1- and 4-day old impairing the vitellogenesis in 2nd reproductive cycle. The results of survivors from monocrotophos treated IV instar showed that 0.004 percent caused a latent action on the ovary thereby more decline in the level of protein which is significant as compared to untreated control. Quinolphos caused more inhibition of protein from 1-day to 7-day old adults as compared to imidacloprid and monocrotophos. Therefore, quinolphos significantly affected the fecundity and fertility. Quinolphos also showed residual toxicity causing more inhibition of ovarian protein in 7-day old in 1- and 4-day old females. This probably significantly affects the 2nd reproductive cycle as well as first also. Survivors derived from oxydemeton-o-methyl treated IV instar showed that there is significant inhibition of ovarian protein in 7-day old females. Therefore, oxydemeton-o-methyl caused a severe impairment of 2nd reproductive cycle. It may be due to more molecules were accumulated in the body and then reappeared to further inhibited the level of protein. While in the first reproductive cycle 0.004 percent oxydemeton-o-methyl significantly reduced the fecundity and fertility.

Zaidi and Khan (1981) also observed a dose dependent effect on the level of ovarian protein after the application of dipterex. Cypermethrin and quinolphos also caused reduction in protein content in adults of *S. litura* (Vijayraghavan and Chitra, 2002). While monocrotophos, dimethoate, methylparathion, quinolphos and endosulfan showed a significant decline in the protein concentration in the alimentary canal of *R. kumarii* (George and Ambrose, 1999). It was suggested by Bharathi and Govindappa (1987a,b) that prolonged insecticidal stress could reduce synthesis of protein by deranging the protein synthetic machinery of insects. Overall conclusion is that quinolphos is most effective insecticide and potent inhibitor of protein for the *D. koenigii*.

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A new report of the myrmecophilous root mealy bug *Xenococcus annandalei* Silvestri (Rhizoecidae: Hemiptera) - a devastating pest

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ABSTRACT: The obligate myrmecophilous root mealybug *Xenococcus annandalei* Silvestri belonging to the family Rhizoecidae and order Hemiptera was recorded on tender roots of a wide range of economically important crop plants and weeds at Idukki, Kerala, India. This is a new distributional report of the pest from South India. Both nymphs and adults are seen congregating the roots and rootlets and suck sap. Ant species *Acropyga acutiventris* Roger was always seen in association with these mealy bugs and helps in the spread of mealy bugs to the healthy plants. Ant nests are seen inside the soil and inside these nests mealy bugs are also seen. The present study identifies the mealybug and the associated ant. Host plants including crops and weed plants are described here. The cryptic habitat of the pest and its association with ants demands detailed examination of the planting materials along with correct management strategies so as to prevent the migration of the pest to other pest free area of the country.

KEY WORDS: Root mealy bug, *Xenococcus annandalei*, *Acropyga acutiventris*, myremecophilous, host plants

INTRODUCTION

Mealybugs are major pests and vectors of many diseases of crop plants. They cause damage by sucking plant sap, by injection of toxins as well as exudation of honey dew resulting in sooty mould formation and reduction in photosynthesis. Among mealybugs, root mealybugs suck sap from the roots and rootlets of host plants resulting in slow growth, stunting, yellowing, lack of vigor and subsequently death of plants. These pests are often found in association with ants. Honey dew secreted by mealybugs is a rich source of nutrient for ants. The ants in turn protect the mealybugs from their natural enemies.

Most of the members of the family Rhizoecidae suck sap from the plant rootlets (Williams 1998), inhabit the soil, leaf litter or rotting logs and are frequently associated with ants. The members of this family were previously included in Pseudococcidae, and recently separated based on the morphology of adult males (Hodgson 2012), molecular sequences (Downie and Gullan 2004 and 2005) and endosymbionts (Gruwell *et al*., 2010). Two sub families, Rhizoecinae Williams and

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Xenococcinae Tang are included under this family. Tang (1992), divided Rhizoecinae into two tribes, Rhizoecini with the hypogaeic mealybugs and Xenococcini with the hypogaeic obligate myrmecophilous mealybugs which feed on the phloem of plant roots. These ant loving mealybugs exhibit a close symbiotic relationship with ants (trophobiosis) where ants give protection to mealybugs from predators and parasitoids and mealybugs in turn provide honey dew secretion which is a rich nutritive source for ants (Scott *et al*., 2011).

The root mealybug *Xenococcus annandalei* Silvestri was first reported from Barkuda Island in Odisha, India, during 1924, infesting roots of coconut palms *Cocos nucifera* L. and *Ficus obtusa* Hassk. and associated with the ant, *Acropyga acutiventris* (Silvestri 1924). First instar of *X. annandalei* collected from the roots of coconut was reported from Mysore in 1937 (William, 1978). Later Rajagopal *et al*. (1997) reported the occurrence of *X. annandalei*, severely infesting roots and rootlets of grapes at Bangalore. Apart from grape vine *X. annandalei* was also reported from roots of *Oxalis latifolia* Kunth., *Euphorbia hirta* L., *Blepharis mollinginifolia* Pers. and *Ageratum conyzoides* L. growing as weeds in the grape gardens. Williams (1998) stated that the previous reports of *X. annandalei* were based on a misconception and he reported that the specimens were actually *X. acropygae*. According to him the type species *X. annandalei* appears to be a local species confining only on Barkuda Island, Odisha, India. The pest was also reported from other parts of the world like Queensland and Northern Territory of Australia (Ben Dov, 1994), Papua New Guinea (Ben Dov, 1994, Williams and Watson, 1988), Hong Kong (Ben Dov, 1994, Williams, 1978), Malaysia (Ben Dov, 1994, Williams, 1978) and Vietnam (Ben Dov, 1994, Danzig, 1993). The pest has now been recorded in Kerala, South India, in Idukki district. The present study was under taken to identify the pest and the ant species associated with the crop plants and to gather information on the alternate host plants harbouring this pest.

MATERIALS AND METHODS

Mealybug specimens were collected during a visit to a farmer's field at Mannakkudi (6.796003^{0}N 77.126545^{0}E), during July 2012. About 50 cents of area was infested by the mealybugs. These mealybugs were always seen in association with ants. The field was a mixed plantation of clove (*Syzygium aromaticum* (L.) Merrill & Perry), cardamom (*Elettaria cardamomum* (L) Maton), coffee (*Coffea arabica* L.), turmeric (*Curcuma longa* L.) and ginger (*Zingiber officinale* Roscoe.). Mealybug samples along with ants were collected from the root zone of plants showing yellowing and wilting symptoms. Mealybug colonies were also obtained from the ant nests. Both the mealybugs and the ants were preserved separately in 70 per cent ethanol for further identification. Mealybug samples were sent to National Bureau of Agricultural Insect Resources, Bengaluru (NBAIR) and the ant specimens were sent to the Department of Entomology, University of Agricultural Sciences (UAS), Gandhi Krishi Vigyan Kendra (GKVK) Campus, Bengaluru for identification. Subsequent field visits were also made to Nedumkandam (9.8363^{0}N 77.1571^{0}E), Thannimoodu (9.828708^{0}N 77.171930^{0}E), Thopramkudi (9.8763^{0}N 77.0566^{0}E), Upputhara (9.6969^{0}N 77.0208^{0}E), Nathukallu (9.7870^{0}N 77.1087^{0}E) and Ettakkanam (9.698170^{0}N 77.019037^{0}E) areas of Idukki district to know the extent of infestation and also to gather information on the crop plants and weed plants harbouring the pest.

RESULTS AND DISCUSSION

The mealybug was identified as *Xenococcus annandalei* Silvestri by Dr. Sunil Joshi, Principal Scientist, NBAIR, Bengaluru and the voucher specimens were deposited at the Bureau. The present finding is the first report of the occurrence of *X. annandalei* in Idukki district of Kerala (Plate Ia). The pest was first observed at Mannakkudi and subsequently from Nedunkandam, Thannimoodu, Thopramkudi, Upputhara, Nathukallukallu and Ettakkanam regions in Idukki

PLATE I

a. Xenococcus annandalei close up view

b. Yellowing and wilting in *Laportia interrupta*

c. Root mealybug on jack root

district. At Mannakkudi, infested field was a mixed plantation of clove, cardamom, coffee, ginger and turmeric. The main weed species was Hen's nettle, *Laportea interrupta* L. Initial symptoms of yellowing and wilting was observed in some plants of ginger, cardamom, turmeric and also on the weed plant *L. interrupta* (Plate Ib). Shedding of leaves and drying was observed in infested plants of clove. A dense population of mealy bugs and ants were observed on the root zone of jack tree, *Artocarpus heterophyllus* Lam (Plate Ic), but symptom of infestation was not observed. Severely infested fields were observed in Thannimoodu, Thopramkudi, Upputhara and Nathukallu and Ettakkanam regions. Banana plants, *Musa* spp. at Thannimoodu showed drying of leaves and stunted growth (Plate IIa). Severe infestation of mealybugs resulted in shedding of leaves and complete drying of plants and these symptoms were observed in black pepper vines (*Piper nigrum* L.) (Plate IIb), tea (*Camellia sinensis* (L.) Kuntze) (Plate IIc), coffee, clove and cardamom. In cardamom, complete drying of the clumps were also observed (Plate IId). Severely infested field gave a sick appearance (Plate IIe) with completely dried plants of crops, weeds and even trees like clove, coral tree a popular standard of black pepper (*Erythrina indica* L.), wild jack (*Artocarpus hirsutus* Lam.) and nut meg (*Myristica fragrans* Houtt.), Congregation of both adults and nymphs of the root mealybug *X. annandalei* along with ants was observed on the roots and root lets of the affected plants.

Immature stages of *X. annandalei* are white in colour and the matured ones are cream coloured and these mealy bugs are always seen in association with ants. The ants were cryptic in habitat and identified as *Acropyga acutiventris* Roger by Department of Entomology, GKVK, Bengaluru. The ant nests are seen inside the soil from a depth of 5-20 cm. Exit holes with loose soil give an indication of the ant nest (Plate IIIa) and these ant nests had tunnels and chambers. The ant nest tunnels moved along the root system of host plants where more population of ants and mealy bugs can be seen. Ant colonies are densely distributed near the root zone of host plants. If there is any disturbance or food is exhausted, the ant will keep the mealybugs in between its mandibles and helps to migrate to more safer places or new healthy plants (Plate IIIb). Honey dew exudation by the mealybugs were also observed in the present study (Plate IIIc).

PLATE II

a. Root mealy bug infested banana

b. Completely dried black pepper

c. Completely dried tea plant

d. Completely dried clump of cardamom

e. Severely infested field

The present study identifies the presence of a devastating root mealy bug and the associated ant from the moist evergreen forests of Idukki district and these findings are in conformity with the findings of Anu and Sabu, 2007 where they reported the presence of the ant *Acropyga* sp. in the moist evergreen forests of Wayanad district. Biodiversity analysis of the litter ant assemblages in the Wayanad district was done in this study and they confirmed that the ant *Acropyga* sp. is present only in

PLATE III

a. Ant nest with exit hole

b. Ant carrying mealy bug in between mandibles

c. Honey dew exudation by *X. annandalei*

evergreen moist forest. There was no indication of the mealy bug species associated with the ant species. Earlier *X. annandalei* was reported only on two host plants (roots of coconut palms *C. nucifera* and *F. obtusa*) and was not a pest of economic importance (Silvestri 1924). This is the first report in which these insects are observed in a wide range of host plants.

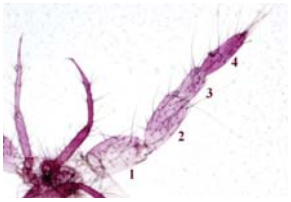
Diagnosis:

Adult females are 1.342 to 1.410 mm long and 0.576 to 0.615 mm broad. Body is broadly oval, with abdomen tapering (Plate IVa). Anal lobes not developed; positions of each lobe recognisable by inner ventral grooves, each apparent lobe with two long ventral setae and one long dorsal seta, forming a group of three. Anal ring formed of a crescentic sclerotized dorsal band without cells, bearing eight setae, each seta about as long as an anal lobe seta; anterior two pairs slender, posterior two pairs stout; posterior most pair of setae transferred to ventral surface. Antennae each four segmented, situated slightly onto dorsal surface, tapering, each about as long as body; articulation between first and second segments well developed, allowing each antenna to fold onto dorsum of body (Plate IVb). Legs well developed, each hind tarsus tapering to long slender claw (Plate IVc). Circuli round, each cupped in centre, two or three present in middle of each of abdominal segments II and III respectively, and sometimes a circulus present in middle of abdominal segment IV (Plate IVd). Body setae present on dorsum and lateral areas of thorax and are minute, slender and abundant. Setae on other areas of body are mostly long and stout. Sickle-shaped setae usually present, at least on thorax (Plate IVe), rarely absent. Eyes and ostioles absent. Pores and ducts absent.

Antenna well defined and the abdomen of the female tapers abruptly and kept in erect position. A special articulation is found in between the enlarged first and second antennal segments and hence the antennae can be folded back along the body. Instead of the mealy wax which is present on most of the mealybugs minute setae is found covering the abdomen. The legs are well developed and the insects move actively. These descriptions are in conformity with the findings of Williams (1978 and 1998).

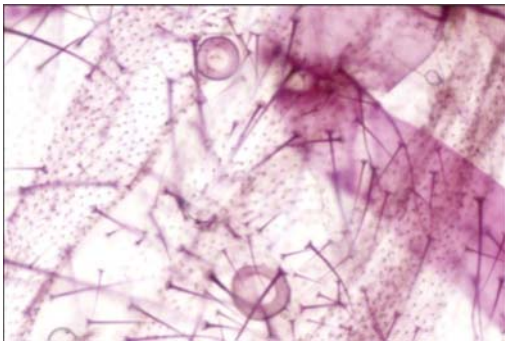
PLATE IV

a. Adult female *X. annadale*

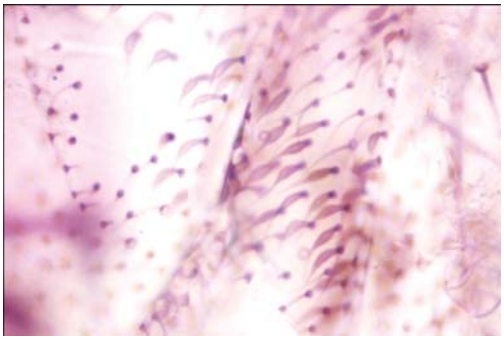


b. Antenna

c. Hind leg



d. Circuli



e. Sickle shaped setae on thorax

The mealybugs were attended by the ant, *A. acutiventris* that formed a mutualistic relationship with *X. annandalei*. The ants of the genus *Acropyga* are all hypogaeic (living entirely underground) and have a mutualistic relationship with root mealybugs (Weber 1944, Williams 1998). The ants help in transport of mealybugs from one place to another and also protect them from natural enemies. Honey dew excreted by the mealybugs is a rich nutrient source to ants.

Nature and extent of damage:

The pest was observed in a wide array of host plants belonging to 14 families and 18 species of which 17 species are new hosts. Economically important crop plants like cardamom (*E. cardamomum*, Zingiberaceae), black pepper (*P. nigrum*, Piperaceae), coffee (*C. arabica*, Rubiaceae), cocoa (*Theobroma cacao* L., Malvaceae), tea (*C. sinensis*, Theaceae), nutmeg (*M. fragrans*, Myristicaceae), banana (*M.* spp., Musaceae), wild jack (*A. hirsutus*, Moraceae), jack tree (*A. heterophyllus*, Moraceae), ginger (*Z. officianale*, Zingiberaceae), turmeric (*C. longa*, Zingiberaceae) and mango (*Mangifera indica* L., Anacardiaceae) were infested with these mealybugs. Yellowing followed by wilting was the general symptom observed on these plants; severe infestations led to complete drying of plants. The infestation was also observed on coral tree (*E. indica*, Fabaceae), garden croton (*Codiaeum variegatum*. L., Euphorbiaceae) and also weed plants like hen's nettle (*L. interrupta* L., Urticaceae), black night shade (*Solanum nigrum* L., Solanaceae), Ficus (*F. obtusa* Euphorbiaceae) and coat buttons (*Tridax procumbens* L., Asteraceae). During the rainy season high population of mealybugs were seen on the upper soil layers (5-15 cm depth) and during cold and dry weather the pest was generally seen in the deep layers of soil (up to 60 cm depth). The cryptic habitat of the pest and its association with ants warrants thorough examination of planting materials along with proper management measures in order to prevent the spread of the pest to other pest free zones of the country. Systematic monitoring of the crop fields is highly essential to diagnose the problem in the early stage itself. Since

the pest is in a highly protective environment and its association with ants makes the pest management more challenging. As the pest is polyphagous causing extensive damage, integrated pest management strategies are to be developed for the management of the pest.

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Oviposition deterrents for the management of citrus leaf miner *Phyllocnistis citrella S*tainton (Lepidoptera: Gracillariidae)

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ABSTRACT: Experiments were carried out to evaluate neem oil (3%), azadirachtin(0.03%), Neem Seed Kernel Extract(7%), Neem cake extract (10%), Horticultural mineral oil (HO)(1%), *Bacillus thuringiensis* (*Bt*) (0.3%), *Bt* (0.3%) + HO (1%), Spinosad 45SC (0.009%), Spinosad 45SC (0.009%) + HO (1%) and imidacloprid 17.8SL (0.009%) for deterrence to citrus leaf miner oviposition on rough lemon seedlings under caged and nursery conditions. Under laboratory conditions(caged), maximum oviposition deterrence of 84.52% was observed with neem oil (3%) followed by imidacloprid 17.8 SL (0.009%) with 83.33 % deterrence. Under nursery conditions, neem oil (3%), spinosad 45 SC (0.009%) + HO (1%) and imidacloprid 17.8 SL (0.009%) were found effective in controlling leaf miner infestation up to 15 days after spraying.

KEYWORDS: Neem products, microbials, oviposition deterrence, *Phyllocnistis citrella*

INTRODUCTION

Citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is one of the serious pests among 27 species of insect and mite species, particularly on nursery and young plantations of citrus (Sharma et al.,2006). The pest attacks mainly plants of the family Rutaceae and within that family, it is mostly attracted to the genus *Citrus* and its commercially grown cultivars. In India, more than 80 % of the *Citrus reticulata* Blanco (Nagpur mandarin) nurseries are found severely affected by leaf miner infestation, especially in Central India (Shivankar *et al.*,2002). The larvae bore through the leaf epidermis, ingesting the sap and produces silvery mined areas. Citrus leaf miner (CLM) may prevent young leaves from expanding; causing them to remain curled and twisted. Leaf mines are characterized by twisted galleries and the epidermis appears as a silvery film. Leaf mining causes retardation of plant growth especially nursery stock ready for field planting and renders the leaves unsightly.

A host of control measures have been deployed for the management of CLM. Among them, biopesticides including botanicals, can offer a safe and effective alternative to conventional insecticides controlling major insect pests. In past literatures, various products like fish oil, rosin soap (3.3%v/v),

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pongamia, mahua (Katole *et al*., 1993), neem seed cake extract (2%) and pongamia seed extracts (2%) were found effective against CLM on lime (Singh and Azam, 1986; Dhara *et al.*, 1990; Jothi *et al.*, 1993). Chakravarthi *et al. (*1998) have reported that use of azadirachtin 0.03% and neem oil gave high level of control of *Diaphorina citri* Kuwayama upto 90%. Spraying *Melia azadirachta* seed oil 0.5% emulsion was effective (93.6% and 96%) against *Planococcus citri* without any sign of phytotoxicity (Well *et al.*, 1989). The efficacy of petroleum spray oils was tested against citrus psylla on calamondin trees (*Citrus madurensis*) and found that 1st and 2nd instars were highly susceptible (Priore and Pandolfo, 1972-73). But still, a limited number of studies have dealt with the use of botanicals against the citrus leaf miner. Hence, the present study was carried out to find out the effect of different botanicals, oilsand microbial formulations in deterring the CLM adults from ovipositing on the young flush leaves and further evaluation of these botanicals/oils along with insecticides on rough lemon (*Citrus jambheri* Lush) seedlings under protected nursery conditions.

MATERIALS AND METHODS

Oviposition deterrence studies were conducted initially to arrive at the optimum dosage of neem based products viz., neem oil (1%,2%,3%), Azadirachtin 1EC (0.01%, 0.02%, 0.03%), NSKE (3%,5%,7%)andHO (0.3%, 0.5%,1.0%) under caged conditions and further evaluation was carried out under nursery (semi-field) conditions during rainy season(season-I), winter(season-II) and spring (season-III), 2014-15 at ICAR-CCRI, Nagpur.

Oviposition deterrence of neem based products and oils against CLM under caged/ semi-field conditions

Rearing techniques for CLM was followed as described by Urbaneja*et al*. (1999). Initially for arriving at the appropriate dosage, five pairs of newly emerged CLM moths were releasedonto one year treatedold rough lemon seedlings inside cages of 3 x1.5 x 1.5 ft size. The different doses tested were Neem oil 1,2& 3%, Azadirachtin 1EC (0.01, 0.02 &0.03%), Neem Seed Kernal Extract (NSKE) (3, 5 & 7%), Horticultural oil (Mak All Season, a product of Bharat Petroleum) (0.3, 0.5 & 1.0%) each along with water spray as control. The concentrations of the respective molecules were prepared freshly, sprayed using a hand held sprayer (one litre capacity) on the seedlings till runoff. Each treatment was replicated four times with10 seedlings in each replication. A multi-vitamin drop mixed with 10 % (w/v) of sucrose solution was also provided as a food for adult feeding. Five pairs of newly emerged CLM moths were released in each cage for egg laying. Observations were taken 48 hours after treatment (HAT) on number of eggs laid on new leaves from shoot tip of 10 cm length from both treated and control seedlings.

