ISSN 0377- 9335

ENTOMON

Volume 41 March 2016 Number 1

FOUR DECADES OF EXCELLENCE

ASSOCIATION FOR ADVANCEMENT OF ENTOMOLOGY
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ENTOMON

ENTOMON is a quarterly journal published by the Association for Advancement of Entomology devoted to the publication of research work on various aspects of insects and related branches of Entomology.

Address all MS and editorial correspondence to the Chief Editor, ENTOMON, Department of Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Thiruvananthapuram 695 522, Kerala, India. E mail: ***editor.entomon@kau.in***

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Note from the Chief Editor

Dear Entomologists,

The Association for Advancement of Entomology (AAE) was established in the Department of Zoology, University of Kerala, at Trivandrum by the reputed Insect Physiologist late Professor K.K. Nayar and a team of Research and Teaching Entomologists of the University of Kerala, the Kerala Agricultural University (KAU), the Kerala Forest Research Institute and the ICAR- Central Tuber Crops Research Institute in 1975. The Founder President of AAE was Professor M.R.G.K. Nair of KAU who continued in that position till his demise (1975 – 1998). AAE became a well known organization in the country and abroad, extending its activities in Entomological Research.

The Head Quarters of the AAE is at present based in the "Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellayani, Trivandrum, Kerala 695522" and we are thankful to the Vice Chancellor, KAU and its authorities for the consent. The management of the Association has been entrusted to a newly elected Executive Committee and Editorial Board with effect from December 2013, with Professor N. Mohandas as the President and Dr K.D. Prathapan as the Secretary.

As we all know AAE has been publishing research journal "ENTOMON" since 1976 and it is one among the top Entomology

journals. We have taken enormous efforts to clear the backlog of ENTOMON ensuring the quality and strict periodicity of this prestigious publication. The National Academy of Agricultural Sciences evaluated the ENTOMON 2015 volume 40 - issues 1, 2, 3 and 4 and has awarded a NAAS score of 4.12 for the journal. It is indeed a moment of rejoice and great step forward achieved after a long lapse.

ENTOMON is completing 40 years of yeomen service in the field. The Executive Committee and the Editorial Board perceived the need for a face lift of the format of the journal and adopted the present format of ENTOMON. I am proud to present ENTOMON 41 in its new form.

Looking forward to your continued support.

"Future beckons sustainable entomological solutions"

Regards

Dr M.S. Palaniswami, Chief Editor

ENTOMON

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Short Communications

ENTOMON 41 (1): 1-10 (2016)
Article No. ent. 41101

Oviposition behavior of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) on four varieties of *Lathyrus sativus* L. seeds

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ABSTRACT: *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae) is an important stored grain pest of *Lathyrus sativus* L. (Leguminosae). Olfactometer assay using the surface waxes of the four varieties, Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds @1, 1, 2 and 2 μg ml^{-1} showed that the surface waxes of all the varieties attracted *C. maculatus* females and the least attraction was to WBK-13-1. Ovipostion by *C. maculatus* was significant on all the varieties with surface waxes in no choice assay. The highest preference was to Bio L 212 Ratan and it was followed by Nirmal B-1, WBK-14-7 and WBK-13-1. The insect did not prefer wax removed seeds for egg laying. The study suggests that WBK-13-1 and WBK-14-7 are the less preferred varieties of *L. sativus* by *C. maculatus* for oviposition, and these varieties might be promoted for cultivation.

KEY WORDS: *Callosobruchus maculatus*, *Lathyrus sativus*, surface waxes, olfactometer assay, ovipositional preference.

INTRODUCTION

In recent years, consumption of *Lathyrus sativus* L. (Leguminosae), commonly known as khesari, has been growing worldwide, because it is now perceived as part of a healthy diet. The crop is cultivated in India, Bangladesh, China, Nepal, Pakistan and Ethiopia (Gaur and Maloo, 2011; Girma and Korbu, 2012). Farmers grow this pulse crop due to low-cost cultivation, and resistance to drought, salinity and stress (Gaur and Maloo, 2011). Earlier khesari seeds are considered as a staple food because of neurotoxin (β-ODAP) which is making a comeback because of new plant varieties (Rao, 2011; Singh and Rao, 2013). Further, the seeds contain both homoarginine and β-ODAP, which are important to human health, in areas of cardiovascular physiology, hypoxia and nutrition (Singh and Rao, 2013).

Callosobruchus maculatus (F.) (Coleoptera: Chrysomelidae: Bruchinae) is a polyphagous pest of stored legumes in tropics and subtropics (Utida, 1972; Fox *et al.*, 2010). A cursory review of literature indicate the existence of the 'active' and 'normal' morphs of *C. maculatus* on different stored legumes, the two forms that are thought to represent adaptations to the two very different environments of field and seed store, respectively (Utida, 1972; Huignard *et al.*, 1985; Messina and Renwick, 1985; Thanthianga and Mitchell, 1990; Fox, 1993; Appleby and Credland, 2001; Zannou *et al.*, 2003; Fox *et al.*, 2010; Arnold *et al.*, 2012; Adhikary *et al.*, 2015; 2016). The active form of

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this insect has an important role in dispersal of populations from stores due to its increased flight activities than normal form (Utida, 1972; Appleby and Credland, 2001; Arnold *et al.*, 2012). However, normal females are the primary target of growers for the management of infestations of legume seeds because they are the principal egg layers (Zannou *et al.*, 2003). Infestation by this insect to cowpea, in three months of storage results in damage up to 30 % (Ouedraogo *et al.,* 1996), and total loss of this seed had been shown to occur within six months of storage (Caswell, 1961). The adults lay eggs on khesari seed surface and the larvae of *C. maculatus* feed for four instars in the seeds and complete their development within 12–16 days (Adhikary and Barik, 2012). Infestation by this insect reduces nutritional quality and viability of the seeds.