Effective doses of each treatment from above cage studies viz.,neem oil 3%, azadirachtin0.03%, NSKE 7%, Neem cake extract 10%, Horticulture mineral oil 1% and imidacloprid 0.009% along with water spray as control were selected and sprayed on 1.5 years old rough lemon seedlings raised under protected nursery sheds(100m X 10 m) at CCRI farm with 15 seedlings per replication inside muslin cloth cages (3 x 1.5 x 1.5 ft size) with four replications. Egg count was taken 48 HAT per seedling by counting number of eggs laid on new leaves from shoot tip of 10 cm length and oviposition deterrence (%) was calculated using the formula of Williams et al.(1986):

$$\frac{\text{No. of eggs in control - No. of eggs in treatment}}{\text{No. of eggs in control}} \times 100$$

Efficacy of botanicals/oils/insecticides against CLM in protected nursery

The best dose of each treatment viz., Neem oil (3%), Azadirachtin 1 EC(0.03%), NSKE(7%) and Horticultural oil 1% along with *Bacillus thuringiensis* (*Bt*) formulation (0.3%), *Bt* + HO (1%), spinosad 45SC (0.009%), spinosad 45SC (0.009%)+ HO (1.0%), imidacloprid 17.8SL (0.009%) on one year old rough lemon seedlings.. The use of spinosad 45SC (Spintor, Bayer Company), a bio-rational insecticide containing natural material active against pest populations has

increased significantly because of eco-friendly mode of action. But CLM is protected inside the leaf, hence the use of mineral oil (HO) was included in our study as a surfactant to increase the penetration of insecticides through the epidermis of the citrus leaf. Imidacloprid 17.8SL (Confidor, Bayer Crop Science Limited) having translaminar mode of action served as a check.

Observations on % infestation by citrus leaf miner were recorded at 7, 10 and 15 days after spraying (DAS). The data generated were statistically analyzed (http://stat.iasri.res.in/sscnarsportal) using completely randomized design (CRD) for cage studies and randomized block design (RBD) for nursery experiments. The data on egg count and % infestation were transformed to square root and arc sine, respectively as per the method followed (Gomez and Gomez, 1984) and means were separated by Duncans Multiple Range Test (DMRT) for inference.

RESULTS

Oviposition deterrence of neem based products and oil against citrus leaf miner under caged/semi-field conditions

Under caged conditions, neem oil 3%, azadirachtin 0.03%, NSKE 7% and HO 1.0% was found significantly the best doses for deterring the CLM adults from oviposition with an egg count/10 cm shoot of 0.79, 0.85,1.50 and 0.80, respectively (Table.1). The best insecticide/botanical dose was furtherevaluated for oviposition deterrence under caged conditions in protected nursery(semi-field condition). Out of the seven treatments, egg count/ 10 cm shoot was significantly low in neem oil 3% (0.60) but was at par with imidacloprid 17.8 SL (0.009%) (0.70) and HMO1% (0.80) treated seedlings (P=0.05). Maximum oviposition deterrence (%) of 84.52 was observed in the case of rough lemon seedlings sprayed with neem oil (3%) followed by imidacloprid 17.8 SL (83.33%) (Table.2). Spray with horticultural mineral oil 1% was found significantly next best in repelling the leaf miner adults from ovipositing the leaves with 80.91% deterrence. Neem cake extract (10%) and NSKE (7%) were found to have the lowestdeterrence of 66.67 and 64.28 %, respectively.

Table1. Oviposition deterrence of different doses of botanicals/oils against citrus leaf miner, *Phyllocnistis citrella*

Treatment	Egg count/ 10cm shoot
T1 Neem oil 1%	1.80(1.32)^{c}
T2 Neem oil 2%	1.20(1.08)^{b}
T3 Neem oil 3%	0.79(0.65)^{a}
T4 Water spray	4.20(2.04)^{d}
CD=0.222(0.01%); CV=13.95	
T1 Azadirachtin 1EC (0.01%	1.90(1.37)^{b}
T2 Azadirachtin 1EC (0.02%)	1.60(1.25)^{b}
T3 Azadirachtin 1EC (0.03%)	0.85(0.91)^{a}
T4 Water spray	4.20(2.04)^{c}
CD=0.199(0.01%); CV-11.75	
T1 NSKE 3%	3.00(1.71)^{b}
T2 NSKE 5%	2.80(1.65)^{b}
T3 NSKE 7%	1.50(1.21)^{a}
T4 Water spray	4.20(2.04)^{c}
CD=0.282(0.01%); CV=15.12	
T1 HO 0.3%	2.10(1.43)^{c}
T2 HO 0.5%	1.30(1.12)^{b}
T3 HO 1.0%	0.80(0.88)^{a}
T4 Water spray	4.20(2.04)^{d}
CD=0.260(0.01%); CV=15.64	

NSKE- Neem Seed Kernel Extract,
HO- Horticulture mineral oil
*Values in parentheses are square root transformed

Efficacy of insecticides/botanicals against citrus leaf miner in the protected nursery

During monsoon 2014, neem oil 3%, spinosad 45 SC + HO (1%) and imidacloprid 17.8 SL @ 0.009% recorded 24.06, 25.36 and 24.39% leaf miner infestation 7 days after spraying (DAS) but was at par with Horticultural oil 1%(28.63). At 10 DAS, neem oil 3% and spinosad 45 SC + HO 1% recorded an infestation of 16.11 and 17.15%, respectively. Similarly, at 15 DAS neem oil 3%,

Table 2. Oviposition deterrence (%) of insecticides/botanicals against citrus leaf miner, *Phyllocnistis citrella* on rough lemon seedlings

Sl.No.	Treatments	Egg count/10 cm shoot (after 48 hours)	Oviposition deterrence (%)
1.	Neem oil (3%)	0.60(0.76)[a]	84.52
2.	Azadirachtin (0.03%)	0.95(0.97)[b]	79.76
3.	NSKE (7%)	1.50(1.20)[c]	64.28
4.	Neemcake extract (10%)	1.40(1.14)[c]	66.67
5.	Horticulture Mineral Oil (1%)	0.80(0.88)[ab]	80.91
6.	Imidacloprid (0.009%)	0.70(0.82)[ab]	83.33
7.	Water spray	4.20(2.04)[d]	-
	CD (P=0.05)	0.16	
	CV	15.42	

*Values in parentheses are square root transformed

spinosad 45 SC + HO 1% and imidacloprid 17.8 SL followed by HO 1% were found significantly effective in reducing leaf miner infestation (Table.3).

During winter 2014, neem oil 3% (24.68), horticultural oil 1% (26.27), spinosad 45 SC + HO (1%) (25.31) and imidacloprid 17.8 SL (24.39) recorded significantly less infestation at 7 DAS (Table 3). At 10 DAS, imidacloprid 17.8 SL, spinosad 45 SC + HO 1% and neem oil 3% were the effective module with significantly lowest infestation of 17.55, 17.16 and 16.12%, respectively. Only imidacloprid 17.8SL (25.16%) was found effective in keeping the leaf miner infestation levels below 30% ETLat 15DAS. But during spring 2015, imidacloprid 17.8 SL recorded lowest infestation at 7 DAS (31.72%) and 15 DAS (29.96). At 10 DAS, an infestation of 29.99% was observed when sprayed with neem oil 3% and spinosad 45 SC + HO 1% (Table 3).

Under nursery conditions over the three seasons (pooled mean data for monsoon and winter, 2014; spring, 2015), at 7 DAS, infestation was significantly low in seedlings treated with imidacloprid 17.8 SL (0.009%) (26.83%) but was at par with neem oil (3%) with 27.03%, spinosad 45 SC + HO (1%) with 28.44%; HO 1% with 29.85% and NSKE (7%) with 33.48%. At 10 DAS, neem oil still recorded an infestation of 20.74 % only and spinosad45 SC(0.009%) + HO (1%) with 21.43 % while imidacloprid 17.8 SL (0.009%) followed by neem oil (3%) and spinosad45 SC (0.009%) + HO (1%) were the effective at 15 DAS. Azadirachtin (1%EC) @ 0.03 % was found less effective as indicated by infestation levels of 40.45 % at 7 DAS to 38.46 %at 15 DAS while NSKE (7%) with only 33.48 to 39.01 % up to 15 DAS. Sprays with *Bt* + HO recorded an infestation which ranged between 28.88 to 35.49% while HO 1 % (alone) with 29.49 to 35.11% indicated that *Bt* as sole (37.63 to 43.58%) insecticide may not be effective in controlling the pest. But in combination with HO, better results were observed in our studies.

DISCUSSION

In the context of several pressures like pesticide resistances accelerated the search for environmentally safe, toxicologically selective and efficacious pesticides (Dias *et al.,* 2005). Neem products have several advantages compared to conventional synthetic pesticides such as their extremely low mammalian toxicity (Thoeming and Poehling, 2006) and a very low probability of pest resistance (Feng and Isman, 1995). It is evident from the above studies that neem oil (3%) has

Table 3. Efficacy of botanicals/insecticides against citrus leaf miner, *Phyllocnistis citrella* during monsoon, 2014, winter, 2014 and spring, 2015 in protected nursery at ICAR- CCRI, Nagpur

Treatment	Per cent infestation															
	Monsoon 2014				Winter 2014				Spring 2015				Pooled mean			
	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS	Pre-treatment	7 DAS	10 DAS	15 DAS
Neem oil (3%)	46.33 (42.89)	24.06 (29.33)^{a}	16.11 (23.65)^{a}	24.29 (29.53)^{a}	45.52 (42.42)	24.68 (29.76)^{a}	16.12 (23.65)^{a}	29.63 (32.97)^{b}	46.00 (42.69)	32.34 (28.67)^{ab}	29.99 (25.00)^{a}	34.22 (31.67)^{c}	45.95 (42.67)	27.03 (31.27)^{ab}	27.03 (26.84)^{a}	29.38 (32.77)^{abc}
Azadirachtin (0.03%)	44.62 (42.02)	39.29 (38.87)^{d}	36.69 (37.27)^{e}	42.66 (40.78)^{d}	44.68 (41.94)	41.28 (39.97)^{cd}	36.69 (36.79)^{c}	35.67 (36.66)^{c}	45.00 (42.12)	40.77 (42.67)^{d}	37.85 (37.66)^{e}	37.06 (36.33)^{d}	51.33 (45.67)	40.45 (39.49)^{c}	37.08 (37.51)^{d}	38.46 (38.31)^{def}
Horticultural oil (HO 1%)	46.66 (43.08)	28.63 (32.27)^{ab}	28.66 (32.33)^{cd}	27.00 (31.25)^{ab}	47.07 (43.31)	26.27 (30.79)^{a}	25.37 (30.21)^{b}	41.67 (40.20)^{de}	47.66 (43.65)	34.65 (32.33)^{bc}	34.44 (32.00)^{c}	36.66 (35.66)^{d}	47.13 (43.35)	29.85 (33.08)^{ab}	29.49 (32.84)^{bc}	35.11 (36.25)^{cde}
NSKE (7%)	38.78 (38.51)	32.39 (34.67)^{bc}	30.15 (33.29)^{cd}	41.00 (39.81)^{d}	37.57 (37.80)	32.39 (35.12)^{b}	27.30 (31.49)^{b}	37.99 (38.04)^{cd}	39.33 (38.84)	35.65 (34.00)^{c}	34.85 (32.66)^{c}	38.05 (38.00)^{d}	38.56 (38.38)	33.48 (35.34)^{abc}	30.77 (33.66)^{cd}	39.01 (38.65)^{ef}
Bacillus thuringiensis (Bt)(0.3%)	40.62 (39.59)	41.00 (39.81)^{d}	37.22 (37.59)^{e}	41.66 (40.18)^{d}	42.38 (40.62)	43.05 (40.99)^{d}	37.22 (37.59)^{c}	46.38 (42.92)^{ef}	43.33 (41.16)	40.19 (41.67)^{d}	38.44 (38.66)^{e}	42.70 (46.00)^{e}	46.56 (43.01)	41.41 (40.05)^{c}	37.63 (37.83)^{d}	43.58 (41.30)^{gf}
Bt (0.3%) + HO (1%) (35.92)^{bcde}	44.70 (41.95)	36.37 (37.06)^{cd}	25.74 (30.47)^{c}	31.48 (34.12)^{c}	44.27 (41.71)	35.67 (36.65)^{bc}	26.47 (30.96)^{b}	35.16 (36.36)^{c}	44.66 (41.93)	34.44 (32.00)^{bc}	34.44 (32.00)^{c}	36.66 (35.66)^{d}	51.21 (45.72)	35.49 (36.55)^{bc}	28.88 (32.46)^{bc}	34.43
Spinosad 45SC (0.009%)	44.03 (41.57)	41.33 (40.00)^{d}	32.39 (34.68)^{de}	30.22 (33.33)^{bc}	44.52 (41.85)	36.52 (34.57)^{b}	34.21 (35.78)^{c}	34.22 (35.77)^{bc}	45.00 (42.12)	34.43 (32.00)^{bc}	36.86 (36.00)^{d}	33.41 (30.33)^{bc}	52.65 (46.60)	40.76 (39.64)^{c}	34.49 (35.95)^{cd}	32.62 (34.82)^{bcd}
Spinosad 45SC (0.009%) + HO (1%)	43.33 (41.16)	25.36 (30.20)^{a}	17.15 (24.45)^{a}	25.71 (30.46)^{a}	46.18 (42.81)	25.31 (30.17)^{a}	17.16 (24.45)^{a}	30.00 (33.18)^{b}	46.00 (42.70)	34.64 (32.33)^{bc}	29.99 (25.00)^{a}	31.30 (27.00)^{ab}	47.95 (43.82)	28.44 (32.16)^{ab}	21.43 (27.38)^{a}	29.00 (32.56)^{ab}
Imidacloprid 17.8 SL (0.009%)	45.49 (42.41	24.39 (29.56)^{a}	21.33 (27.44)^{b}	24.47 (29.61)^{a}	45.22 (42.25)	24.39 (29.56)^{a}	17.55 (24.75)^{a}	25.16 (30.07)^{a}	45.33 (42.31)	31.72 (27.67)^{a}	30.86 (26.33)^{b}	29.96 (25.00)^{a}	45.67 (42.51)	26.83 (31.15)^{a}	23.25 (28.67)^{ab}	26.53 (30.98)^{a}
Control	45.57 (42.45)	63.11 (52.61)^{e}	49.00 (44.42)^{f}	47.66 (43.66)^{e}	45.58 (42.46)	63.12 (52.61)^{e}	47.76 (43.71)^{d}	48.89 (44.37)^{f}	45.66 (42.50)	44.61 (49.33)^{e}	41.94 (44.67)^{f}	45.76 (51.33)^{f}	48.82 (44.32)	56.95 (49.03)^{d}	46.23 (42.83)^{e}	47.44 (43.53)^{g}
CD(P=0.05)	NS	4.09	2.91	2.79	NS	3.34	2.34	2.82	NS	2.65	0.66	2.33	NS	5.34	4.22	3.58
CV		6.54	5.22	4.62		5.37	4.27	4.44		4.25	1.10	3.72		8.46	7.31	5.71

DAS-Days after spraying; Figures in the parentheses are arc sine transformed values; In a column, means followed by same letter (s) are not significantly different at p=0.05 by DMRT

oviposition deterrent effect on CLM adults. The efficiency of neem based products, including emulsified neem oil, NSKE etc for citrus leaf miner management has been confirmed in earlier studies (Jayanthi and Verghese, 2004; Nath and Sinha, 2011). Katole *et al.* (1993) tested the efficacy of some botanicals and synthetic insecticides against infestation of CLM on Nagpur mandarin and found that neem oil was effective in the control of CLM which is in line with our findingthat neem oil (3%) waseffective in deterring the leaf miner adults from ovipositing within 48 hours after spray.Efficacy of Neem oil (1%) has been reported against citrus leaf miner to cause a percent reduction of 33.1, 28.7 and 26.4 at 3, 7 and 14 DAS, respectively(Patil, 2013). Among synthetic insecticides, imidacloprid 17.8 SL (0.009%) was significantly effective in deterring the oviposition by adults as well as checking the pest population up to 15 DAS. Feeding deterrence of sub-lethal concentrations of imidacloprid in plant tissue has been reported for adult Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama on citrus(Boina *et al.*, 2009). According to them,sublethal concentrations of imidacloprid in plant tissue negatively affectsthe development, reproduction, survival and longevity of ACP, which contributes to population reductions over time . Reduction in citrus leaf miner infestation may be due to either feeding deterrence or oviposition deterrence effect of imidacloprid.

Horticultural oil (HO) has shown to work as a temporary oviposition deterrent in nursery seedlings upto 10 DAS (32.84%) in our studies as sole sprays or in combination. Oviposition deterrence as a result of oil deposits as physical barrier has also been demonstrated with Asian citrus psyllid, *D. citri* and citrus leaf miner earlier (Rae et al., 1996). Horticultural mineral oil (Mak all Season) @1.5% reduced the infestation of citrus up to 11 days, @ 2.0 % against psylla up to 7 days and @ 2.0 % against citrus thrips and mites in citrus nurseries. Only limitation with horticultural oils is that they should be used with care to avoid phytotoxicity on the seedlings when temperature is high (>40^{o}C) (Rao et al., 2014).

The botanicals/oils with oviposition deterrent and ovicidal activity are really a valuable weapon to protect the seedlings under nursery conditions before the plants are damaged by the pests. The present findings of this study indicated that timely intervention with selective botanicals /oils/ insecticides during the active adult emergence targeting oviposition may help to combat the rapid multiplication of citrus leaf miner and thereby protect the young flush leaves in citrus during the peak flushing seasons.

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Population dynamics of *Melanagromyza obtusa* (Malloch) (Diptera: Agromyzidae) and its natural parasitization in pigeonpea

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ABSTRACT: Population dynamics of pigeonpea pod fly *Melanagromyza obtusa* (Malloch) and its natural parasitization on Cv. ICP-8863 (Maruthi) studies revealed that the larval population of attained a peak level during 51st standard meteorological week (SMW) with 60 larvae per 100 pods and pupal population on 4th SMW with 47 pupae per 100 pods. During the same period pod damage was at its peak with 81.00 per cent and causing grain damage 54.34 per cent, which subsequently declined to 5.18 per cent during 10th SMW. During the investigation, parasitoids belonging to six families could be recorded on the immature stages of the pod fly. The peak level of natural larval, pupal and total [= overall (larvae + pupae)] parasitization of pod fly was observed during 2nd SMW with 60.00, 51.61 and 55.81 per cent, respectively. Analysis of weather parameters relationship indicated negative correlation between larval population and grain damage *vis-a-vis* maximum temperature and evaporation. The correlation matrix among larval and pupal population; pod and grain damage; and larval, pupal and total parasitization exhibited positive correlation.

KEY WORDS: *Melanagromyza obtusa*, parasitization, population, pigeonpea pod fly

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is grown throughout the tropics but most widely in South and South - East Asia, where it is a major source of vegetable protein. Pigeonpea is the second important pulse crop of India after chickpea being grown in an area of 3.71 million hectares with an annual production of about 2.78 million tonnes resulting in an average productivity of 750 kg per ha during 2014-15 (Anonymous, 2015). Nearly 300 species of insects are known to infest pigeonpea crop at its various growth stages in India. Among the major pod infesting insects pod fly, *Melanagromyza obtusa* (Malloch) (Diptera: Agromyzidae), has emerged as a key pest causing 10.00 per cent to 80.00 per cent damage (Shanower *et al*., 1999; Kumar and Nath, 2003) which is estimated to cause a monitory annual loss of US$ 256 million [= Rs. 1500.00 Crores approx.] (Sharma *et al*., 2011; Arbind *et al*., 2013). In the earliest record of this pest from India at Nagpur, estimated damage to tur-pods was at 12.50 per cent of the whole crop (Ahmad, 1938). A single larva destroys minimum of one complete seed in its lifetime and sometimes it has been seen to move to adjacent

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seed of the same pod to continue the feeding, making seeds unfit for human consumption and germination.

The pest being internal feeder difficult to notice its damage outside poses problems to manage with chemical insecticides (Srinivasan and Durairaj, 2007; Sharma *et al*., 2011). Currently pest management strategies for the pigeonpea pod fly emphasize on chemical control and host plant resistance (Shanower *et al*., 1998). So far, many molecules have been evaluated against this pest, but the significant control is not obtained. Host plant resistance based on physico-chemical traits of pod especially pod wall thickness, trichome density, reducing and non-reducing sugars, total phenols, tannins, and crude fiber hold promises as an important tool for selection of varieties to fit for the management of this pest (Moudgal *et al*., 2008). More than 20 Hymenopteran parasitoids have been reported on this pest (Sharma, 2007). Hence, considering the fact that bio-control agents could play an important role in the natural management of the pest, present investigations were under taken to study the population dynamics of pod fly, *M. obtusa vis-a-vis* level of natural parasitization to enable designing of strategies for its management based on ecological and natural parasitization principles of integrated pest management for resource poor farmers in India.

MATERIALS AND METHODS

The field experiment was conducted at Agricultural Entomology Unit, Agricultural Research Station, Badnapur; (VNMKV, Parbhani) to assess the population dynamics of *M. obtusa* and its natural parasitization. The pigeonpea variety, ICP-8863 (Maruthi) was raised with standard agronomical practices during *Kharif* season 2014-15 under normal field conditions with plant to plant and row to row distance of 30 cm and 60 cm, respectively. No insecticides was applied to protect the crop from the natural infestation of pod fly and left to natural conditions. The population of pod fly, *M. obtusa* was recorded from pod initiation till harvest of the crop by destructive method. The larval and pupal population along with its pod and grain damage were recorded on randomly collected 100 pods covering all the plants at weekly intervals till harvest of crop. The per cent pod damage and per cent grain damage was calculated using the following formula as suggested by Naresh and Singh (1984).

Per cent pod/grain damage =

$$\frac{\text{Number of infected pods/grains}}{\text{Total number of pods/grains}} \times 100$$

The collected larvae and pupae were maintained at the rate of one per vial (plastic vials with 30 ml capacity) and reared at ambient temperature for observing the emergence of different parasitoids. These beneficials were later on identified based on taxonomic features and grouped into different families. The per cent larval, pupal and total (larvae + pupae) parasitization was calculated using the following formulae.

Per cent larval/pupal parasitization =

$$\frac{\text{Number of infected larvae/pupae}}{\text{Total number of larvae/pupae}} \times 100$$

Per cent total (larvae + pupae) parasitization =

$$\frac{\text{Number of infected larvae + pupae}}{\text{Total number of larvae + pupae}} \times 100$$

Correlation analysis was also studied to know the role of weather parameters relationship with parasitization for its influence. The strength of the correlation was described using the guide suggested by Evans (1996) as –

0.00-0.19: “very weak”; 0.20-0.39: “weak”; 0.40-0.59: “moderate”; 0.60-0.79: “strong” And 0.80-1.0: “very strong”

RESULTS AND DISCUSSION

Observations revealed that the larval population of *M. obtusa* was active from 48th SMW (22 larvae / 100 pods) which increased gradually and attained a peak on 51st SMW with 60 larvae per 100 pods. The larval population declined later to 2 larvae in

10th SMW. *M. obtusa* pupal population could be observed from 48th SMW (4 pupae /100 pods), which also increased gradually and attaining a peak on 4th SMW with 47 pupae per 100 pods thereafter declining to 9 pupae in 10th SMW. The pod damage could be observed from 48th SMW (26.00 %) which increased gradually and attained a peak of 81.00 per cent on 3rd SMW, which later declined to 13.00 per cent as observed on 10th SMW. The grain damage due to *M. obtusa* was observed from 48th SMW (15.43 %) which increased gradually to reach a peak on 3rd SMW with 54.34 per cent and started declining to 5.18 per cent as observed on 10th SMW (Table 1). The results obtained in the present investigation in relation to population dynamics of pod fly, *M. obtusa* and its damage on pigeonpea are in conformity with the earlier workers, Pillai and Agnihotri (2013) where in the peak activity was reported during 46th standard week while the population of *M. obtusa* was minimum (31 per 100 pods) during 49th standard week. Similarly, Das and Katyar (1998) reported that the pod fly was first noticed in the 43rd SMW, while maximum pods (16.00 %) infestation with larvae were observed during 5th SMW. The studies of Subharani and Singh (2007) during 2002-04 revealed that the damage commenced at pod filling stage (1.23 and 2.00 %) in the third week of January in both years (2002-03 and 2003-04, respectively). The maximum infestation (15.56 %) of the pest was recorded during third week of February in the first year, whereas it was observed a week earlier, i.e. during second week of February as 13.72 per cent in the second year. Paul *et al*. (2005) reported that 10.00 per cent seed damage from approximately 20.00 per cent pod infestation due to *M. obtusa,* could be considered as the threshold level.

In present investigations, six parasitoid families i.e. Eulophidae, Torymidae, Pteromalidae, Ormyridae, Eurytomidae and Chalcididae emerged from the

Table 1. Population dynamics and natural parasitization level of pod fly, *M. obtusa* in relation to weather parameters

SMW	Rainfall (mm)	Temperature (°C)		Humidity (%)		Damage (%)		Population No. / 100 pods		Parasitization (%)		
		Max.	Min.	AM	PM	Pod	Grain	Larva	Pupa	Larva	Pupa	Total (Larvae + Pupae)
48	0.0	31.3	10.8	80	23	26	15.43	22	4	0.00	0.00	0.00
49	0.0	31.1	9.9	81	25	34	18.24	20	4	0.00	0.00	0.00
50	0.0	29.9	14.8	80	43	40	21.75	34	8	8.82	0.00	4.41
51	0.0	27.3	6.3	74	23	67	38.56	60	37	15.00	2.70	8.85
52	0.0	28.8	8.9	72	24	71	39.36	47	20	27.66	40.00	33.83
1	9.2	27.0	15.1	89	52	74	38.51	59	41	33.90	31.71	32.80
2	0.0	28.3	5.8	76	20	77	45.86	45	31	60.00	51.61	55.81
3	0.0	28.9	10.2	72	29	81	54.34	59	41	54.24	48.78	51.51
4	0.0	31.1	14.1	76	26	61	33.53	41	47	43.90	36.17	40.04
5	0.0	30.8	13.0	71	27	64	43.18	57	41	26.32	24.39	25.35
6	0.0	32.2	14.1	65	27	69	40.51	54	38	14.81	15.79	15.30
7	0.0	33.1	12.3	73	18	60	36.01	30	22	23.33	13.64	18.48
8	0.0	35.0	14.6	66	18	24	12.77	14	9	28.57	11.11	19.84
9	24.3	30.9	15.0	79	38	15	7.98	13	4	7.69	0.00	3.85
10	16.6	33.4	16.0	77	29	13	5.18	2	9	0.00	0.00	0.00

immature stages of the host pod fly, *M. obtusa*. Eulophidae, Torymidae and Pteromalidae families restricted to larval parasitism while Ormyridae, Eurytomidae and Chalcididae families to pupal stage. Earlier report (Yadav *et al*., 2012) revealing the parasitoid-complex of four hymenopteran parasitoids *viz*., larval parasitoid, *Euderus lividus* (Ashmead) (Eulophidae) and pupal parasitoids, *Ormyrus orientalis* (Walker) (Ormyridae), *Eurytoma* sp. (Eurytomidae) and *Pseudotorymus* sp. (Torymidae) is in support of the present finding as the four of six families corroborated. Makinson *et al*. (2005) for the first time reared two parasitoids *viz*., *Callitula* sp. (Hymenoptera: Pteromalidae) and *Ormyrus* sp. (Hymenoptera: Ormyridae) from *M. obtusa* on *Cajanus latisepalus* pods in Australia.

The natural larval parasitization level of pod fly was observed from 50th SMW (8.82 %) which increased gradually to reach a peak on 2nd SMW with 60.00 per cent. The larval parasitization got then declined to nil on 10th SMW. The pupal parasitization level of *M. obtusa* was observed from 51st SMW (2.70 %) which increased gradually and attained a peak on 2nd SMW with 51.61 per cent. The pupal parasitization level was then totally declined as observed on 9th and 10th SMW. The total (larvae + pupae) parasitization level of *M. obtusa* was recorded from 50th SMW (4.41 per cent) which increased gradually and attained peak activity on 2nd SMW with 55.81 per cent and then the parasitization level was totally declined as observed on 10th SMW. The results obtained in the present investigation in relation to natural parasitization of pod fly, *M obtusa* on pigeonpea is in conformity with the earlier workers, Pillai and Agnihotri (2013) wherein peak level of weekly per cent parasitization (18.18 %) was observed during 51st SMW while minimum level of weekly per cent parasitization (6.52 %) was observed during 47th SMW. Similarly, Meena *et al*. (2010) reported maximum parasitization of *M. obtusa* by *Ormyrus* sp. during 14th SW (21.00 %) while, Moudgal *et al*. (2005) reported parasitization range of larval - pupal parasitoid *Euderus lividus* Ashmead and the pupal parasitoid *Eurytoma* sp. on *M. obtusa* from 5.45 to 10.00 per cent and 3.69 to 5.00 per cent, respectively. Durairaj (2005) reported *Ormyrus* sp., *Eurytoma* sp. and *Eupelmus* sp. as pupal parasitoids. A high level of parasitism was recorded in August (87.50 %), followed by May and June. More than 50.00 per cent parasitism was recorded in April, June, September and October while a low level of parasitism (2.50 %) in December.

The simple correlation between larval and pupal population; per cent pod and grain damage; per cent larval, pupal and total (larvae + pupae) parasitization of *M. obtusa* infesting pigeonpea with weather parameters during *Kharif* season 2014-15 indicate negative correlation between larval population of *M. obtusa* with maximum temperature (r = 0.7045). Similarly, pod damage with rainfall and maximum temperature was observed to be moderately negatively correlated (r = -0.5339 and -0.6285). Similarly, grain damage with maximum temperature and rainfall was also found negatively correlated (r = -0.5765 and -0.5490, respectively). The results obtained in the present investigation in relation to simple correlation between *M. obtusa* population and weather parameters is in conformity with the earlier workers, Akhauri *et al*. (1997) where in negative correlation was revealed with minimum temperature (r = -0.270) and relative humidity (r = -0.271), indicating that weather does not play important role in infestation. Similarly, Naresh and Singh (1984) reported a negative correlation between larval and pupal population of pod fly with temperature, having its regression coefficient -0.490 and whereas the pod fly population with relative humidity (r = 0.922), wind velocity (r = 0.354) and rainfall (r = 0.542) indicate positive correlation (Table 2).

The correlation between larval population pupal population; pod damage and grain damage; larval and pupal parasitization of *M. obtusa* infesting pigeonpea indicate positive correlation with coefficient of 0.8534, 0.9276 and 0.9195, respectively. There was a strong positive correlation between pupal population with pod damage and grain damage having its regression coefficient 0.847 and 0.8394, respectively. The correlation between pod with grain damage and pupal parasitization of pod fly exhibited significant correlation with

Table 2. Correlation matrix between larval and pupal population, pod and grain damage, larval, pupal and total (Larvae + Pupae) parasitization of pod fly and weather parameters

	Rainfall (mm)	Temperature (°C)		Humidity (%)		Evapo-ration	Wind Velocity (kmph)
		Max.	Min.	AM	PM		
Larva Population	-0.4768	-0.7045*	-0.3929	-0.1127	0.1528	-0.5363*	0.0147
Pupal Population	-0.3305	-0.453	-0.1697	-0.1873	0.0549	-0.3238	0.1913
Pod Damage	-0.5339*	-0.6285*	-0.4775	-0.158	-0.0145	-0.494	-0.0525
Grain Damage	-0.549*	-0.5765*	-0.4912	-0.238	-0.0787	-0.4055	-0.0395
Larval Parasitization	-0.3251	-0.3647	-0.3184	-0.185	-0.1118	-0.2296	-0.1286
Pupal Parasitization	-0.3169	-0.4409	-0.3452	-0.1421	-0.0691	-0.3564	-0.0931
Total (larvae + pupae) Parasitization	-0.3266	-0.4098	-0.3376	-0.1665	-0.0921	-0.2979	-0.1129

Significant at 5% ; * Negative Moderate Correlation.

Table 3. Correlation matrix among larval and pupal population, per cent pod and grain damage, per cent larval, pupal and total (Larvae + Pupae) parasitization

Para-meters	Population		Damage (%)		Parasitization (%)		
	Larva	Pupa	Pod	Grain	Larva	Pupa	Total
Larva	1	0.8534*	0.9276*	0.9195*	0.5618	0.6129	0.5975
Pupa		1	0.847*	0.8394*	0.6836	0.6802	0.6939
Pod			1	0.982*	0.7252	0.7845*	0.7679*
Grain				1	0.7341	0.7825*	0.7715*
Larval					1	0.9314*	0.9828*
Pupal						1	0.9826*
Total							1

Significant at 5%; *Strong positive correlation

coefficient of 0.982 and 0.7845, respectively, indicating that with availability of food, the population of *M. obtusa* increases leading to more pod and ultimately the grain damage. Moderate positive correlation was observed with larval parasitization (0.7252) indicating such increased population also increases the chances of its parasitization naturally. The correlation between grain damage and pupal parasitization of pod fly showed strong positive correlation having its regression coefficient 0.7825, while moderate positive correlation was observed with larval parasitization (0.7341) indicating that with availability of adequate food, the population of *M. obtusa* increases leading to more chances of parasitization. The correlation between larval parasitization with pupal parasitization shows strong positive correlation with its regression coefficient 0.9314, representing that availability of adequate host increases the level of parasitization (Table 3).

Pod fly, *Melanagromyza obtusa* is a major emerging constraint playing an important role in pigeonpea yield reduction. So far various chemical molecules have been evaluated against this pest, but the significant control is not obtained. Many parasitoids are reported on this pest which plays major role in natural control of this pest with natural parasitization varying from 2.00 per cent to 90.00 per cent. Therefore, even on resistant/ tolerant genotype, the need based use of newer botanical or chemical molecules taking care of the environment as well as the parasitoid complex of this pest cannot be a sole panacea for management of pod fly, but the bioagents can rally round to greater extent if explored properly.

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Seasonal occurrence of chiku moth *[Nephopteryx eugraphella]* (Ragonot) and bud borer *[Anarsia achrasella]* Bradley on sapota

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ABSTRACT: Seasonal occurrence of bud boring insect pests *viz.*, chiku moth (*Nephopteryx eugraphella*) and bud borer (*Anarsia achrasella*) on eight varieties of sapota as PKM-1, PKM-3, PKM-4, DHS-1, DHS-2, Kalipatti, Cricket ball and CO-3 revealed that the maximum bud damage due to chiku moth was observed during second fortnight of April to first fortnight of June to the extent of 9.93 to 10.78 per cent, while bud borer showed maximum infestation of 9.03 and 9.47 per cent during month of May and then decline from June onwards. The temperature and evaporation rate had significant influence on chiku moth and bud borer incedence. Among the varieties, CO-3 had comparatively low bud in pest infestation due to both pests. DHS-2 showed higher bud damage by chiku moth, whereas bud borer damage was higher in Kalipatti.

KEY WORD: Seasonal occurrence, *Nephopteryx eugraphella*, *Anarsia achrasella*, sapota

INTRODUCTION

In India, sapota or sapodilla is an important fruit of tropical region and gaining importance among fruit crops. Gujarat shared 16% sapota area and 17% production of the country and ranked third position after Maharashtra and Karnataka (Anonymous, 2014b). In Gujarat, cultivation of sapota coupled with intensive monoculture of Kalipatti variety supported by changing environmental condition as well as unchecked pest population caused outburst of insect pests in wider area. Among different bud boring insect pests, chiku moth (leaf webber), *Nephopteryx eugraphella* (Ragonot) (Lepidoptera: Pyrilidae) and bud borer (bud worm), *Anarsia achrasella* Bradley (Lepidoptera: Gelechiidae) are foremost pests of sapota. They damages up to 20 to 30 per cent of flowers/buds and therefore, are considered to be key factors affecting the yield potential of sapota in Gujarat (Jhala *et al*., 1986 and Patel, 2001). About 25-28 per cent yield losses due to bud borer and chiku moth can be avoided in protected condition (Anonymous, 2014 a and 2015 b). The caterpillar of chiku moth feeds on leaves, buds and flowers and sometimes on tender fruits. The caterpillars clump the leaves together by webbing and feed within on the chlorophyll and leaving behind a network of veins. The buds were bored and ultimately wither away. The bud borer bores through the upper tapering part of the flower bud of sapota and eats up inner content leading to no flower setting or retention.

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In way forward of developing pest management strategy detail study on chiku moth and bud borer incidence was studied on different varieties of sapota in changing ecological condition of South Gujarat.

MATERIALS AND METHODS

The experiment on bud boring pest activity was carried out during 2014-15 in sapota orchard of Fruit Research Station, Navsari Agricultural University, Gandevi. The seasonal occurrence study of chiku moth and bud borer based on per cent bud damage on eight varieties of sapota *viz.*, PKM-1, PKM-3, PKM-4, DHS-1, DHS-2, Kalipatti, Cricket ball and CO-3 was examined on three replicated trees planted at 5 x 5 m spacing under high density plantation. In sapota orchard, randomly selected 5 twig of each variety was selected at fortnightly interval for the incidence of chiku moth and bud borer in new buds. The total numbers of new buds as well as buds damaged by chiku moth and bud borer were recorded separately from each twig. The per cent bud infestation was calculated from the data of bud damage. To evaluate the influence of various ecological factors on progression of bud boring pest, the bud damage was correlated with different meteorological parameters *viz.*, maximum and minimum temperature; morning and evening relative humidity; bright sunshine hrs; rainfall and evaporation.

RESULTS AND DISCUSSION

Chiku moth (*N. eugraphella*):

The seasonal occurrence of chiku moth showed a varying degree of bud infestation throughout the year in the different varieties of sapota (Table 1). The average maximum infestation of chiku moth on buds was 9.93 to 10.78 per cent observed between second fortnight of April to first fortnight of June at peak flowering period and then decline onwards. It was minimum (3.98-4.17%) during January and increased towards February and again reached maximum in April at beginning of crest flowering span. Among varieties, early peak bud infestation was commenced from March in PKM-3, PKM-4, DHS-1, DHS-2 and Kalipatti, while it was observed from April onwards in PKM-1, Cricket ball and CO-3 variety. In Kalipatti, second peak bud damage was occurred during October-November between 6.23 to 7.52 per cent incidence, while in Cricket ball, it was observed in second fortnight of November (7.86%) and first fortnight of December (6.48%). The lowest incidence intensity was also vary among varieties, wherein minimum bud damage was reported during August in DHS-2 (4.44%), November in DHS-1 (2.78%), December in PKM-4 (3.14%) and February in Cricket ball (2.56%) as well as other varieties (PKM-1, PKM-3, Kalipatti and CO-3) had lowest in January as similar with mean incidence data (2.20-4.31%).

The varietal evaluation of sapota varieties (Table 1) indicated that none of the variety was found completely free from the damage of chiku moth during the year 2014-15. There was no much difference among varieties of sapota. However, the average lowest bud infestation was recorded on variety CO-3 (4.94%) comparable with PKM-1 (5.95%) and PKM-4 (6.36%). The variety DHS-2 had the highest mean damage (7.39%) and comparable to Kalipatti (7.25%). Rest of varieties *viz.*, DHS-1, Cricket ball and PKM-3 were recorded 6.42, 6.56 and 6.60 per cent average bud damage, respectively.

The correlation data of average chiku moth damage in sapota (Table 3) indicated highly significant positive association with maximum temperature (r = 0.696), minimum temperature (r = 0.470) and evaporation rate (r = 0.903), but significant negative association with morning relative humidity (r = -0.664). The similar type of trend was observed in all varieties with more or less relationship except in PKM-1, PKM-4 and Kalipatti, wherein minimum temperature didn't have role in increasing chiku moth bud damage, but closely related.

Bud borer (*A. achrasella*):

The seasonal incidence of bud borer (Table 2) showed diverse degree of infestation throughout the year in the different varieties of sapota from

Table 1: Seasonal occurrence of chiku moth (*N. eugraphella*) in different varieties of sapota (2014-15)

Tr. No.	Per cent bud damage*											
	MARCH, 14		APRIL, 14		MAY, 14		JUNE, 14		JULY, 14		AUG., 14	
	I	II	I	II	I	II	I	II	I	II	I	II
T_1: PKM-1	5.72 (13.84)	7.62 (16.02)	6.97 (15.28)	11.97 (20.23)	10.49 (18.88)	10.46 (18.97)	8.83 (17.29)	6.09 (14.27)	5.86 (13.99)	4.05 (11.63)	4.36 (12.02)	5.17 (13.11)
T_2: PKM-3	9.37 (17.82)	10.95 (19.32)	8.06 (16.49)	10.07 (18.50)	10.79 (19.18)	11.98 (20.26)	10.37 (18.78)	8.08 (16.49)	6.90 (15.23)	4.76 (12.58)	3.78 (11.21)	3.92 (11.43)
T_3: PKM-4	7.64 (16.4)	8.86 (17.31)	8.41 (16.86)	10.93 (19.29)	10.51 (18.93)	10.59 (18.99)	9.86 (18.29)	8.23 (16.67)	5.23 (13.20)	4.58 (12.37)	3.20 (10.31)	4.56 (12.32)
T_4: DHS-1	8.52 (16.96)	10.17 (18.58)	7.44 (15.84)	9.02 (17.48)	9.63 (18.06)	11.67 (19.97)	10.57 (18.97)	9.49 (17.93)	8.98 (17.43)	4.61 (12.38)	5.93 (14.08)	6.67 (14.98)
T_5: DHS-2	10.90 (10.28)	11.31 (19.68)	9.33 (17.78)	10.44 (18.84)	10.60 (18.97)	10.93 (19.31)	9.98 (18.39)	8.77 (17.23)	8.57 (17.04)	7.77 (16.17)	6.04 (14.21)	4.44 (12.17)
T_6: Kalipatti	8.93 (17.37)	10.13 (18.54)	8.71 (17.16)	10.62 (19.01)	10.21 (18.64)	10.09 (18.51)	9.98 (15.82)	7.65 (16.06)	5.15 (13.11)	4.53 (12.28)	6.72 (15.01)	6.70 (15.01)
T_7: Cricket ball	6.38 (14.63)	7.78 (16.19)	8.31 (16.74)	9.27 (17.71)	9.56 (18.00)	11.68 (19.97)	10.37 (19.16)	9.90 (18.31)	6.82 (15.18)	5.96 (14.10)	5.28 (13.27)	4.76 (12.60)
T_8: CO-3	4.76 (12.59)	5.08 (13.03)	6.35 (14.57)	7.10 (15.34)	6.11 (14.30)	8.81 (17.24)	9.86 (16.97)	6.99 (15.32)	4.63 (12.41)	4.30 (11.96)	5.86 (14.00)	2.08 (8.31)
Avg.	7.78	8.99	7.95	9.93	9.74	10.78	10.57	8.15	6.52	5.07	5.15	4.79
S.E.m±	0.46	0.57	0.30	0.43	0.45	0.40	0.41	0.36	0.28	0.27	0.34	0.25
CD at 5%	0.60	1.08	0.91	1.29	1.36	1.22	1.25	1.09	0.84	0.81	1.04	0.77
CV%	6.15	5.57	3.18	4.03	4.28	3.64	3.99	3.76	8.25	7.55	4.54	3.52

* Figures in parentheses are arc sin transformed values.

Cont. Table 1

Tr. No.	Per cent bud damage*												
	SEP., 14		OCT., 14		NOV., 14		DEC., 14		JAN., 15		FEB., 15		Avg.
	I	II	I	II	I	II	I	II	I	II	I	II	
T1: PKM-1	6.80 (15.07)	6.67 (14.96)	6.67 (14.96)	4.84 (12.71)	4.38 (12.07)	3.08 (10.06)	4.34 (12.01)	3.90 (11.39)	2.37 (8.87)	3.84 (11.28)	4.59 (12.36)	3.67 (11.04)	5.95
T2: PKM-3	4.78 (12.55)	6.33 (14.56)	6.52 (14.78)	4.78 (12.57)	6.14 (14.35)	4.85 (12.730)	5.61 (13.72)	4.58 (12.34)	3.22 (10.34)	3.07 (10.10)	4.91 (12.82)	4.60 (12.38)	6.60
T3: PKM-4	5.14 (13.07)	3.70 (11.08)	4.78 (12.58)	5.56 (13.63)	5.44 (13.50)	5.00 (12.92)	3.17 (10.24)	5.17 (13.15)	4.95 (12.81)	5.53 (13.59)	6.44 (14.69)	5.10 (13.04)	6.36
T4: DHS-1	6.33 (14.54)	4.76 (12.57)	3.33 (10.53)	4.28 (11.90)	3.84 (11.25)	2.78 (9.97)	4.19 (11.77)	5.27 (13.28)	4.57 (12.33)	3.49 (10.75)	4.20 (11.79)	4.23 (11.83)	6.42
T5: DHS-2	5.32 (13.34)	6.73 (15.04)	5.50 (13.51)	4.76 (12.60)	6.70 (15.00)	5.52 (13.60)	6.30 (14.52)	6.38 (14.64)	5.77 (13.89)	4.64 (12.41)	5.66 (13.75)	4.96 (12.84)	7.39
T6: Kalipatti	7.33 (15.71)	6.60 (14.87)	7.52 (15.92)	6.23 (14.46)	6.62 (14.92)	7.18 (15.52)	5.73 (13.83)	5.67 (13.75)	4.31 (11.93)	6.22 (14.43)	6.00 (14.18)	7.81 (16.23)	7.25
T7: Cricket ball	5.52 (13.57)	5.20 (13.17)	5.90 (14.01)	5.32 (13.32)	5.96 (14.12)	7.86 (16.27)	6.48 (14.73)	5.22 (13.21)	3.51 (10.79)	4.38 (12.07)	3.03 (9.99)	2.56 (9.23)	6.56
T8: CO-3	3.00 (9.97)	5.71 (13.82)	4.10 (11.70)	4.08 (11.65)	5.33 (13.36)	3.97 (11.47)	4.52 (12.27)	4.56 (12.33)	3.14 (10.22)	2.20 (8.53)	3.17 (10.18)	4.17 (11.78)	4.94
Avg.	5.53	5.71	5.54	4.98	5.55	5.03	5.04	5.09	3.98	4.17	4.75	4.64	6.43
S.E.m±	0.50	0.39	0.54	0.29	0.31	0.39	0.35	0.32	0.42	0.30	0.47	0.36	--
CD at 5%	1.52	1.19	1.63	0.88	0.94	1.19	1.06	0.98	1.27	0.91	1.42	1.10	--
CV%	6.42	4.95	6.89	3.90	3.94	5.33	4.72	4.31	6.38	4.45	6.52	5.13	--

* Figures in parentheses are arc sin transformed values.

Table 2: Seasonal occurrence of bud borer (*A. achrasella*) in different varieties of sapota (2014-15)

Tr. No.	Per cent bud damage*											
	MARCH, 14		APRIL, 14		MAY, 14		JUNE, 14		JULY, 14		AUG., 14	
	I	II	I	II	I	II	I	II	I	II	I	II
T1: PKM-1	4.22 (11.87)	6.64 (14.95)	7.81 (16.21)	8.22 (16.65)	8.09 (16.50)	7.93 (16.35)	8.17 (16.61)	5.60 (13.69)	3.81 (11.26)	4.28 (11.94)	1.38 (6.750	1.10 (6.03)
T2: PKM-3	2.06 (8.28)	3.86 (11.31)	5.33 (13.33)	7.87 (16.29)	10.17 (18.57)	10.43 (18.84)	7.78 (16.19)	6.25 (14.48)	4.15 (11.76)	5.24 (13.23)	3.94 (11.43)	5.29 (13.28)
T3: PKM-4	6.67 (14.97)	5.56 (13.65)	7.79 (16.19)	8.37 (16.80)	11.37 (19.70)	11.11 (19.47)	8.28 (16.72)	5.87 (14.02)	3.33 (10.51)	4.49 (12.23)	4.81 (12.66)	2.00 (8.13)
T4: DHS-1	7.41 (15.80)	6.24 (14.47)	7.17 (15.54)	8.17 (16.58)	11.25 (19.60)	11.25 (19.60)	8.89 (17.34)	6.53 (14.80)	4.26 (11.92)	4.67 (12.48)	2.11 (8.35)	1.30 (6.58)
T5: DHS-2	4.78 (12.62)	6.02 (14.20)	5.56 (13.65)	6.50 (14.77)	7.70 (16.11)	7.14 (15.50)	9.66 (18.11)	7.71 (16.12)	5.79 (13.91)	3.47 (10.72)	3.56 (10.84)	2.27 (8.65)
T6: Kalipatti	5.60 (13.68)	6.39 (14.63)	7.63 (16.02)	8.04 (16.45)	10.32 (18.75)	10.42 (18.83)	7.44 (15.83)	9.71 (18.16)	7.95 (16.37)	4.42 (12.15)	2.21 (8.57)	1.80 (7.71)
T7: Cricket ball	3.50 (10.81)	4.10 (11.68)	6.72 (15.04)	7.79 (16.19)	8.33 (16.77)	9.75 (18.19)	7.38 (15.76)	5.86 (14.00)	3.65 (11.00)	5.33 (13.36)	5.66 (13.77)	1.05 (5.87)
T8: CO-3	2.40 (6.90)	3.20 (10.30)	6.03 (14.21)	4.76 (12.61)	5.00 (12.92)	7.75 (16.16)	7.94 (16.36)	6.08 (14.28)	4.42 (12.14)	5.80 (13.94)	3.69 (11.08)	1.42 (6.80)
Avg.	4.58	5.25	6.76	7.47	9.03	9.47	8.19	6.70	4.67	4.71	3.42	2.03
S.E.m±	0.46	0.52	0.35	0.44	0.74	0.89	0.55	0.82	0.47	0.68	0.79	0.60
CD at 5%	0.51	0.50	0.61	1.33	0.96	1.24	0.66	0.65	0.99	0.79	1.38	1.24
CV%	5.38	7.17	6.32	4.82	3.14	6.09	7.51	5.46	6.89	6.57	8.42	9.76

* Figures in parentheses are arc sin transformed values.

Cont. Table 2

Tr. No.	Per cent bud damage*												Avg.
	SEP., 14		OCT., 14		NOV., 14		DEC., 14		JAN., 15		FEB., 15		
	I	II	I	II	I	II	I	II	I	II	I	II	
T1: PKM-1	1.40 (6.76)	1.50 (7.00)	2.42 (8.95)	3.17 (10.24)	5.07 (13.03)	3.20 (10.29)	4.30 (11.97)	5.63 (13.72)	5.42 (13.42)	4.38 (12.06)	4.86 (12.72)	5.68 (13.79)	4.76
T2: PKM-3	2.00 (8.13)	2.06 (8.26)	2.80 (9.61)	3.20 (10.30)	4.62 (12.43)	4.30 (11.97)	4.70 (12.46)	5.21 (13.21)	5.73 (13.77)	5.80 (13.93)	6.66 (14.97)	6.90 (15.25)	5.26
T3: PKM-4	3.05 (10.07)	3.30 (10.47)	3.80 (11.22)	3.70 (11.10)	3.42 (10.65)	2.60 (9.29)	1.96 (8.02)	2.33 (8.62)	3.64 (10.97)	4.11 (11.66)	3.06 (10.10)	4.17 (11.80)	4.95
T4: DHS-1	1.75 (7.62)	1.40 (6.78)	2.00 (8.13)	4.55 (12.31)	4.71 (12.52)	3.06 (10.11)	2.78 (9.58)	3.23 (10.38)	4.79 (12.61)	5.66 (13.77)	5.08 (13.02)	5.56 (13.64)	5.16
T5: DHS-2	1.96 (8.01)	1.60 (7.25)	2.90 (9.80)	3.67 (11.04)	2.67 (9.42)	2.04 (8.21)	2.08 (8.12)	3.63 (10.93)	3.02 (9.96)	4.09 (11.65)	5.09 (13.03)	5.53 (13.61)	4.52
T6: Kalipatti	2.17 (8.49)	2.06 (8.24)	2.10 (8.35)	2.76 (9.56)	3.78 (11.20)	5.95 (14.14)	4.66 (12.44)	3.06 (9.99)	4.21 (11.85)	5.93 (14.07)	5.76 (13.89)	7.06 (15.43)	5.48
T7: Cricket ball1.	39 (6.74)	1.20 (6.27)	1.30 (6.56)	2.14 (8.42)	3.25 (10.40)	2.28 (8.65)	2.40 (8.88)	3.86 (11.31)	4.23 (11.73)	4.23 (11.87)	5.55 (13.62)	4.39 (12.08)	4.39
T8: CO-3	1.10 (6.02)	1.60 (7.25)	1.40 (6.82)	2.35 (8.83)	3.33 (10.51)	1.19 (6.26)	2.33 (8.75)	3.63 (10.99)	3.70 (11.02)	4.49 (12.19)	2.44 (9.01)	4.32 (11.98)	3.77
Avg.	1.85	1.84	2.34	3.19	3.86	3.08	3.15	3.82	4.34	4.84	4.81	5.45	4.79
S.E.m±	0.72	0.53	0.48	0.58	0.74	0.62	0.50	0.55	0.90	0.50	0.70	0.32	--
CD at 5%	1.11	1.01	0.66	0.96	1.32	1.12	1.52	1.68	2.73	1.50	2.11	0.97	--
CV%	5.67	6.68	5.02	6.78	8.34	7.56	8.63	8.61	13.10	6.79	9.64	4.11	--

* Figures in parentheses are arc sin transformed values.

Table 3: Correlation of chiku moth (*N. eugraphella*) seasonal occurrence with ecological parameters (2014-15)

Tr. No. / Weather Parameter	Temperature (°C)		Relative Humidity (%)		Bright Sunshine hrs	Rainfall (mm)	Evaporation (mm/day)
	Tmax.	Tmin.	Mor. RH	Eve. RH			
T_1: PKM-1	0.546**	0.308	-0.608**	0.059	-0.102	-0.071	0.756**
T_2: PKM-3	0.753**	0.385**	-0.656**	-0.021	0.024	-0.254	0.882**
T_3: PKM-4	0.683**	0.247	-0.721**	-0.128	0.141	-0.296	0.897**
T_4: DSH-1	0.436*	0.501**	-0.545**	0.269	-0.182	-0.077	0.766**
T_5: DSH-2	0.673**	0.540**	-0.511**	0.169	0.087	-0.162	0.792**
T_6: Kalipatti	0.724**	0.314	-0.536**	-0.079	0.238	-0.392	0.888**
T_7: Cricket ball	0.635**	0.567**	-0.573**	0.173	-0.175	-0.116	0.755**
T_8: CO-3	0.582**	0.509**	-0.633**	0.099	-0.206	-0.177	0.777**
Avg.	0.696**	0.470*	-0.664**	0.081	-0.028	-0.208	0.903**

*Significant at 5% level and ** at 1% level.

Table 4: Correlation of bud borer (*A. achrasella*) seasonal occurrence with ecological parameters (2014-15)

Tr. No. / Weather Parameter	Temperature (°C)		Relative Humidity (%)		Bright Sunshine hrs	Rainfall (mm)	Evaporation (mm/day)
	Tmax.	Tmin.	Mor. RH	Eve. RH			
T_1: PKM-1	0.530**	-0.083	-0.639**	-0.395*	0.152	-0.301	0.719**
T_2: PKM-3	0.294	0.104	-0.411*	-0.165	-0.087	-0.165	0.542**
T_3: PKM-4	0.576**	0.372	-0.611**	0.022	-0.003	-0.158	0.850**
T_4: DSH-1	0.541**	0.118	-0.685**	-0.220	0.055	-0.239	0.799**
T_5: DSH-2	0.496**	0.299	-0.616**	-0.035	-0.100	-0.197	0.817**
T_6: Kalipatti	0.459*	0.159	-0.451*	-0.156	-0.005	-0.217	0.682**
T_7: Cricket ball	0.344	0.203	-0.547**	-0.033	-0.116	-0.055	0.670**
T_8: CO-3	0.224	0.309	-0.468*	0.100	-0.323	0.086	0.579**
Avg.	0.489*	0.203	-0.615**	-0.127	-0.048	-0.180	0.788**

*Significant at 5% level and ** at 1% level.

March 2014 to February 2015. The bud borer damage was increased from March onwards and reached maximum at peak flowering period in May (9.03-9.47%) and in first fortnight of June (8.19%). The bud infestation decline July onwards and observed minimum during monsoon period of September (1.84-1.85%). Among varietal differences in seasonal occurrence, higher bud damage was commenced from March in DHS-1, while in May in DHS-2 and CO-3 and other five varieties had similar higher damage from April. The minimum bud damage was also vary in different varieties like August in PKM-1 (1.10%); September in PKM-3 (2.00%), DHS-1 (1.40%), DHS-2 (1.60%), Kalipatti (2.06%), Cricket ball (1.20%) and CO-3 (1.10%) as well as December in PKM-4 (1.96%). From the data presented in Table 2 indicated that none of the variety was found

completely free from the attack of bud borer during the year 2014-15. However, the lowest average seasonal occurrence was recorded on variety CO-3 (3.77%), which was followed by Cricket ball (4.39%) and DHS-2 (4.52%). The variety Kalipatti had the highest mean seasonal occurrence (5.48%) and nearer to variety PKM-3 (5.26%). Rest of varieties *viz*., PKM-1, PKM-4 and DHS–1 were recorded 4.76, 4.95 and 5.16 per cent bud damage, respectively.

The correlation study on average incidence of bud borer on sapota (Table 4) indicated significant positive association with maximum temperature (r = 0.489) and evaporation rate (r = 0.788) as well as negative correlation with morning relative humidity (r = -0.615). This type of trend was also reported in five sapota varieties, but slightly differs in PKM-3, Cricket ball and CO-3, wherein the maximum temperature didn't have any influence on bud borer damage. In contrast, evening relative humidity had slight correlation with bud damage in PKM-1.

The current chiku moth bud infestation level is found similar with previous findings of South Gujarat condition (Anonymous, 2009) and Periyakulam (T.N.) locations (Anonymous, 2015a). In earlier few reports, chiku moth was reported highest during September onwards under South Gujarat (Patel *et al.*, 1993, Anonymous, 1998 and Deshmukh, 2001), while under middle Gujarat, its peak activity was reported in July (Patel, 1996) as well as during September in North Gujarat location (Hajare *et al.*, 2012). These data were recorded on Kalipatti variety and difference may be due to variability in ecological conditions. Susceptibility of Kalipatti to chiku moth corroborates with earlier reports (Anonymous, 1995 and 1998). Patel (1996) reported that cricket ball was more susceptible. The variety PKM 1 was reported as least susceptible (Anonymous, 2001). Chiku moth correlation findings in the present investigation are more or less contradicting with the findings of Patel *et al.* (1993), Patel (1996) and in earlier data recorded on Kalipatti variety in South Gujarat condition (Anonymous, 1995, 1998, 2009, 2014a, 2015b). The findings on the peak activity of bud borer damage in sapota cv. Kalipatti under South Gujarat condition corroborates with the results of Jhala *et al.* (1986) and the data recorded at same sapota orchard (Anonymous, 1998, 2001 and 2009). This trend showed there is much fluctuations in intensity of bud damage during last two decades. Similarly, the same trend of bud borer infestation was also reported at Periyakulam area of Tamil Nadu (Anonymous, 2013) and hill zone of Karnataka by Ravulapenta *et al.* (2014) as well as in Gujarat at Bharuch by Patel *et al.* (2014) and at Anand by Thumar *et al.* (2015) in different varieties of sapota. In earlier reports, Kalipatti and DSH-1 were found more prone to bud borer damage as compare to PKM-1 under South Gujarat condition (Anonymous, 1995, 1998, 2001, 2014a, 2015b). The present bud borer correlation findings are also supported by the results of Jayanthi *et al.* (2008) as well as previous research carried on Kalipatti at same locality with more or less relationship with ecological factors (Anonymous, 1998, 2009, 2014a, 2015b).

With respect to bud boring insect pests, the maximum abundance of chiku moth and bud borer was observed in May-June at peak flowering stage of sapota orchard. The results revealed increase maximum temperature and ultimately evaporation rate as well as decrease morning relative humidity caused higher bud borer damage in sapota. On evaluation of eight varieties, CO-3 had least bud damage due to chiku moth and bud borer, whereas Kalipatti, DHS-2 and PKM-3 were highly infested. However, the rest of the varieties viz., PKM-1, PKM-4, DHS-1 and Cricket ball were found moderately susceptible to both pests.

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Contact toxicity of synthetic pyrethroid insecticides to honey bees *Apis cerana indica* Fab., *Apis mellifera* Linnaeus and *Trigona iridipennis* Smith in laboratory condition

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ABSTRACT: The toxicity of insecticides was assessed to worker honey bees of Indian bee *Apis cerana indica* Fab., Italian bee *Apis mellifera* Linnaeus and Stingless bee *Trigona iridipennis* Smith by contact toxicity method and observations made recorded 6, 12 and 24 Hours after treatment (HAT) and the per cent mortality were worked out. The different treatment of insecticides *viz.*, newer formulation of bifenthrin 10 EC at 40, 80, 120 and 160 a.i. ha^{-1}, Wilthrin® 10 EC at 80 a.i. ha^{-1} and Lambda-cyhalothrin 5 EC at 25 a.i. ha^{-1} was used to determine the toxicity level and each treatment was replicated three times. Results revealed that, bifenthrin 10 EC was highly toxic to workers of all three species of honey bees tested. At 24 HAT, bifenthrin 10 EC at 160 g a.i ha^{-1} was highly toxic to honey bee which was evident from the observation of Indian bees (93.33% mortality), Italian bees (90.00%) and stingless bees (96.67%).

KEYWORDS: Insecticides, toxicity, *Apis cerana indica*, *Apis mellifera*, *Trigona iridipennis*

INTRODUCTION

Honey bees are the most important pollinator of many field crops, vegetables and fruit crops (Husain *et al.*, 2014) and play an important role in the creation and conservation of biodiversity (Hegde, 1999) and increases the crop yield (Chan *et al.*, 2006). For decades, pesticides used for control of crop pests have caused honey bee mortality and morbidity (Johnson *et al.*, 2010). The population decline in honey bees was detected due to unintended exposure of agricultural pesticides (Nabti *et al.*, 2014). The present investigations were undertaken to assess the safety of newer formulation of synthetic pyrethroid insecticide bifenthrin 10 EC in comparison with Wilthrin® 10 EC and lambda-cyhalothrin 5 EC on three species of worker honey bees namely Indian bee *Apis cerana indica* Fab., Italian bee *Apis mellifera* Linnaeus and stingless bee, *Trigona iridipennis* Smith under laboratory conditions. If insecticides are used in an appropriate manner, they can control the target organisms without negatively affecting the pollinator populations and also conserving the ecosystem (Davis, 1989). Hence, considering the importance of honey bees are pollinating agent on agro ecosystem we evaluated the effect of insecticides on mortality of honey bee workers.

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MATERIALS AND METHODS

According to modification of Busvine (1980) the safety of bifenthrin 10 EC to the worker bees of different bee species *viz.*, Indian bee, *A. cerana indica*, Italian bee, *A. mellifera* and stingless bee, *T. iridipennis* was carried out in the laboratory by indirect contact test using filter paper (Stanley *et.al.*, 2009). The different concentration of insecticides were prepared using distilled water *viz.*, newer formulation of bifenthrin 10 EC at 40, 80, 120 and 160 g a.i. ha^{-1}, Wilthrin® 10 EC at 80 g a.i. ha^{-1} and Lambda-cyhalothrin 5 EC at 25 g a.i. ha^{-1} and each treatment was replicated thrice. Plastic containers were used for the bioassay and there were adequate perforations in the upper lid in order to provide proper aeration for the bees. The filter paper discs were cut according to the size of the plastic container. Then the filter paper discs were sprayed with one ml of different concentration of insecticides dissolved in distilled water using atomizer. The wetted filter paper discs were allowed to shade dried by hanging for about 10 minutes. The shade dried filter papers were placed in the bioassay container and honey bees were released at the rate of 10 per container. The honey bees were kept in the refrigerator for one minute prior to release so as to calm them for an easy transfer. After exposure for one hour, the bees were allowed in perforated polythene bags and were provided with 40 per cent sucrose solution soaked in cotton wool as feed. The mortality of bees was recorded at 6, 12 and 24 h after treatment and the per cent mortality was worked out as following.

$$\text{Per cent mortality} = \frac{\text{Number of honey bees dead}}{\text{Total number of honey bees released}} \times 100$$

RESULTS AND DISCUSSION

Indian bee, *A. cerana indica:*

The results of bifenthrin 10 EC on Indian bees, *A. cerana indica* is presented in Table 1. Bifenthrin 10 EC at 40 g a.i. ha^{-1} produced the least mortality

Table 1. Effect of bifenthrin 10 EC on Indian bees - *Apis cerana indica* Fabricius

Treatment	Dose (g a.i. ha^{-1})	6 HAT*		12 HAT*		24 HAT*		Mean (%)
		Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	
Bifenthrin 10 EC	40	26.67 (31.09) ^{b}	18.52	40.00 (39.23)^{b}	27.78	66.67 (54.74)^{b}	46.83	44.44
Bifenthrin 10 EC	80	30.00 (33.21)^{bc}	22.22	46.67 (43.09)^{bc}	35.65	73.33 (58.91)^{bc}	57.94	50.00
Bifenthrin 10 EC	120	36.67 (37.27)^{cd}	29.63	53.33 (46.91)^{c}	43.98	86.67 (68.58)^{d}	78.57	58.89
Bifenthrin 10 EC	160	43.33 (41.17)^{de}	37.04	66.67 (54.74)^{d}	60.19	93.33 (75.04)^{de}	88.89	67.78
Wilthrin® 10 EC	80	33.33 (35.26)^{bc}	25.93	50.00 (45.00)^{c}	39.81	76.67 (61.12)^{c}	63.49	53.33
Lambda-cyhalothrin 5 EC	25	50.0 (45.00)^{e}	44.44	70.00 (56.79)^{d}	64.35	96.67 (79.48)^{e}	95.24	72.22
Untreated check	-	10.00 (18.43)^{a}	0.00	16.67 (24.09)^{a}	0.00	36.67 (37.27)^{a}	0.00	21.11

*Mean of three observations; HAT- Hours After Treatment: Figures in parentheses are *arc sin*e values.
In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT

Table 2. Effect of bifenthrin 10 EC on Italian bees - *Apis mellifera* Linnaeus

Treatment	Dose (g a.i. ha^{-1})	6 HAT*		12 HAT*		24 HAT*		Mean (%)
		Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	
Bifenthrin 10 EC	40	23.33 (28.88)^{b}	14.81	36.67 (37.27)^{b}	20.83	66.67 (54.74)^{b}	49.21	42.22
Bifenthrin 10 EC	80	26.67 (31.09)^{b}	18.52	40.00 (39.23)^{bc}	25.00	76.67 (61.12)^{bc}	64.29	47.78
Bifenthrin 10 EC	120	30.00 (33.21)^{bc}	22.22	46.67 (43.09)^{c}	33.33	83.33 (65.91)^{cde}	74.60	53.33
Bifenthrin 10 EC	160	36.67 (37.27)^{cd}	29.63	56.67 (48.83)^{d}	45.83	90.00 (71.57)^{de}	84.92	61.11
Wilthrin® 10 EC	80	26.67 (31.09)^{b}	18.52	43.33 (41.17)^{bc}	29.17	80.00 (63.43)^{cd}	69.84	50.00
Lambda-cyhalothrin 5 EC	25	43.33 (41.17)^{d}	37.04	63.33 (52.73)^{d}	54.17	93.33 (75.04)^{e}	89.68	66.67
Untreated check	-	10.00 (18.43)^{a}	0.00	20.00 (26.57)^{a}	0.00	33.33 (35.26)^{a}	0.00	21.11

*Mean of three observations; HAT- Hours After Treatment. Figures in parentheses are *arc sine* values.
In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT.

of 26.67, 40.00 and 66.67 per cent at 6, 12, and 24 HAT, respectively. Recommended dose of bifenthrin 10 EC at 80 g a.i. ha^{-1} (30.00, 46.67 and 73.33%) was on par with Wilthrin® 10 EC at 80 g a.i. ha^{-1} (33.33, 50.00 and 76.67%) at 6, 12 and 24 HAT, respectively. After 24 h of exposure, all the insecticidal treatments had the highest mortality (>50%) compared to other intervals. The highest mortality was recorded in treatment lambda-cyhalothrin (50.00, 70.00 and 96.67%) at 6, 12 and 24 HAT, respectively.

Italian bee, *A. mellifera* :

Similar to Indian bees, the insecticides tested were significantly toxic to Italian bees (Table 2). The per cent mortality of bees were ranged between 10.00 to 43.33 at 6 HAT and 20.00 to 63.33 at 12 HAT. After 24 h treatment, the bifenthrin 10 EC at 40 g a.i ha^{-1} recorded 66.67 per cent mortality followed by bifenthrin 10 EC at 80 g a.i ha^{-1} (76.67%), Wilthrin® 10 EC at 80 g a.i ha^{-1} (80.00%), bifenthrin 10 EC at 120 g a.i ha^{-1} (83.33%) and bifenthrin 10 EC at 160 g a.i ha^{-1} (90.00%). The maximum mortality of 93.33 per cent was observed in lambda-cyhalothrin 5 EC at 25 g a.i. ha^{-1} at 24 HAT.

Stingless bee, *T. iridipennis:*

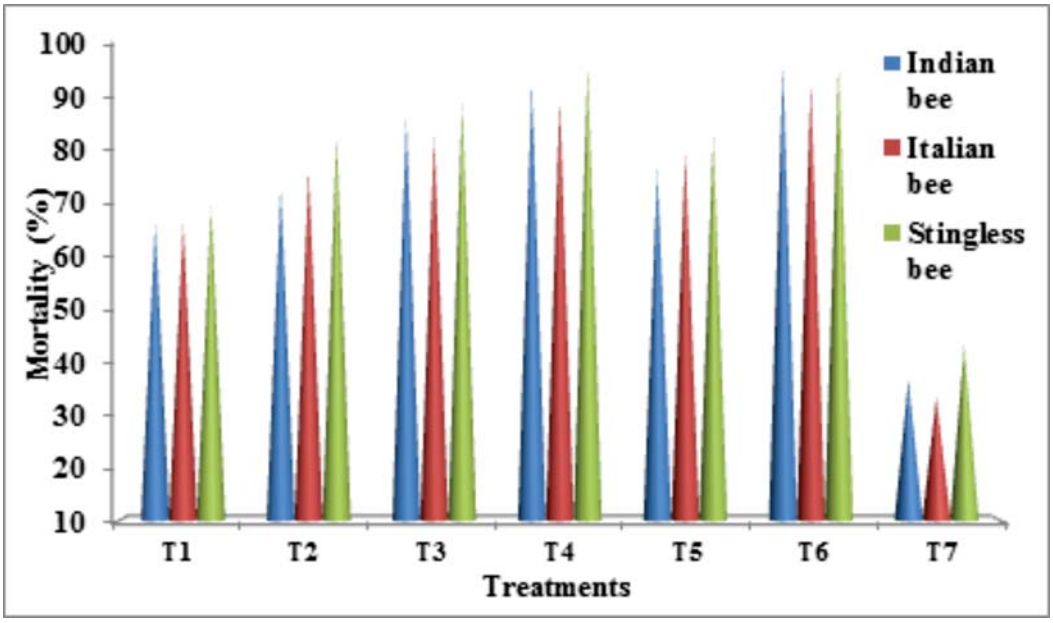
In case of stingless bees also, significant mortality was observed with bifenthrin 10 EC. The bifenthrin 10 EC at 40, 80, 120 and 160 g a.i. ha^{-1} recorded 26.67, 36.67, 50.00 and 56.67 per cent mortality at 6 HAT, respectively (Table 3). At 24 HAT, the honey bee mortality was observed in bifenthrin 10 EC at 40 g a.i ha^{-1} (70.00%) followed by bifenthrin 10 EC at 80 g a.i ha^{-1} (83.33%) which was on par with Wilthrin® 10 EC at 80 g a.i ha^{-1} (83.33%) and maximum per cent mortality was observed in lambda-cyhalothrin 5 EC at 25 g a.i ha^{-1} (96.67%).

The insecticidal effects on non-target organisms can be cat-egorized as harmless (<50% mortality), slightly harmful (50 to 79% mortality), moderately harmful (80 to 89% mortality) and harmful (>90% mortality) when test-ed at the field recommended

Table 3. Effect of bifenthrin 10 EC on stingless bees - *Trigona iridipennis* Smith

Treatment	Dose (g a.i. ha^{-1})	6 HAT*		12 HAT*		24 HAT*		Mean (%)
		Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	Per cent mortality	Corrected mortality (%)	
Bifenthrin 10 EC	40	26.67 (31.09)^{ab}	11.57	46.67 (43.09)^{b}	30.36	70.00 (56.79)^{b}	46.67	47.78
Bifenthrin 10 EC	80	36.67 (37.27)^{bc}	24.07	53.33 (46.91)^{b}	38.69	83.33 (65.91)^{c}	71.11	57.78
Bifenthrin 10 EC	120	50.00 (45.00)^{cde}	39.81	66.67 (54.74)^{cd}	56.55	90.00 (71.57)^{cd}	83.33	68.89
Bifenthrin 10 EC	160	56.67 (48.83)^{de}	47.69	76.67 (61.12)^{de}	69.64	96.67 (79.48)^{d}	93.33	76.67
Wilthrin® 10 EC	80	40.00 (39.23)^{bcd}	27.31	56.67 (48.83)^{bc}	43.45	83.33 (65.91)^{c}	70.00	60.00
Lambda-cyhalothrin 5 EC	25	60.00 (50.77)^{e}	51.39	80.00 (63.43)^{e}	74.40	96.67 (79.48)^{d}	94.44	78.89
Untreated check	-	16.67 (24.09)^{a}	0.00	23.33 (28.88)^{a}	0. 00	43.33 (41.17)^{a}	0.00	27.78

*Mean of three observations; HAT- Hours After Treatment. Figures in parentheses are *arc sine* values. In a column means followed by a common letter are not significantly different at P = 0.05 by DMRT.



T_1- Bifenthrin 10 EC 40 g a.i. ha^{-1}
T_2- Bifenthrin 10 EC 80 g a.i. ha^{-1}
T_3- Bifenthrin 10 EC 120 g a.i. ha^{-1}
T_4- Bifenthrin 10 EC 160 g a.i. ha^{-1}
T_5- Wilthrin® 10 EC 80 g a.i. ha^{-1}
T_6- Lambda- cyhalothrin 5 EC 25 g a.i. ha^{-1}
T_7- Untreated check

Fig. 1. Effect of bifenthrin 10 EC on three species of honey bees at 24 Hours after Treatment (HAT)

dose (Nasreen *et al*., 2007). In the present studies the effect of bifenthrin 10 EC was harmful to all three species of worker honey bees. The higher dose of bifenthrin 10 EC at 160 g a.i. $ha^{-1}$was highly toxic to honey bees which was evident from the observations of Indian bee (93.33% mortality), Italian bee (90.00%) and stingless bee (96.67%) at 24 h after treatment (Fig 1). Lambda-cyhalothrin was also equally toxic to honey bees as that of bifenthrin. After 24 h of exposure, all the insecticidal treatments had the highest mortality (>40%) compared to other intervals. The present findings agree with Thomazoni *et al.* (2009) who reported cent per cent mortality to honey bee workers in Talstar® 100 EC at 1000 ml ha^{-1}. Earlier, Ellis *et al.* (1997) also found honey bees exhibiting greater susceptibility to bifenthrin under laboratory conditions. Gough and Wilkinson (1984) observed that lambda-cyhalothrin had higher contact toxicity to honey bees. Husain *et al.* (2014) observed bifenthrin was highly toxic to honey bees (*A. dorsata*, *A. florea* and *A. mellifera*) with low LT_{50}. Also Rigotti (2005) found that pyrethroids caused cent per cent mortality of honey bee adults under laboratory condition. Similarly, Dai *et al.* (2010) observed that pyrethroids including bifenthrin were highly toxic to honey bees. Since bifenthrin 10 EC at recommended doses proved toxic to bees, the application should be resorted to in hours of low bee activity in field.

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Expression of the heat shock protein genes in the adults of *Callosobruchus chinensis* due to *Centella asiatica*

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ABSTRACT: Medicinally important plant *Centella asiatica* was ckecked against the stored product pest *Callosobruchuschinensis*. HSP gene is more expressed in the treated adult insects, indicating the development of stress condition in the treated insects due to plant extract treatment. The presence of insecticidal compounds like rotenoids and steroids present in the plant *Centella asiatica* may stimulated the immune system of the insect and caused over expression of HSPs.

KEY WORDS: heat shock protein genes, *Centella asiatica*, *Callosobruchuschinensis*

INTRODUCTION

Different kinds of pesticides are used for checking the stored product pest *Callosobruchus chinensis.* Chemical pesticides are the most important among them.Injudicious use of chemicals as a pest management method have lead to the development of resistance in insects towards them and have a high degree of residual effect due to their nondegradable nature (Dwivedi and Sonia Venugopalan, 1998).

Plants and plant compounds are proved to have pesticidal activity against various pests.Many medicinal plants are proved to be effective in controlling the stored pest *Callosobruchus chinensis.* These plants produce a stress condition in the insects and there is the expression of certain heat shock proteins. Heat Shock Proteins (HSPs) are a family of proteins that are produced by cells in response to exposure to stressful conditions. Extracellular and membrane bound heat-shock proteins, especially Hsp70 are involved in binding antigens and presenting them to the immune system (Nishikawa *et al.,* 2008). HSP70 is a key protein that is closely related to the molecular mechanism of insect resistance to the environment. Thus, understanding the differential expression of HSP70 may provide insight into how insects react to the stress environment and provide specific information about the mechanisms of resistance to various stress (Ling Wang *et al.,* 2015).More reports about insecticide inducible HSPs in insects have been published (Sharma *et al*.. 2008, Gupta *et al*., 2007). Many populations of *Frankliniella occidentalis* have developed resistance to various classes of insecticides, including avermectin (Immaraju *et al.,* 1992) which is now widely used for thrips control in China (Gao *et al.,* 2012).

A well-known mechanism used by organisms to cope with environmental stresses is the expression of heat shock proteins HSPs. HSPs usually act as molecular chaperones that promote protein folding

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and assembly, and prevent the aggregation of denatured proteins or newly-synthesized polypeptides (Gehring and Wehner, 1995; Sorensen *et al*., 2003; Feder and Hofmann, 1999). HSPs may be induced when a cell or organism undergoes any number of diverse environmental stresses, such as exposure to heat, cold, metal ions, pesticides, desiccation, or hypoxia (Kregel, 2002; Morrow *et al*., 2004; Joanisse *et al*., 1998).

Present study is an attempt to analyze the expression of HSP 70 genes in *Centella asiatica* acetone extract treated adult insects of *Callosobruchus chinensis*. The plant is widely used for medicinal purposes. Centella is mildly antibacterial, antiviral, anti-inflammatory and anti ulcerogenic. *Centella asiatica* extracts have been used traditionally for wound healing, asiaticoside, a constituent in *Centella asiatica*, has been reported to possess wound healing activity by increasing collagen formation and angiogenesis.

MATERIALS AND METHODS

Experiments were conducted in the Entomology Research Laboratory, Department of Zoology, University College Thiruvananthapuram. Pulse beetle, *Callosobruchus chinensis* adults were reared at normal room temperature and a relative humidity of 40% on clean and un-infested green gram (*Vigna radiata* L).The seeds were made pesticide free by washing with clean water. Newly emerged adults were used for the study.

Acetone extract of *Centella asiatica* was prepared with 20 g of powdered leaves of the plant was weighed and tied in a thin cloth and placed in the soxhlet apparatus. 200 ml acetone was taken in the glass flask and boiled at 55^0 C continuously. Boiling was continued for six to eight hours till the extract become pale green. On completing the boiling, the extract was allowed to cool and stored in air tight containers for further use under refrigerated condition.

The effect of acetone extract was analyzed by using residual film method. No.1 Whatman filter paper were cut in round shape and placed in the plastic containers. Sub lethal dose of the extract (2% or 20 g) was selected by analyzing the lethal dose by probit analysis and the dose below lethal dose was taken as sub lethal dose (lethal dose for acetone extract of the plant is 25 g - Table 2,3 and Fig. 2) was applied to these filter papers using a micropipette and allowed to dry so that the solvent may evaporate completely. Then 50 g of feed was weighed out and twenty five one day old adult insects were placed in the containers so that each would get about 2g of feed. For each treatment control was also set up without applying plant extract. Solvent (acetone) alone was used in the control. Five replicates were kept for each treatment and its control. 100 mg of treated and control insects were homogenized in Trizol and taken for RNA isolation. LD_{50} was calculated using probit analysis (Muhammad Akram Randhawa, 1944).

Total RNA was isolated using the total RNA isolation kit per the manufacture instruction (Invitrogen, USA). Addition of Trizol solution causes the disruption of cells and the release of RNA. Chloroform extraction following centrifugation, exclusively in the aqueous phase whereas proteins are in the interphase and organic phase. On mixing with isopropanol, RNA gets precipitated as a white pellet on the side and the bottom of the tube. 1ml of trizol reagent was added to the 100mg tissue sample and homogenized until it formed a fine paste. 200 µl of chloroform was added and shaken vigorously for 15 seconds and incubated for 2-3minutes at room temperature. Then the sample was centrifuged at 14000 rpm for 15 minutes at $4^{æ\%}$C. The aqueous layer was collected and 100% 500 µl of isopropanol was added. It was incubated for 10 minutes at room temperature. Supernatant was discarded and pellet was collected, washed with 1ml of 75% of ethanol (Merck). It was then centrifuged at 10000 rpm for 5 minutes at $4^{æ\%}$C in a cooling centrifuge (Remi). The RNA pellet was dried and dissolved in TE buffer. The purity of extracted RNA was determined using flurimeter Qubit 3.1 (Life Technologies, USA)

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction laboratory commonly used in molecular biology to generate many copies of a DNA sequence, a process termed "amplification". In RT-PCR, however an RNA strand is first reverse transcribed into its DNA complement (Complementary DNA or cDNA) using the enzyme reverse transcriptase and the resulting cDNA is amplified using PCR or real time PCR. RT-PCR technique was performed using primer designed specifically for amplified gene. Verso One step RT PCR kit of Thermoscientific, USA was used for the cDNA synthesis and amplification. About 5 ìl of RNA, 1ìl of enzyme mix, 2.5ìl of RT Enhancer, 2ìl of forward primer and reverse primer were added to an RNAse free tube. To this mixture 25 ìl of primer RT PCR premix was added. Then the total reaction volume was made up to 50 ìl with the addition of sterile distilled water. The solution was mixed by pipetting gently up and down. The thermal cycler (Eppendorf Master Cycler) was programmed to undergo cDNA synthesis and amplification. The cycling conditions followed were as per -

Gene	Primer sequence
Callosobruchus HSp70 F	GGCATCGGAATGAACAGACA
Callosobruchus HSp70 R	GGATTTCCAGACACAAGACTCC

	Temperature	Time	No. cycles
cDNA synthesis	50^0C	15 min	1
Verso inactivation	95^0C	15min	1
Denaturation	95^0C	20sec	
Annealing	50-60^0C	30sec	35-45
Extension	72^0C	1min	
Final extension	72^0C	5min	1

Gene fragments were separated by charge and size and move through agarose gel matrix, when subjected to an electric field through agarose gel electrophoresis method. The electric field is generated by applying potential across an electrolyte solution (buffer). When boiled in an aqueous buffer, agar dissolve and upon cooling solidifies to a gel. Agarose gelelectrophoresis was performed to check the purity of isolated mRNA· 1% agarose gel was prepared in 1x TE buffer and melted in hot water bath at 90^0C. Then the melted agarose was cooled down to 45^0C. 6µl of 10mg/ml of ethidium bromide was added and poured in to gel casting apparatus with the gel comb. After setting, the comb was removed from the gel.The electrophoresis buffer was poured in the gel tank and the platform with the gel was placed in it to immerse the gel. The amplified RNA sample was switched on and it was observed that RNA bands started migrating towards the anode. The stained gel was visualized using a gel documentation system (E gel imager, Invitrogen) and the mean density was determined using Image J analysis software.

RESULTS AND DISCUSSION

HSP gene is more expressed in treated insects compared to control. It indicates the development of stress condition in the treated insects due to the presence of insecticidal compounds in the plant extract (Fig. 1). The band is distinct in the treated insects compared to control. In the relative expression of HSP 70 in *C. chinensis* the mean intensity was higher (5610.26) in the treated individuals and it was low (3278.66) in the control.

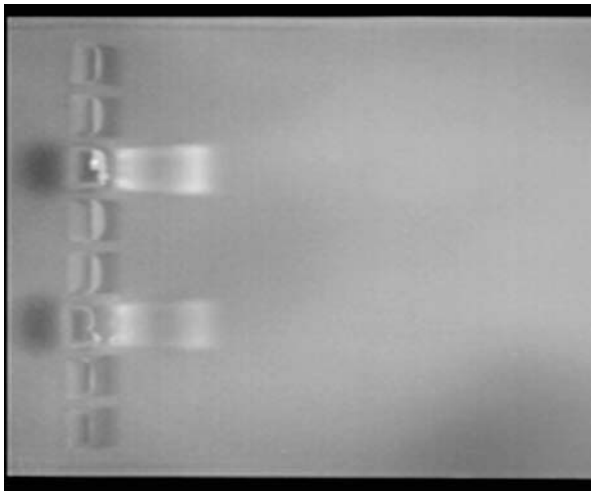

Fig.1. Expression of HSP gene in treatment and control

LD_{50} was calculated using probit analysis (Table 2, 3 and Fig. 2). Log LD 50 is 1.37 and LD 50 is 25 g.

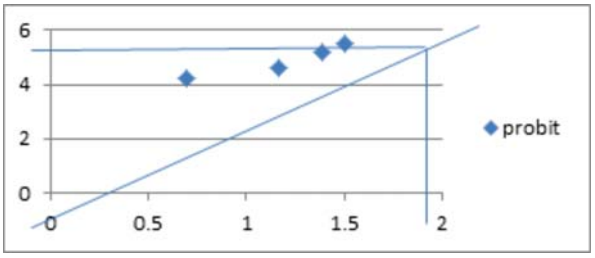

Fig. 2 LD_{50} using probit analysis

Table 2. Mortality of adults with *C. asiatica*

Dose(%)	Mortality (%)
0.5	22±0.02
1.5	38±0.01
2.5	58±0.03
3.5	70±0.02

Table 3. Probit analysis of adult insects on treating with *C. asiatica*

Group	Dose g/kg	Log dose	% dead	% corre-cted	Probit
1	5g	0.69	22	22	4.2
2	15g	1.17	38	38	4.6
3	25g	1.37	58	58	5.2
4	35g	1.50	70	70	5.5

In the present study there is a significant expression of HSP gene in the plant extract treated insects. It suggests that plants represent a promising source of insecticidal compounds. Over expression of HSP indicates the resistance developed in insects to overcome the stress aroused due to plant extract treatment. Previous studies have suggested that insect HSPs play protective roles in response to abiotic and biotic stresses (Zhao and Jones, 2012). The presence of insecticidal compounds like rotenoids and steroids present in the plant *C. asiatica* may stimulated the immune system of the insect and caused over expression of HSPs. The expression of HSPs has been reported to be induced and modulated in response to pesticides (Sonoda and Tsumuki, 2007). Wang Hai-hong *et al.* (2013) investigated the expression of HSPs in thrips after exposed to a pesticide avermectin. They found that avermectin is seemed to regulate the expression of *F. occidentalis* HSPs in two different ways. Five Fo-HSPs were induced at low concentrations of avermectin. The upregulation was approximately 12-fold for Fo-HSP28.9 for *F. occidentalis* exposed to bean discs treated at 0.002 ppm compared with the untreated control. Jatuporn Tungjitwitayakul *et al.* (2015) studied the expression of heat shock protein genes in different developmental stages after temperature stress in the maize weevil. Yasir H Siddique *et al.* (2013) reported the the effect of chemical, cyclophosphamide in the expression of HSP 70 in *Drosophila melanogaster*. Tang *et al.* (2012) reported that Stress-induced HSP70 from *Musca domestica* plays a functionally significant role in the immune system.

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Predatory potential of two species of *Monomorium* on the developing stages of silkworm *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae)

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ABSTRACT: Among the predators that attack tasar silkworm, *Antheraea mylitta, Monomorium destructor* and *M.minimum* are serious on early larval instars as well as pupae of *A. mylitta*. Host-predator interactions were studied, including all the predatory events of the predation by a single as well as groups of ants on *A. mylitta*. Predatory risk of these ants in the field is discussed.

KEYWORDS: *Antheraea mylitta*, *Monomorium*, predatory behavior, tasar silkworm

INTRODUCTION

The tropical silkworm, *Antheraea mylitta* (Drury) (Lepidoptera: Saturniidae) is primarily reared on *Terminalia tomentosa* synonym *T. elliptica* Willd. and *T. Arjuna* (Roxb.) W. & A and it produces a unique variety of wild '*Tasar*' silk (Jolly *et al.*, 1968, 1979). It has three crops in a year, and though it is wild by nature, it is being exposed to several threats during its life span (Jolly *et al.*, 1968, Singh and Thangavelu, 1991). The abundance of the predators in the tasar rearing sites directly affects the wild tasar silk production (Singh and Thangavelu, 1991). However, among the predators, the ants are also affecting the Indian sericulture industry (Negi *et al.*, 1993; Gathalkar and Barsagade, 2016 a), as well as several other commercially important insects (Gosswald, 1990; Hölldobler and Wilson, 1990; Petal, 1978; Risch and Carroll, 1982). Similarly, the ant species, viz. *Pheidolegeton diversus* (Jerdon), *Monomorium minutum* (Mayr) and *Myrmicaria brunnea* (Saunders) are also documented as a predator of tasar and muga silkworms both (Negi *et al.*, 1993; Gathalkar and Barsagade, 2016 a). Whereas, *Monomorium minimum* (Buckley), and *Pheidole* sp. are known to attack the temperate tasar silkworm, *Antheraea proylei* (Jolly) (Negi *et al.*, 1993). Similarly, the ant *Tapinoma melanocephalum* (Fabricius) is attacking the pupae and adults of the muga silkworm (Singh 1991, Negi *et al.*, 1993). While, *Polyrhachis bicolor* (Smith) recognized to drag the spinning larvae, in a group (Bidyapati *et al.*, 1994). The ant species such as *Tetraponera rufonigra* (Jerdon), *Camponotus compressus* (Fabricius) and *Oecophylla smaragdina* (Fabricius) are very frequent foragers in the tasar rearing fields (Singh, 1991; Gathalkar and Barsagade, 2016 a,b; Negi *et al.*, 1993) by which the tasar silk production is being reduced.

The myrmicine genus *Monomorium* is one of the most influential groups of ants regarding its abundant diversity, intra-morphological and biological

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variability (Aslam *et al.*, 2006). Of these, *Monomorium pharaonis* (Linnaeus), *Monomorium destructor* (Jerdon), and *Monomorium floricola* (Jerdon) are well-known domestic pests (Williams, 1994). As predators of various pest species, they also are used in pest management system. In addition, some ants are essentialfor the pollination, predation, scavenging, soil improvement, nutrient cycling as well as plant dispersal (Gotwald, 1986; Folgrait, 1998; Lach *et al.*, 2010). There are 358 species, and 27 subspecies have been listed in the genus *Monomorium* (Bolton, 2016). Mostly the several species of ant are acting like a pest in the various fields and urban habitats (Vega and Rust, 2003). In urban populations, ants cause frequent pest problems where they destroy the aesthetic and economic value of many products of human consumption (Hölldobler and Wilson, 1990; Lee, 2002). These ant species also act as vectors of various plant diseases, whereas, the attack of some ant species is quite painful to domestic animals as well as human beings (Vinson, 1986; Goddard and de Shazo, 2004). However, the ant species are also used as an ecological indicator, to assess the ecological status, concerning species diversity and the impact of invasive species (Bharti *et al.*, 2016), rather most of them are standard generalized predators of many tropical crops (Aslam *et al.*, 1994). Subsequently, the weaver ant *O. smaragdina* is the highly aggressive predator in tasar sericulture, as well as it is also used as a biological control agent in many commercial crops (Way and Khoo, 1991; Paulson and Akre, 1992). Similarly, these predatory ants are also helpful in controlling a variety of insect pests of various crops in temperate and tropical areas, such as cocoa, pears, cotton and rice (Way and Khoo, 1991; Paulson and Akre, 1992). Generally, the soil-dwelling ant species are known to feed on many like earthworm, acarid, isopod, myriapod, collembolan, termite, beetle, bark lice and lepidopteran species (Cerda and Dejean, 2011). Subsequently, many studies have been conducted on the foraging behavior of various ant species (Sudd, 1968; Gotwald, 1986). The ant species such as *O. smaragdina*, *Monomorium* sp. and *Pheidole* sp. are the well-known predators of the *A. mylitta* (Jolly *et al.*, 1979; Singh and Thangavelu, 1991; Singh, 1991). However, the predation biology of these ants is poorly known in the field of tasar-culture. Therefore, the present study was carried out to explore more about the predatory potential of these tiny ants especially, *Monomorium destructor* and *M. minimum* and their invasive impact on tasar-culture.

MATERIALS AND METHODS

The tasar silkworm, *A. mylitta*, is cultivated in the tropical forests of India, and primarily reared on *Terminalia tomentosa* (Yen), *T. arjuna* (Arjun) and several other secondary food plants. *Antheraea mylitta* is the principal non-mulberry silk producing insect in the tropical forest of Vidarbha in Maharashtra. The life cycle of *A. mylitta* undergoes into the egg, five larval stages, pupa, and adult (Gathalkar and Barsagade 2016a). There are three crops, *viz.*, crop I, crop II and crop III in the months of June–August, August–October, and October– January respectively. During the study, various eco-zones of Bhandara and its adjacent districts were investigated during 2010-2013, to know the occurrences and the predation risk of pest species. In addition, all the predatory behaviour of ants including host-predator interaction and host damage were observed visually and video-graphed. Further more, the identification of ant species was made in Department of Zoology, RTM Nagpur University, Nagpur with the help of an online catalog (Bolton, 2014) and morphological characteristics.

RESULTS

The predator belonging to family Formicidae, such as *Monomorium* (*M. minimum* and *M. destructor*) are abundant in tasar rearing fields and affecting the total silk production by attacking the defenseless stages of *A. mylitta.* These reddish-brown small sized ants are active throughout the year on the tasar host plants with their nest under the tree at ground and tree crest. The ants (workers) attack the first to third instar larvae, as well as the pupa of *A. mylitta*, through the cocoon shell by making small holes and feed complete pupa/seed (Figs 1 a-d), and causes the tasar mortality.

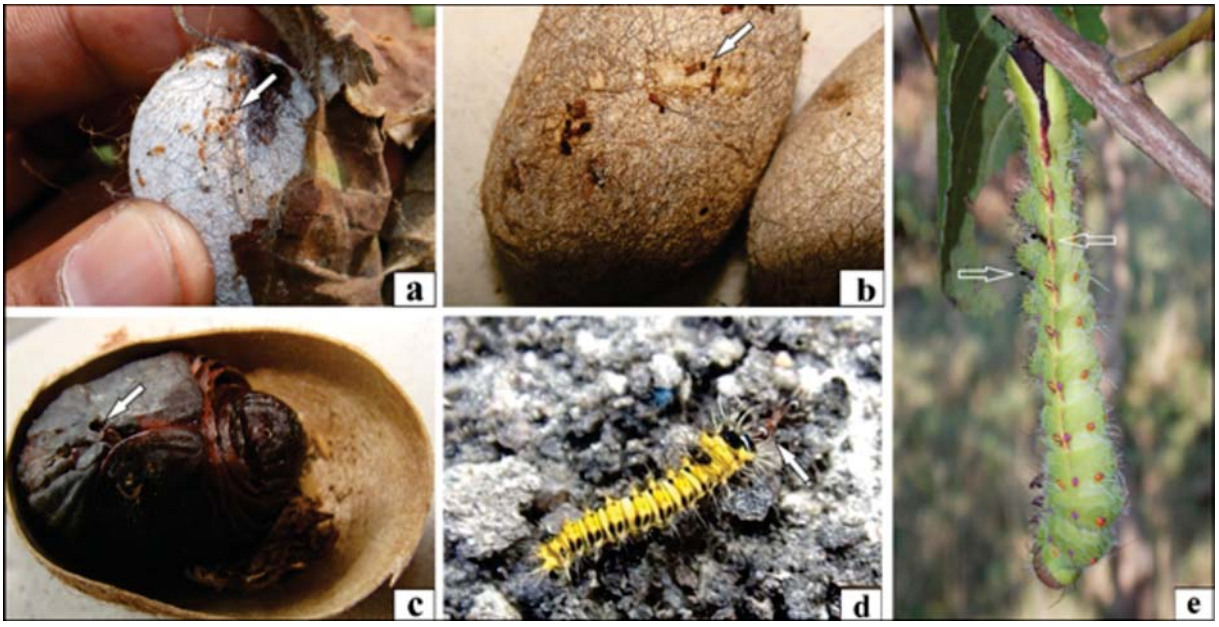

Figure 1 Predation of *Antheraea mylitta* by tiny ants showing, a-b: attack of *Monomorium destructor* on the cocoon, c: damaged pupa, d: attack of *M. minimum* transporting the first instar larva e: feeding activity of *M. minimum* on the fifth instar larva of *A. mylitta.*

Damage level:

These tiny ants revealed the aggressive predation on the early larval instar as well as the pupae (through the cocoon shell) of the tasar silkworm, *A. mylitta.* The average mortality of all the three crops/year suggested that the early instar stages are more vulnerable to predation while the fourth and fifth stages showed very rare predation by these predators. The pupae of the silkworm also totally destructed by the predation and pupa of silkworm became dead. The tasar mortality by these predators concerning its crop-wise mortality it is estimated up to 2-4% of crop damage, due to which production of silk is being affected.

Behaviour Study:

Feeding habits and prey distraction(Field invasion)

In tasar rearing areas, the as the ants *Monomorium minimum* and *M. destructor* have their terrestrial nests as well as the conspicuous trail on plants, including *Terminalia tomentosa* and *T. arjuna* where they feed the tasar larva. The worker ants of these species attack many larvae of *A. mylitta*, including pupae, and kill a broad range of host stages (Figs 1 a-d). Similarly, the weaver ant *O. smaragdina* is a dangerous larval predator of *A. mylitta* was also reported during the present study. These ants have aggressive predatory habit, they attack the early instars and the pupae of silkworm. Whereas, the attack on late instar disturbed from their normal development or the entire spinning process. The highly organized, aggressive predatory behavior, combined with extensive foraging throughout the area occupied by a colony, explains the success of tiny ant species in killing or driving away many tasar silkworms. Due to the attack, early larval instar, as well as the pupae of the silkworm, get damaged totally, which affects the raw silk production. Being a predator of concealed pupae of tasar silkworm and the pores of the cocoon shell made by *Monomorium destructor* (workers), the quality of cocoons also affected (Fig. 1a-c). Some of the ants also carry their prey to their colony. Similarly, we also recorded the dare of this tiny ant *i.e.* by *M.minimum* which was carrying the first instar larva of silkworm (Fig.1d) (Sup. Info. 1: https://youtu.be/jSycX5tAuMg). During the

predation, the host larva trying to escape many times, but the grips of ant mandibles make the tasar larva effortless. Surprisingly, the single ant can drag the whole first instar larva of the silkworm, where the larvae trying to escape many times but the predator does not allow its single move. Sometimes, they also feed the late instar larva of *A. mylitta*, either the larva may be previously damaged by another predator, dead or diseased, where they can get an easy source of food (Fig. 1e). Due to the predation of this tiny ant, the larvae of tasar silkworm become sluggish. Further more, the death of the larva occurs. However, the pupa remains into the dead shell of the cocoon with complete destruction or dead pupa. These observations are somewhat serious and the care should be taken while the rearing of the tasar silkworm, to explore the benefit of nature blessed tasar silkworm *A. mylitta*, which provides a unique yarn for economic excellence, through the tasar-culture.

DISCUSSION

The parasite–predator complex of the silkworm *A. mylitta* results in loss of wild tasar silk production (Gathalkar and Barsagade, 2016 a). Among the predators, the ants are also the risk factor in the tasar rearing fields. However, in the present study, it has been observed that the *Monomorium* species *viz.*, *M. destructor* and *M. minimum*are also affecting the larval as well as pupal stages of the tasar silkworm. These ant species attack the silkworm larvae while they are feeding on the host plants, whereas, the pupae, adults and eggs are primarily affected at grainage. However, it is well-documented that the most arboreal and some terrestrial taxa forage extensively for carbohydrate-rich plant secretions and insect exudates (Hölldobler and Wilson, 1990; Davidson, 1997). Subsequently, the predatory habit of the ants has a major influence in many habitats (Wilson, 1971; Carroll and Janzen, 1973). Similarly, *O. smaragdina*is a well-known predator of *A. mylitta*. Nevertheless, it is being used as a biological control agent in various agricultural crops (Way and Khoo, 1991; Paulson and Akre, 1992; Way *et al*., 2002). Based on ant-prey inter relationships and their foraging habit, the predacious ants can be classified as specialists or generalists (Wilson, 1959). Most of the species are scavengers where they prey on small organisms, including the insect eggs. The specialist ant does not seem to be significant in biological control, though some must have an impact, on certain pest (Way and Khoo, 1991). The generalist ant predators include those that are recognized as important in biological control (Petal, 1978; Risch and Carroll, 1982). Most of the invasive ants are usually habitat generalists, can invade and establish themselves in undisturbed habitats (Passera, 1994). Indigenous generalist predators have been controlling pests on crops since the dawn of agriculture, and the Chinese have used ant nests into citrus orchards to monitor the pest population (Symondson *et al.*, 2002). Ant as a predator of many pests of the commercially important crops, they are also useful in pest management. It is also well documented as the ants prey on eggs as well as larvae of numerous pest species in many different countries and habitats (Way *et al*., 1989; Weseloh, 1989; Way and Khoo, 1991).

The small red ant, *Formica rufa* (Linnaeus) also known to kill many different defoliating pests in European forests (Pascovici, 1979; Gosswald, 1990). Thus, these ants are acting as biological-control agents, some ants are important in pollination, soil improvement, and nutrient cycling (Gotwald, 1986). In contrast, some feed on/or disturb the plants and may act as vectors of some plant diseases, while their attack also causing the skin irritation of human being, domestic animals, and other beneficial organisms (Vinson, 1986; Goddard and de Shazo, 2004). In contrast, the predacious ants affect the behavior of prey directly and depress the size of potential pest populations (Rico-Gray and Oliveira, 2007). Whereas, most are the scavenger ant species prey on small organisms, including insect eggs (Way and Khoo, 1991, 1992). As a predator ants are important in biological control, and the ranges of prey species captured by these ant species (Petal, 1978; Risch and Carroll, 1982). Many insects possess generalized defense mechanisms such as flight, jumping away, or dropping off the plant when threatened, but these may not be effective against ants that forage at different levels of the ecosystem (Heads and

Lawton, 1985). Size and other physical attributes aid in prey defense (Way and Khoo, 1992). However, in terms of commercially important crop, like a silkworm rearing, the occurrence of ant species is problematic to larval as well as the pupal stage of silkworms in the tasar rearing field and grainage. The ants around and in the households, they feed any food available (Smith, 1965). *Monomorium destructor* is a small ant, and it also exhibits polymorphism and varies in size from 1.8 to 3.5 mm (Harris *et al*., 2005). These are the common household pests, and the foragers are slow to find food compared with other tramp ants (Lee, 2002; Lee *et al*., 2002). *M. destructor* was recorded primarily foraging in the crown of coconut trees, but it was also seen at the base of trees in Sri Lanka (Way *et al.,* 1989). They were a minor component of the ant fauna, with *M. floricola* (Jerdon), *O. smaragdina*, *Crematogaster* sp. and *Paratrechina longicornis* (Latreille) the most common ants (Way *et al.*, 1989). The attack by *O. smaragdina* (workers) is severe in tasar sericulture, where they completely tear the early larval stages of *A. mylitta.* Also, they transport their prey to their colony as observed earlier (Gathalkar and Barsagade, 2016 a,b). *Monomorium destructor* forms large polygyne colonies (Smith, 1965), where they form their nest predominantly in trees in hollow twigs and branches as well as in the soil in tropical regions (Smith, 1965). Different species adopt different foraging patterns or strategies (Ayre, 1962) with a proportion of foragers feeding on liquid food and demonstrating high trophallaxis rates (Stradling, 1978). Previous work reported that foraging workers of *Monomoriurn* sp. are passive-movers unlike the erratic foragers from the *Tapinorna* or *Paratrechina* genera (Edwards, 1986). Similarly, *Pheidole* sp. are the major predators of *Alabama argillacea* egg (Gravena and Pazetto, 1987). Certain cultural practices benefited with the predation some ant species, e.g., *Monomorium, Solenopsis*, as predators and/or scavengers of eggs and other life stages of pests (Way and Khoo, 1992), and these small ants can flourish even where other ants dominate like *O. smaragdina* (Way *et al.,* 1989). The tasar larval destruction by the ant predators is severe, as well as the small sized pores on the cocoon caused by *M. destructor*, with broken silk thread, which is the ultimate root of the valueless cocoon. Similarly, the damaged tasar pupae, could not develop further, and next generation where the seed cocoons get permanently vanished. Therefore, the tasar mortality by these predators with respect to its crop-wise mortality it is estimated up to 2-4% of crop damage studied earlier (Gathalkar and Barsagade, 2016 a), and the production of silk is being affected. A behavioral study on *Monomorium* shows its predatory potential with the power of grasping, whereas, the larva of *A. mylitta* became defenseless. Therefore, an abundance of these ants in tasar growing areas, hamper the tasar crop production and need to have very careful about the risk. Also, the techniques related to its eradication from the tasar rearing sites need to explore further, and an electrophysiological study may be helpful in this regard to control the damage.

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Evaluation of ajwain and mustard seed extract on susceptibility of *Anopheles stephensi* Liston

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ABSTRACT: The most effective method to manage mosquito population is to kill aquatic stages (egg, larva and pupa) of mosquito than the aerial adult stage. The susceptibility test was evaluated against larvae of *Anopheles stephensi* in the laboratory conditions maintaining the temperature of 27±2ºC and 70±80% Relative Humidity. The combination of the seed extracts of *Trachyspermum ammi* (ajwain), mustard oil and naphthalene were taken in the ratio 1:2:1. Results of the larval susceptibility test after 25 hours of treatment revealed that the combination of the three components are significant in killing the larval population of *Anopheles stephensi* to some extent. It has also been noticed that the rise in dose from 1ml/199ml to 7ml/99ml of water increased the larval mortality.

KEY WORDS: Susceptibility test; *Anopheles stephensi* larvae; ajwain; mustard

Mosquito and man relationship lies on earth from millions of year ago. Major focus is on killing adult stages of mosquito but larval stages are easy to manage as they are in known areas, do not fly, do not bite, do not spread diseases, safer, easy to kill by various integrated managements. The three stages of mosquito egg, larvae and pupae are aquatic while only the adult stage is aerial. So we can manage and control three stages at a time. Three strains of *Aedes aegpti* and a single species of *Culex molestus* were studied to determine susceptibility of larvae to various insecticides by George (1957). *Anopheles stephensi* is a well known urban vector found in tropic regions of India (Dash *et al*, 2007) breeds in man-made sites such as overhead tanks, wells, masonry tanks, water coolers, barrels, discarded tyres, tins, intradomestic containers, garden pots, curing water in construction sites etc (Kumar and Thavaselvam, 1992). There are many ways of larval control like application of tablet, pellet and granular formulation of larvicidal chemicals, introduction of larvivorous fishes like *Gambusia affinis, Poecilia reticulate* in large breeding sites (ponds and pools), use of insect growth regulators, use of mineral oils and other microbial control agents such as *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus* (Khyami-Horani, 1995). A combination of T*rachyspermum ammi*, mustard oil and naphthalene was tested on larval susceptibility *Anopheles stephensi* Liston in the laboratory.

For conducting the test 250ml glassbeakers, dropper to transfer larvae, soxhlet apparatus for extraction of T*rachyspermum ammi*, mustard oil and naphthalene powder, third or fourth instar larvae, cooling incubator for maintain temperature were used. Larval susceptibility test was accomplished by taking the help of WHO guidelines (2005) and Khalil *et al.* (2015). Thirty larvae (basically third

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or fourth larval instar) were taken from the stock culture and transferred to the each beaker containing 199 ml of water with the help of the dropper. Five ml or gm of each of botanical oil extract of T*rachyspermum ammi* (Ajwain) seeds, oil of mustard and naphthalene was taken and mixed with 95ml of acetone to make the stock solution. These three components were mixed in 1:2:1 ratio. Variable doses 1ml, 2ml, 4ml, 5ml and 7ml were applied to the beakers starting from the lowest to highest and each experiment was conducted in triplicate. The beakers were then kept in the incubator maintaining the temperature of 28ºC. After 24 hours the larval mortality was counted with the number of moribund larvae. The mortality was also seen in control beaker having only the solvent acetone. Therefore, the mortality percentages for treated groups were corrected by Abott's formula (Abott, 1925).

Results of the larval susceptibility test are presented in Table 1 which reveals that the combination of the three components are significant in killing the larvae population of *Anopheles stephensi* and the corrected mortality percentage ranged from 29.85% to 75.42%. It had also been noticed that the rise in dose from 1ml/199ml to 7ml/199ml of water increased the larval mortality. Khalil et al. (2015), reported that corrected mortality percentage varies between 98-100% shows susceptibility; between 80-97% suggests probability of resistance and below 80% as resistant type. The mortality percentage ranged from 29.85% to 75.42% i.e resistant type.

Data was analyzed by probit analysis and the probit equation of the research work is as follows:

$$y = 4.3774 + 1.2384\ x$$

Table 1: Combination of the three components in the ratio 1:2:1

Doses in %	Replicate	Larval mortality	Average	% Mortality	Corrected Mortality
1	R1	6			
1	R2	7	6.67	33.35	29.85
1	R3	7			
2	R1	9			
2	R2	9	8.67	43.35	40.36
2	R3	8			
4	R1	8			
4	R2	10	9.33	46.65	43.84
4	R3	10			
5	R1	12			
5	R2	11	12	60	57.89
5	R3	13			
7	R1	15			
7	R2	17	15.33	76.65	75.42
7	R3	14			
Control	0	1		5	

The values for LD 50, LD 90, LD 95 are 3.1824, 34.4959 and 67.8075 with their confidence intervals ranging from 4.00 to 0.00, 8.7456 to 0.00 and 12.3287 to 0.00.

Choubey (2007) reported the insecticidal activity of *Trachyspermum ammi, Anethum graveolens and Nigella sativa* essential oils against stored product beetle *Tribolium castaneum* Herbst. Horowitz *et al*. (1998) evaluated the pesticidal resistance of *Culex pipens* against chemicals Chlorpyrifos, Cypermethrin and Permethrin. Nazni *et al*. (2005) conducted experiments on adult and larval susceptibility against *Culex quinquefasicatus* (Say) using multiple concentrations of insecticides malathion, temephos and permethrin in Malaysia. According to WHO (2013) if the test conducted with 100 larvae showing below 90% mortality need not to be confirmed for existence of resistant test.

Further test are needed to study about susceptibility of the above combination against larvae of *Anopheles stephensi* in large area. Currently malaria control is based on drugs and insecticides but the problem comes for resistance to insecticides therefore environment management offers new approach for sustainable malaria control (Lindsay *et al*., 2004). The significance of the above test is that it is a simple method to apply and all the three components are easily available at home. Vasquez *et al*. (2009) also evaluated the susceptibility test of temephos, chlorpyrifos and permethrin against *Culex pipens* which showed significant resistant ratios.

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Satellite nest architecture and demography of the plant - visiting ant, *Camponotus compressus* (Fabricius)

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ABSTRACT: In the present study the satellite nest architecture and demography of the common and widespread sugar-loving carpenter ant, *Camponotus compressus,* were determined. The nests were located in soft and moist soil. The dental plaster casts revealed that the vertically oriented satellite nests harbouring brood (42.8 ± 21.12) and worker ants (29.2 ± 8.94) were 51.2 ± 8.17 cm deep. The nests were characterised by the concentration of 4 ± 1.09 chambers, in the upper part of the nest and a single narrow shaft at the lower end. We suggest that the location of the nests chambers close to the nest exit/entrance hole may facilitate rapid communication among the *C. compressus* worker ants on discovery of extra floral-nectary bearing or homopteran-harbouring plants by a colony member. This study can lead to a better understanding of nest construction mechanisms and the effect of nest architecture on foraging behaviour and organization of an ant colony.

KEYWORDS: Satellite nest architecture, demography, carpenter ants, polydomy

Ground dwelling ant species excavate species-typical subterranean nests (Tschinkel, 1987, 1999, 2003, 2005; Mikheyev and Tschinkel, 2004; Moreira *et al*., 2004) which are made by an active soil removal process (Tschinkel, 2005). The subterranean nest constructed by an ant colony is a functional part of the superorganism (Tschinkel, 2011). The underground nests provide a protected environment and stable microclimatic conditions to the queen and the brood of the ant colony (Frouz, 2000). The worker ants locate, forage and retrieve food from the surrounding environment which is then carried either singly or in groups of 2 or more to the nest. Hence, the colony's success in finding food such as plant-derived extra floral nectar (Agarwal and Rastogi, 2008 a) and honeydew from the sap sucking insects (Way, 1963) may be affected by the nest site and structure. Moreover, since worker ants recruit colony members by short or long-range recruitment strategies (Rastogi *et al*., 1997) the position and location of the nest chambers may be important for social interactions and speed of food retrieval. Consequently, the nest architecture may be an important regulator of social activity in an ant colony (Stickland and Franks, 1994).

Variations in the shape, size, number and arrangement of chambers within ant nests gives rise to species-typical architecture (Tschinkel, 2005). However, the study of the subterranean ant-nest architecture is still in its infancy. Ant nests of most species studied till now consist of two basic elements: the vertical shafts and the horizontal

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chambers (Tschinkel, 2003). A few descriptive studies of *Pogonomyrmex badius*, *Camponotus socius* and *Odontomachus brunneus* have outlined the range of architectural variations commonly found within and among these ant species (Tschinkel, 2004, 2005; Cerquera and Tschinkel, 2010).

Carpenter ants belong to the hyper disperse genus *Camponotus* which is ubiquitous in distribution (Bolton *et al.,* 2007) and includes polydomous species (Pfeiffer and Linsenmair, 1998; Buczkowski, 2011). Polydomous ant species have multiple spatially separated but socially connected colonies (Robinson, 2014). *Camponotus compressus* (Fabricius, 1787) is widespread in Asia (Nettimi and Iyer, 2015) and is common in many parts of India (Agarwal and Rastogi, 2008 a; Bharti *et al*., 2016). This ant species frequents a variety of habitats including forests, grasslands, agricultural land and even urban areas (Sonune and Chavan, 2016). Surprisingly, these ants abound in the ephemeral, annual cropping systems where the primary nests are constructed at the bases of trees or shrubs located at the field boundaries (pers. obs.) and the satellite nests are located in the irrigations channels and the crop-growing central field zone (Agarwal *et al.,* 2008 a). *Camponotus compressus* colonies construct two types of nests: the primary nests, usually at the base of a tree (within which they make galleries) and the associated satellite nests (Orr *et al*., 1996; Kumari *et al.*, 2016). A recent study indicates that *C. compressus* colonies modify soil pH and also soil nutrients (Kumari *et al.*, 2016). It is also well known that these ants visits extra floral nectary-bearing plants (Agarwal and Rastogi, 2008b; 2010) and tend homopterans for honeydew (Way, 1963). No information is available on its nest architecture till date. This is the first study providing a description of the architecture and demography of the satellite nests of *C. compressus*.

The study was carried out in the Botanical Garden of Banaras Hindu University, Varanasi, India, during the winter season (October, 2016 to February, 2017).Following the method of Tschinkel (2010) a thin slurry of dental stone plaster in water (in 1:1 ratio) was poured into the nest entrance hole of actively used (Shukla *et al.,* 2013) satellite nests (n = 5) and this was allowed to set overnight. The hardened cast was gently and systematically excavated after a 24 hr period with the help of a small spade. Since dental plaster casts are only moderately hard, the casts very often broke during the excavation process so the pieces were carefully and systematically labeled numerically (the labels were kept in position with the help of an adhesive tape), while digging and were sequentially assembled later. The dental stone plaster casting method offers an advantage because the casting material flows downward and fills all the nooks and cavities of a nest and occupies the entire inner space, something that is difficult to achieve during direct excavation of an uncast ant nest. For descriptive purposes, the shaft is defined as a more or less vertical length while a chamber is defined as a horizontal feature of the nest (Tschinkel, 2005). The nest dental plaster cast (along with its broken segments) was carefully transferred to a tray and brought to the laboratory where each nest was reassembled and the dimensions of each were carefully measured (in cm). The nest's volume was estimated by dividing the nests' cast weight by the density of dental stone plaster (Mikheyev and Tschinkel, 2004). The demography of the actively used satellite nests was examined by carefully excavating another set of satellite nests (n = 5) and sorting out the brood (larvae and pupae) and worker ants present within each nest.

Satellite nests were located in soft, slightly moist ground, covered with *Cynodon dactylon* grass. Casts of the 5 satellite nests of *C. compressus,* shown in the photograph (Fig. 1) reveal that the nests are mainly vertically oriented. Each nest is found to contain well-demarcated chambers and a single shaft. The chambers (Mean ± SEM; 4 ± 1.09; range: 2-8) were present predominantly in the upper part of each nest, just beneath the nest hole. The lower part of each nest consisted of a single, long, shaft.The satellite nests were moderately deep (51.2 ± 8.17 cm) and the nest volume was 246.56 ± 66.76 cm^3. The variations in nest depth (32 to 78 cm) and volume (115.20 to 425.60 cm^3) are suggested to be due to the variation in the satellite

Fig.1. Representative dental plaster casts (n =5) of the satellite nests of *Camponotus compressus*

nest life span (from 15 days to 4 months; Kumari *et al*., 2016). Only worker ants (29.2 ± 8.94) and brood (42.8 ± 21.12) were recorded within the satellite nests. The brood comprised of only late instar larvae (24.2 ± 12.18) and pupae (18.6 ± 9.80) and were recorded only in the top 1 to 2 chambers. The worker ants were however found throughout the nest including the shaft region.

Each satellite nest of *C. compressus* was characterized by a single vertical shaft connecting simple horizontal chambers. This is a widespread architectural unit among the subterranean ant nests. Chambers were typically in the upper part of the nest, near the surface and the lower part of the nest shaft was tunnel-like without any distinguishable chambers. In contrast, the nests of *P. badius*, *C. socius* and *O. brunneus* ant colonies have chambers along the entire nest depth (Tschinkel, 2004, 2005; Cerquera and Tschinkel, 2010). Examination of the nest demography and the volume of the nest cast reveals that the satellite ant colony of *C. compressus* is small as compared to the nest of *C. socius* (Tschinkel, 2005). Population size of an ant nest is suggested to be directly correlated with the complexity of the colony architecture (Franks and Deneubourg, 1997). Since larger colonies excavate larger nests as a result of nest deepening, chamber enlargement and the addition of new vertical series of chambers (Tschinkel, 2004). The presence of late larval and pupal stages and the complete absence of the eggs and early larval stages in the satellite nests indicate that the early stages remain confined to the wooden, tree-based galleries of the primary nests (Bristow *et al.*, 1992). Our study reveals a well-defined demographic structure and a simple nest architecture of *C. compressus* satellite nests. This appears to be similar to the simple nests built by *Leptothorax* ants, whose colony size ranges between 50 and 500 individuals (Franks and Deneubourg, 1997).

Being sugar-loving *C. compressus* worker ants visit a diverse assemblage of plants and forage mainly on extrafloral nectar and homopteran honeydew (Agarwal and Rastogi, 2008 a; Nettimi and Ayer, 2015). The availability of both of these is expected to undergo periodical changes depending on the season, homopteran density fluctuations and plant phenology. The satellite nest architecture of *C. compressus* reveals that a minimum number of two chambers are usually located just beneath the nest hole. The presence of the brood in the topmost chambers may be conducive towards their exposure to more favourable temperature conditions, during the winter season. The positioning of chambers very close to the exit/entrance hole may influence the

foraging performance and thus the speed at which information about a new food source spreads across the colony as the recruitment speed is found to be positively correlated with the connectivity of all chambers. Recent studies indicate that the structure of the top part of a nest, and not the number of ants the chambers can hold, determines the dynamics of collective foraging (Pinter-Wollman, 2015). The upper chambers of satellite nests may therefore facilitate rapid communication among the *C. compressus* worker ants on discovery of extra floral nectary-bearing or homopteran-harbouring plant(s) by a scout ant, although further field-based experiments are required to study this aspect. Thus, ant nest architectural design can contribute not only to our understanding of nest construction mechanisms (Perna and Theraulaz, 2017), but also reveal how nest structure affects the foraging behaviour and the organization of activities within an ant colony.

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New record of *Aleuroclava citrifolii* (Corbett) (Hemiptera: Aleyrodidae) from India

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ABSTRACT: The whitefly *Aleuroclava citrifolii* (Corbett) has been reported for the first time from India on *Memecylon umbellatum* and *Exocoecaria agallocha*.

KEY WORDS: *Aleuroclava citrifolii, Memecylon umbellatum, Exocoecaria agallocha*

The Indian whitefly fauna comprises 444 species under 64 genera. Among the whitefly genera of India the genus *Aleuroclava* Singh is represented by 68 species (Revathi and Sundararaj, 2016). During the survey a species of *Aleuroclava, A. citrifolii* (Corbett) was found breeding on *Memecylon umbellatum and Exocoecaria agallocha* and it has been redescribed with illustrations. This species so far known from Pakistan is reported for the first time from India.

Aleuroclava citrifolii (Corbett) (Fig.1 – 5)

Aleurolobus citrifolii Corbett 1935**,** *Stylops*, 4: 8-10.

Aleurotuberculatus citrifolii (Corbett) Mound and Halsey, 1978: 81.

Aleuroclava citrifolii (Corbett) Martin, 1999: 32.

Puparium: Black, without any wax secretion; elliptical, broadest at metathoracic segment region, tapering at anterior and caudal end, 0.56 - 0.70 mm long, 0.40 - 0.54 mm wide; found singly on under surfaces of leaves.

Margin: Smooth, thoracic tracheal pores indicated by invaginated clefts while caudal tracheal pore distinct. Anterior and posterior marginal setae invisible.

Dorsum: Entire dorsum densely and finely granulated; submargin distinctly separated from dorsal disc by a prominent ventral fold, dense granules forming papillae-like structures (about 34 pairs); abdominal and cephalic segments without median tubercles; prothorax with a pair of small submedian tubercle with trilobed structure. Thoracic and abdominal segment sutures distinct, extending beyond submedian area; dense granules along all segment sutures and form faint rhachis. Longitudinal moulting suture reaching margin and transverse moulting suture reaching submargin. Thoracic tracheal furrows indicated, caudal tracheal furrow funnel shaped, with irregular structures, 63 μm long, 22 μm wide at its broadest end. Pores and porettes discernible.

Chaetotaxy: Four pairs of pointed setae- cephalic setae 4 μm long, first abdominal setae 9 μm long, eighth abdominal setae 9 μm long and submarginal caudal setae 25 μm.

Vasiform orifice: Subcordate wider than long, 40-42 μm long and 45-47 μm wide; with granules at

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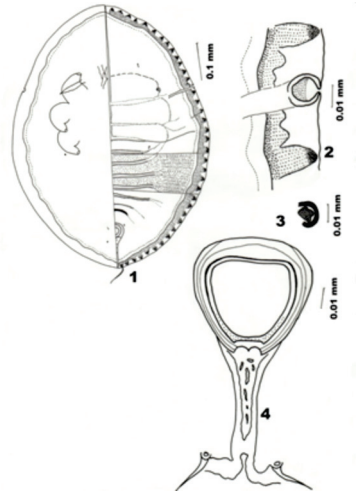

Fig.1-4-Line diagrams: *Aleuroclava citrifolii* (Corbett): 1. Puparium; 2. Margin at thoracic tracheal pore region; 3. submedian tubercle on prothorax; 4. Vasiform orifice

Fig.5: Mounted puparium of *Aleuroclava citrifolii* (Corbett)

the posterior-lateral region; operculum similarly shaped (30-32 µm long and 35 -36 µm wide), filling entirely the orifice and obscuring lingula.

Venter: A pair of ventral abdominal setae 4 µm long, 20 µm apart. Thoracic and caudal tracheal folds not discernible. Antennae reaching base of prothoracic legs. I and VIII abdominal spiracles visible.

Material examined: India: Odisha: Bitharkani National Park, seven puparia on *Exocoecaria agallocha*, 7.iii.2012, T. G. Revathi; Bitharkani National Park, three puparia on *Memecylon umbellatum,* 7.iii.2012, T.G. Revathi.

Hosts: *Citrus* sp**. (Rutaceae)** (Corbett, 1935), *Murraya exotica* (Rutaceae) (Hussain and Khan, 1945), *Morus alba* (Moraceae) and *Rosa indica* (Rosaceae) (Qureshi, 1982); *Memecylon umbellatum* (Melastomataceae) and *Exocoecaria agallocha* (Euphorbiaceae) (new host records).

Distribution: Pakistan: Faisalabad (Corbett, 1935); Jhelum Lahore, Multan, Muzaffargarh, Sialkot (Hussain and Khan, 1945); Peshawar (Qureshi, 1982); India: Odisha (new distribution record).

ACKNOWLEDGEMENTS

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ENTOMON 42(3): 247-250 (2017)
Short Communication No. ent. 42312

Field evaluation of different modules against yellow stem borer *Scirpophaga incertulas* and its effect on natural enemies in rice

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ABSTRACT: Field evaluation of different modules against yellow stem borer, *Scirpophaga incertulas* and effects on natural enemies in rice ecosystem during *kharif* and *rabi* seasons with five modules namely chemical module, bio intensive module, neem based module, integrated module and farmers practice module evaluated revealed that the chemical module had significantly less stem borer damage 2.36 per cent followed by bio intensive module 3.56 per cent and found to be superior than the farmers practice module (8.95 %) in kharif season. The per cent damage was low in the treatment with a chemical module (3.25 %) followed by integrated module (4.61 %) and found to be superior to the farmers practice module (8.84 %) in rabi season. The overall mean population of spiders ranged from 0.14 to 0.25/hill and there was no significant difference among the treatments in both the field experiments and the highest population was observed in farmers practice module. The integrated module recorded a maximum yield of 5513 and 5563 kg/ha while the farmers practice module recorded the lower yield of 3200 and 3063 kg/ha in the khariff and rabi respectively.

KEYWORDS: Modules, rice, *Scirpophaga incertulas*, natural enemies

In India, yellow stem borer, *Scirpophaga incertulas* has assumed the number one pest status as national pest (Pasalu *et al*., 2002). It was reported that the extent of damage caused by the yellow stem borer in rice ranged from 3 to 95 per cent (Ghose *et al*., 1960). For many decades, insecticides have been widely used to control this pest. However the continuous use of pesticides has caused many side effects including loss of biodiversity, residual toxicity, the resurgence of insect pests and environmental pollution (Heinrich and Mochida, 1984; Ganeshkumar and Velusamy, 1996 and Holland *et al*., 2000). Due to these constraints, researchers developed an alternative, economical and eco-friendly method of insect control (Chatterjee *et al*., 2009). The progressive modernization of Indian agriculture involving the use of integrated pest management practices gaining more popularity in recent years due to their effectiveness in controlling pests and environment friendliness; hence the present study was undertaken.

The field trials were conducted in a RBD with five treatments and four replications at PAJANCOA & RI, Karaikal, Puducherry in two consecutive

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seasons *i.e.*, June – September (*kharif*) and November to March (*rabi*) in 2012-13. The plot size was 5 x 4 m with spacing of 15 x 10 cm. There were ADT 45 variety sown in both the seasons. The treatments of experiment are given in the table 1. The recommended dose of fertilizer is 120:40:40 kg / ha of N: P_2O_5: K_2O, respectively. 25 per cent N and K_2O, 100 per cent P_2O_5 as basal, remaining amount of N and K_2O applied in three equal doses at tillering, panicle initiation and flowering stages. The incidence was recorded at weekly intervals. The observation on the damage symptom was recorded on ten randomly selected plants at weekly intervals from 7 DAT and continued upto harvest. The foliar treatments were given using high volume sprayer (Hand operated knapsack sprayer) and the release of parasitoids, pheromone traps were set as per the module (Table 1). Assessment of dead heart and white ears damage symptom caused by yellow stem borer, *S. incertulas* in each of the treatment were made on ten randomly selected plants per plot and the damage was worked out as below:

$$\text{per cent dead hearts} = \frac{\text{No. of damaged tillers}}{\text{Total No. of tillers}} \times 100$$

$$\text{per cent white ears} = \frac{\text{No. of damaged productive tillers}}{\text{Total No. of productive tillers}} \times 100$$

(Heinrichs *et al*., 1985)

The population of natural enemies mostly coccinellids (*Brumoides suturalis* F., *Cheilomenes sexmaculata* F., *Coccinella transversalis* F., *Coccinella transversalis* F., *Harmonia*

Table 1. Management of yellow stem borer, *Scirpophaga incertulas* (Walker) in rice

Sl. No.	T_1 Chemical module	T_2 Bio intensive module	T_3 Neem based module	T_4 Integrated module	T_5 Farmers practice (control)
1.	Application of Carbofuran 3G in nursery at 72 g a.i./20 cents	Set up Pheromone traps at 30 DAT and subsequent at 15 days intervals	Neem cake half dose (125 kg/ha) at basal application	Clipping of terminal leaves at the time of transplanting	Clipping of terminal leaves at transplanting
2.	Spraying Cartap hydrochloride 50 SP @ 250 g a.i./ ha at 30 DAT	Release of *Trichogramma japonicum* at 30 DAT and subsequent at 15 days intervals	Spray of NSKE 5% at 30 DAT	Application of Carbofuran 3G in nursery at 72 g a.i./ 20 cents	At ETL spray recommended insecticide (Fenthion 100 EC at 500 g a.i. / ha)
3.	Application of Cartap hydrochloride 4 G @ 800g a.i./ha at 45 DAT	Spray of *Bacillus thuringiencis* 1 lit/ha at 45 and 60 DAT	Application of neem cake remaining dose at 45 DAT	Set up Pheromone traps at 30 DAT and subsequent at 15 days intervals	-
4.	If one more spray needed spray Cartap hydrochloride 50 SP at 250 g a.i./ha	-	Spray of neem oil 3% at 60 DAT	Release of *Trichogrammajapo-nicum* at 30 DAT and subsequent at 15 days intervals	-
5.	-	-	-	Application of neem cake ¼ dose at 45 DAT	-
6.	-	-	-	Spray of Fipronil 5 SP at 50 g a.i./ha based on the ETL.	-

Table 2. Efficacy of different modules against *Scirpophaga incertulas*, spiders and coccinellids on rice (Mean of four replications)

Treatments	Mean % stem borer damage #		Mean number of coccinellids/hill #		Mean number of spiders / hill #	
	Field experiment I	Field experiment II	Field experiment I	Field experiment II	Field experiment I	Field experiment II
Chemical module	2.36(8.78)[a]	3.25(10.26)[a]	1.01(0.89)[c]	0.82(0.85)[c]	0.14(0.43)[c]	0.33(0.61)[d]
Bio intensive module	3.56(10.80)[b]	5.21(13.10)[b]	1.09(0.98)[bc]	1.64(1.23)[b]	0.18(0.48)[b]	0.54(0.76)[c]
Neem based module	4.00(11.37)[bc]	4.65(12.32)[b]	1.28(1.08)[ab]	1.52(1.19)[b]	0.24(0.53)[a]	0.57(0.77)[c]
Integrated module	4.36(11.94)[c]	4.61(12.32)[b]	1.14(0.99)[bc]	1.72(1.26)[b]	0.19(0.48)[b]	0.75(0.88)[b]
Farmers practice	8.95(17.28)[d]	8.84(17.10)[c]	1.68(1.16)[a]	4.17(1.91)[a]	0.25(0.54)[a]	1.18(1.08)[a]
CD (P= 0.05)	1.58**	1.52**	0.25**	1.32**	0.09**	0.14**

- Mean of 10 hills; Values in parantheses are arc sine transformed values
**- Significant at 1% level
In a column, means followed by common letters are not significantly different by DMRT (P = 0.05)

octomaculata F., *Micraspis discolor* F. and *Propylea dissecta* M.) and spiders (*Araneus* spp. C., *Argiope catenulata* D., *Argiope pulchella* T., *Callitrichia formosana* Oi., *Clubiona japonicola* Bosenberg & Strand, *Leucage decorata* W., *Lycosa* spp., *Oxyopes javanus* T. and *Tetragnatha javana* T.) were recorded. *In situ* counts were taken at weekly intervals on ten randomly selected plants leaving the border rows. The total number of natural enemies presents in plant and they were counted and expressed as numbers per hill.

Among the treatments, the chemical modules recorded significantly less stem borer damage 2.36 per cent followed by bio intensive module 3.56 per cent and found to be superior than the farmers practice module (8.95 %) in field experiment I. The per cent damage was low in the treatment with a chemical module (3.25 %) followed by integrated module (4.61 %) and found to be superior than the farmers practice module (8.84 %) in field experiment II. These results are comparable with the findings of Saljoqi *et al.* (2002); Suresh *et al.* (2011); Tej Kumar (2001) and Karthikeyan *et al.* (2007) who observed the significant decrease of pest infestation. In the present investigation, natural enemies *viz.*, spiders and coccinellids were more abundant in farmers practice module in both the trials.It was found that the overall mean population of predatory coccinellids was high in the farmers practice module (1.68/hill) compared to the other modules. Similar trend was observed in the field experiment II.The overall mean population of spiders ranged from 0.14 to 0.25/hill and there was no significant difference among the treatments in both the field experiments and highest population was observed in farmers practice module. These results are comparable with the findings of Elakkiya (2011) and Punithavalli *et al.* (2011) who reported

that the natural enemies *viz.*, coccinellids and spiders were found more in untreated control compared to other treatments.

The integrated module recorded a maximum yield of 5513 and 5563 kg/ha while the farmers practice module recorded the lowest yield of 3200 and 3063 kg/ha in the field experiment I and II respectively. These are also in agreement with findings of Dash *et al*. (2006); Ramandeep *et al*. (2007) and Elakkiya (2011). Among the management practices, even though the chemical module showed a significant reduction of the infestation by rice yellow stem borer, *S. incertulas*, the integrated module recorded a higher yield and benefit cost ratio and also safer to natural enemies.

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Short Communication No. ent. 42313

A new species of the genus *Anumanniola* Narendran (Chalcidoidea: Eulophidae) from India

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ABSTRACT: *Anumanniola narendrani* **sp. nov.** is described as new species from India. A diagnosis for the genus *Anumanniola* Narendran, with some additional characters is also discussed.

KEYWORDS: Eulophidae, *Anumaniola narendrani* sp. nov , India

The genus *Anumanniola* was erected by Narendran (in Narendran and Sinu, 2003) for species *Anumanniola lasallei* Narendran, based on the single specimen collected from Karnataka. After a gap of thirteen years, we describe here another new species *A. narendrani* **sp. nov.**, based on specimens collected from Indian States of Andhra Pradesh and Karnataka. A diagnosis of the genus is provided with some other important additional characters.

The present study is based on specimens collected mainly by sweep net from Indian States of Andhra Pradesh and Karnataka. Body colour was noted from card mounted specimens before clearing and mounting the specimens on slides in Canada balsam. The body length for the new species is given in millimetres. All other measurements are relative taken from the divisions of a linear scale of an ocular-micrometer. These measurements were taken at 100× magnification of the microscope (one ocular micrometer division = 0.01 mm).

The photomacrographs of card mounted specimens were taken with digital camera (Nikon DS-Fi2) attached to a stereo zoom microscope (Nikon SMZ25) and the photomicrographs of slide mounted parts were taken with a digital camera (Nikon DS-Fi1c) attached to a compound microscope (Nikon Eclipse Ci).

The following abbreviations are used in the text:

C1, C2, etc. = Clava segments 1, 2, .. etc.

F1, F2, .. etc. = Funicle segments 1, 2, .. etc.

T1, T2, .. etc. = Gastral tergites 1, 2, .. etc.

The following acronyms are used for the depositories:

NBAIR = National Bureau of Agricultural Insect Resources, Bengaluru, India.

ZDAMU = Insect collections, Department of Zoology, Aligarh Muslim University, Aligarh, India.

Genus *Anumanniola* Narendran

Anumanniola Narendran, 2003: 1031. Type species *Anumanniola lasallei* Narendran, by monotopy and original designation.

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Diagnosis: Female. Head broader than high; occipital carina, frontal suture and antennal scrobe indistinct; toruli slightly above to lower eye margin; antenna with scape flattened, dialated apically; annuli ?1- or 2-segmented, funicle 4-segmented and clava 2-segmented; pronotum somewhat bell-shaped; mesoscutum sculptured with 2 pairs of thick setae; scutellum medially smooth, with or without a shallow groove, with two pairs of setae, sides longitudinally reticulate (Figs 2 & 4); propodeum with a pair of submedian carinae diverging posteriorly; dorsellum with bulging sculptures on anterior margin; fore wing with apex infuscate; postmarginal vein longer than stigmal vein; coastal cell shorter than marginal vein; apex of metasoma may be tilted upwards (Fig. 1). Legs with mid femur with a characteristic spine (Fig. 10).

Species: World, 2; India, 2.

Anumanniola narendrani sp. nov. (Figures 3–11)

LSID urn:lsid:zoobank.org:act:955C0215-73FF-4483-8764-1E70FE3175E2

Female: Length 0.8–0.9 mm (Holotype, 0.8). Head dark brown. Antenna brown to dark brown except last claval segment white to almost translucent. Mesosoma dark brown. Fore wing (Fig. 8) subhyaline, apically infuscate; fore legs, including coxa, pale white except tarsi pale brown; mid and hind legs pale brown with coxae pale white. Gaster pale brown to brown, with a black line in the middle running from T2–T6, distinct in carded specimen.

Head (Fig. 5) narrower than mesosoma, in frontal view, 1.55× as broad as long, 1.6 × frontovertex width; 4 setae in a row near lateral margins of eyes. Eye height 2.86× as long as malar space, a deep groove arising posteriorly from inner eye margin. Antennal (Fig. 6, 7) toruli slightly above to lower eye margin; scape flattened, dilated apically, 2.5× as long as broad; pedicel 1.25× as long as broad, 0.25× scape length; flagellum with 2 annuli, transverse; distal annulus broader than proximal annulus, and with long setae; funicle 4-segmented, F1 invariably longer than F2 –F4 individually; clava 2-segmented, 3.42× as long as broad; second claval segment conical with apical spicula.

Mesosoma (Fig. 4) 1.94 × as long as broad; pronotum 0.56× length of mesoscutum, transversly reticulate, more prominent in anterior third and with a pair of thick setae posteriorly; notauli distinct reaching less than half length of mesoscutum; mesocutum with raised polygonal reticulate sculpture; mid lobe of mesoscutum with 2 pairs of setae; each side lobe with one thick seta at posterior margin; scutellum subquadrate, medially smooth, laterally with longitudinal reticulation and with 2 pairs of setae; dorsellum smooth with bulging sculpture on anterior margin; propodeum smooth, and with two submedial carinae, slightly diverging posteriorly but not reaching to margin. Fore wing (Fig. 8) 3.28× as long as broad; submarginal vein + parastigma 1.06× length of marginal vein and 8.83× as long as stigmal vein; post marginal vein 2.5× as long as stigmal vein; longest marginal seta 0.33× maximum wing width. Hind wing (Fig. 9) 8.28× as long as broad; longest marginal seta 1.07× maximum wing width. Mid femur with a characteristic spine (Fig. 10).

Metasoma (Fig. 11) - Petiole 2.2× as broad as long with, one pair of lateral spines; gaster longer than mesosoma; ovipositor occupying more than two-third length of gaster, slightly exserted beyond apex of gaster and 1.24× as long as hind tibia.

Relative measurements (holotype): Head length: width, 18:28; frontovertex width, 17; eye height, 13; malar space, 4.5; Antennal segments length: width – scape, 15:6; pedicel: 3.75: 3; F1, 10: 5.75; F2, 7.5: 6.75; F3, 7: 6.75; F4, 5.5: 5.75, C1, 5.75: 3.5, C2, 5.75: 2.25; spicula, 1.5. Fore wing length: width, 69: 21; longest marginal seta, 7; submarginal vein length, 23; parastigma length, 3.5; marginal vein length, 25; stigmal vein length, 3; postmarginal vein length, 7.5. Hind wing length: width, 58: 7; longest marginal seta, 7.5; Mesosoma length: width, 37: 19; hind tibia, 29. Metasoma. Petiole length: width, 2.25: 5; gaster length, 39 ovipositor length, 36.

Male: Unknown.

Material examined: Holotype, female (on slide under four cover slips) labelled:

INDIA: ANDHRA PRADESH, East Godawri, Kakinada, Thimmapuram, 07.ii.2014, Coll. M.T. Khan". (ZDAMU, Reg. No. HYM. CH.774).

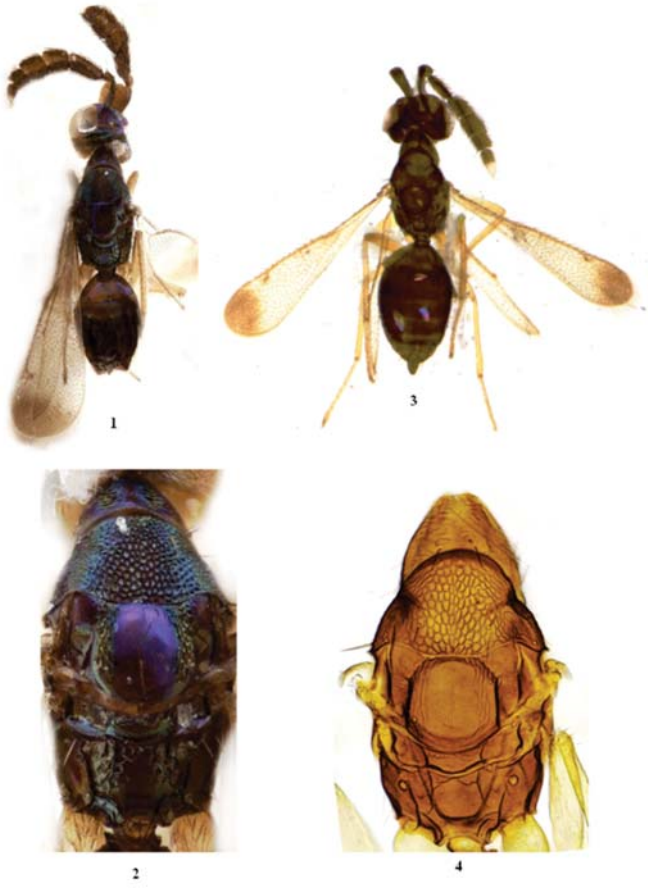

Fig. 1 & 2: *Anumanniola lasallei* Narendran, holotype ♀. 1, habitus; 2, mesosoma showing sculptures.
Fig. 3 & 4: *Anumanniola narendrani* sp. nov., holotype ♀. 3, habitus; 4, mesosoma

Paratype. 1female (slide No. EUL.165) INDIA: KARNATAKA, Tumkur, Kunigal, 9.ix.2014, Coll. K. Veenakumari. (ICAR/NBAIR/EULP.102).

Distribution: India - Andhra Pradesh (**new record**), Karnataka, Odisha; Sri Lanka.

Etymology: The species is named after late Prof. T.C. Narendran, who erected the genus *Anumanniola*.

Comments: The new species *Anumanniola narendrani* **sp. nov.** comes close to *A. lasallei* Narendran, but it differs from the latter by the following characters: antenna with 2-annuli, last claval segments with a spicula; pronotum transversely reticulate, more prominent anteriorly; mesocutum with raised polygonal reticulate sculpture; scutellum almost subquadrate, without shallow median groove, laterally with longitudinal reticulation; propodeum smooth and with two submedian carinae slightly diverging posterioly, not reaching to posterior margin; submarginal vein with 5 dorsal setae; hind wing 8.2× as long as maximum wing width; metasoma longer than mesosoma; ovipositor sheath straight. In *A. lasallei*: mandible bidentate (Narendran, 2003), ? without denticles; antenna with ? 1-annulus, last claval segments without spicula; pronotum with transversely reticulate completely; mesocutum with deep setigerous punctures; scutellum not subquadrate, with a median shallow groove, laterally with longitudinal setigerous punctures; propodeum with two submedian carinae slightly diverging posteriorly, reaching up to posterior margin, basal part and area around submedian carinae with thick reticulation; submarginal vein with 6 dorsal setae; hind wing 7.1× as long as its maximum width; metasoma shorter than mesosoma; ovipositor sheath tilted upwards.

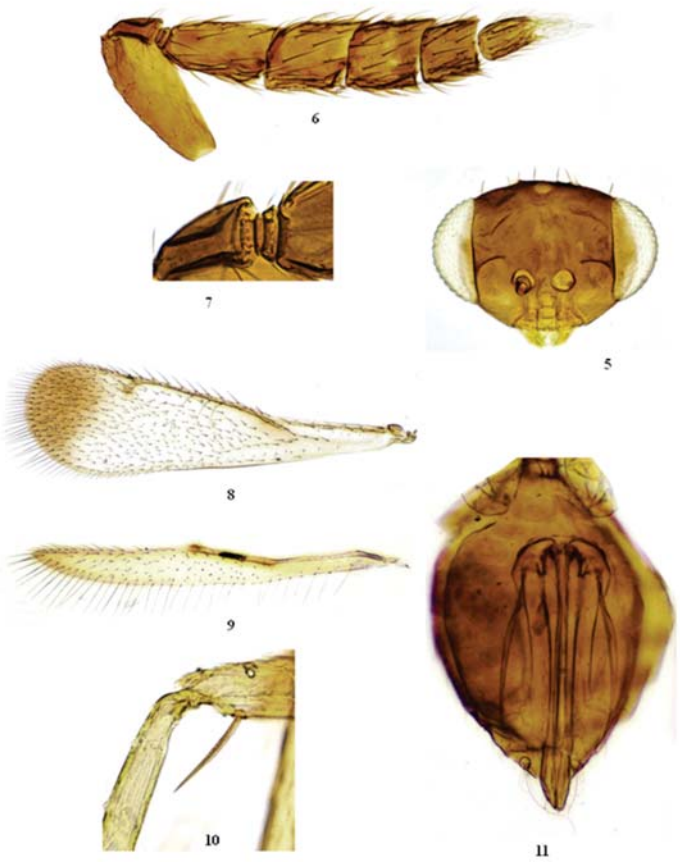

Fig. 5–11: *Anumanniola narendrani* sp. nov., holotype ♀. 5, head; 6, antenna; 7, antennal annuli; 8, fore wing; 9, hind wing; 10, mid femur with a charactersic seta; 11, metasoma

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Ultrastructure of cephalic organs of *Laemobothrion maximum* (Phthiraptera : Amblycera) infesting black kite, *Milvus migrans*

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ABSTRACT: Present report supplements information on the ultrastructure of cephalic organs viz. mandibles, maxillary palps, labial palps and cephalic ctenidia located on the ventral side of head of amblyceran louse, *Laemobothrion maximum* (on the basis of SEM). Sharply pointed mandibles of the louse might be involved in process of blood intake. © 2017 Association for Advancement of Entomology

KEY WORDS: Phthiraptera, Amblycera, Cephalic organ, *Laemobothrion maximum*

Impact of parasitism of avian Phthiraptera mainly depends upon their feeding habits and population density. Genus *Laemobothrion* encompass large sized swiftly moving amblyceran species occurring on many avian Orders. It most commonly parasitizes the birds belonging to Falconiformes (Nelson and Price, 1965). Perez *et al.* (1995) have recorded the morphological features of *L. maximum* infesting buzzard, *Buteo buteo* and also indicated its haemetophagous nature. Srivastva (1974) reported that the nymphs and adult females of *L. percnopteri*, Gervais (infesting white scavanger vulture, *Neophron percnopterus* actively feed on host blood which forms a significant part of their diet. Zlotorzycka and Danecki (1969) reported the death of a lammergeier due to heavy infestation of *L. vulturis daneckii*. In order to expedite the mechanism of blood intake, it was found worthwhile to observe the nature of cephalic organs *L. maximum.*

Selected workers have performed SEM studies on the morphological features of cephalic organs of few phthirapteran species (Miller, 1971; Eichler and Sixl, 1974; Stendel and Holm, 1975; Eichler *et al.*, 1976; Zlotorzycka, 1990). In the present study, Scanning Electron Microscopic studies were performed on the ventral side of head of *L. maximum* parasitizing black kite, *Milvus migrans* to provide supplementary information on the cephalic structure of its head.Adult lice (3 males, 2 females) were collected from an accidently electrocuted black kite, *Milvus migrans*, encountered in District Rampur (U.P.) The head of an adult louse was separated from thorax and subjected to cleaning (0.1M Phosphate buffer), dehydration (ethanol series) and air drying followed by gold coating with palladium in Neo Coater 100-240V and examined under SEM (Neo JCM-6000).

On the basis of morphological characters i.e. sitophore sclerite of hypopharynx with two holes, prominent preocular swellings in front of eye and presence of four stout spiniform setae on proximodorsal part of femure second, the specimens were distinguished as *L. maximum* (Nelson and

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Plate I

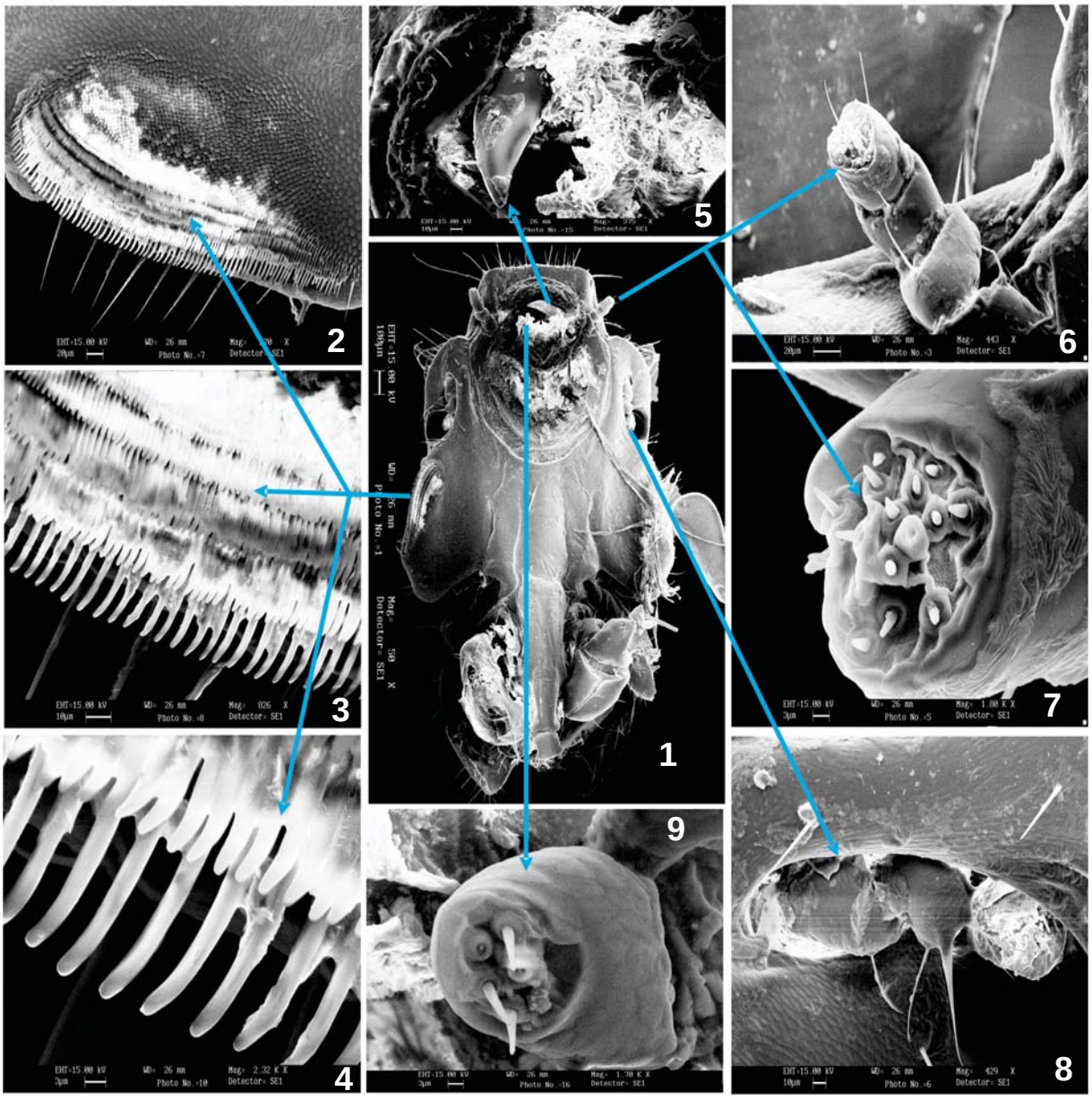

Fig. 1. SEM of ventral part of head of *Leamobothrion maximum*

Fig. 2. SEM of pleural view of head at the level eye, showing the location of cephalic ctenidia

Fig. 3, 4. Enlarged view of cephalic ctenidia. More enlarged view of cephalic ctenidia

Fig. 5. SEM of mandible of *L. maximum*

Fig. 6. SEM of maxillary palp of *L. maximum*

Fig. 7. Enlarged view of apex of terminal segment of maxillary palp segment of *L. maximum*

Fig. 8. SEM of antenna of *L. maximum*

Fig. 9. SEM of tip of terminal segment of labial palp of *L. maximum*

Price, 1965; Dik, 2006). The long head was broadest at the temple (Fig.1). The small antennae were concealed in pit like depressions on the ventral side of the swellings. Each antenna arose from the clavate apical segment (Fig. 8) and projected out of depression. The round temple gave the head a characteristic appearance. Roughly triangular mandible appeared to have pointed tip (Fig. 5). Small maxillary palpi (4 jointed) occured at the junction of rectangular pre-antennal region, arising from capsule like swelling. The maxillary palp (Fig. 6) bore several trichoid sensilla. The apex of terminal flegellomere (Fig. 7) carried five basiconic sensilla. Most of sensilla appeared normal but 2-3 remain partially folded. One sensillum appeared globular in nature. The tips of labial palps bore 5 basiconic sensilla (Fig. 9). Two of the sensilla appeared basally folded. Pleural view of head exhibited (below the level of eyes; Fig. 2, 3 and 4) the presence of at least 6 rows of rigid spine shaped structures, arranged in comb like pattern. Each row contained at least 70-80 spines (often termed as cephalic ctinidia). However, the exact role of cephalic ctenidia deserves investigation.

Haematophagous amblycerans are of great concern to veterinarians/ parasitologists, as they do not only affect the vitality and productivity of the host birds but often act as carrrier and transmitter of pathogens (Clayton *et al.*, 2016). Since, avian lice are telemophages and cannot cannulate the host blood (Lavoipierre, 1967), the mechanism of intake of host blood deserves further investigation. Attempt to correlate the presence of host blood in the gut with the nature of mandibles of a dog louse *Trichodectes canis* has been made by Bouvier (1945). Clay (1948) recorded three closely associated stylet like structures (which may be used for piercing) in a louse *Tricholoctes,* occurring on humming birds. Rao *et al.* (1975) indicated that strong teeth and denticles located on lateral process of lateral lobe of amblyceran louse, *Gliricola porcelli* may also help in haematophagy. Sharp mandibles of *L. maximum* might be used for injuring the skin to obtain the host blood. Perez *et al.* (1995) further noted that indented rigid prolongations located near mandible may also help in the process. Thus, the mechanism through which haemetophagous Amblycera imbibe the host blood exhibit considerable variation.

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