It is well established that successful use of varieties with good resistance has a number of merits over control by chemical insecticides as these insecticides are not safe to the users as well as to the environment. Further, application of chemical insecticides need periodic repetition and consequently it is not cost effective. Methyl bromide which is used in the developing countries for disinfestations of the stored grain will be restricted worldwide by 2015 under the terms of Montreal Protocol (United Nations Environment programme, 1998). Further, resistance to treatments with insecticides *viz*. dimethoate, permethrin, carbosulfan and to the fumigant phosphine has been reported in *C. maculatus* (Bogamuwa *et al.*, 2002). Therefore, it is necessary to find suitable varieties which are resistant to the bruchid attack. The first physical contact between the insect and seed occurs on the seed coat surface. Hence, surface wax plays an important role in egg laying by *C. maculatus*. So, an attempt has been made to find whether *C. maculatus* display any differences on egg laying behavior on four varieties of khesari seeds, two cultivars, Bio L 212 Ratan and Nirmal B-1 currently grown by farmers and another two cultivars, WBK-14-7 and WBK-13-1 that are being considered for commercialization. The purpose of the investigation was to study variations in the surface waxes of four varieties of khesari seeds, analyse the role of surface waxes in short range attraction of *C. maculatus* and oviposition by the bruchid on the four varieties of khesari seeds.

MATERIALS AND METHODS

Seed

Four varieties (Bio L 212 Rattan, Nirmal B-1, WBK-14-7 and WBK-13-1) of khesari seeds were collected from Pulses and Oilseeds Research Station, Behrampore, West Bengal, India.

SEM study of seed coat

Uninfested whole seeds were broken cautiously in the laboratory to separate the seed coats for scanning electron microscope (SEM) study. The seed coat surface of each variety of khesari seed sample was mounted on aluminium holders (stabs) coated with gold-palladium (2 nm thickness) using Hitachi made Scanning Electron Microscope (Model: S 530 with IB 2 ion cotter, Japan). The SEM study was replicated thrice.

Extraction of waxes from seed coat

Hundred grams of uninfested seeds of Bio L 212 Ratan (number = 1226 ± 6.5), Nirmal B-1 (number = 1332 ± 8.3), WBK-14-7 (number = 1242 ± 5.9) and WBK-13-1 (number = 1314 ± 3.4) (mean ± SE) were separately immersed in 1 L *n*-hexane for 5 min under room temperature (27°C). The extract was filtered through Whatman No. 41 filter paper, and the solvent was removed under reduced pressure. The dried crude extract was used for olfactory assay. Five replications for each variety were maintained. Wax removed seeds were used for ovipositional assays to find any role of wax.

Test insects

C. maculatus infesting chickpea seeds (variety: Radhey) were collected from local stores at Burdwan, West Bengal, India during June 2014. They were maintained in 1 L glass jars containing seeds of Radhey for one generation, and were covered with fine-mesh nylon nets at 14 L: 10 D photoperiod, 27 ± 1ºC and 70 ± 3% relative humidity

in a BOD incubator (ADS instruments and Tech., Calcutta). Active/inactive male and female forms were determined by flight activity, elytra size and intensity of pigmentation on elytra. Newly emerged F_2 inactive males and females (male: antennae long and deeply serrated, and pygidium uniformly covered with golden setae; female: antennae short and subserrated, and pygidium with a pair of black postero-lateral spots) were separated morphologically from the stock cultures everyday at 8 AM and 8 PM and were kept in separate glass jars without chickpea seeds. For mating, virgin inactive females collected within 12 h of adult emergence were provided with a single inactive male in a 60 mm Petri dish. After a single copulation, inactive females were transferred to a 15 × 8 cm glass jar containing a small Petri dish (2 × 1 cm) with water (Howe and Currie, 1964; Fox, 1993). The behavioral of 4–6 day-old mated inactive females was observed in olfactory assays; whereas mated females were used for ovipositional assays.

Olfactory assay

Surface waxes (2 mg) from each variety of khesari seed were dissolved in petroleum ether to prepare four concentrations of 0.5, 1, 2 and 4 μg/ml for olfactory assays. The dose of the waxes was lowered until the insect did not produce any response. Further, dose of the wax was tested until the insect produced highest significant ($P < 0.0001$) attraction. The insect displayed highest attraction ($P < 0.0001$) to 2 μg ml^{-1} of surface waxes from Bio L 212 Ratan and Nirmal B-1 seeds, 4 μg ml^{-1} of surface waxes were not tested for these varieties. Further, response of the insect to 2 μg ml^{-1} surface waxes was tested as following combinations: Bio L 212 Ratan vs. Nirmal B-1, Bio L 212 Ratan vs. WBK-14-7, Bio L 212 Ratan vs. WBK-13-1, Nirmal B-1 vs. WBK-14-7, Nirmal B-1 vs. WBK-13-1 and WBK-14-7 vs. WBK-13-1 to find which variety of seed surface wax was most attractive.

The behavioral responses of adult *C. maculatus* females were observed in a short glass Y-tube olfactometer (5 cm long stem and arms; 0.6 cm radius, 60º Y-angle) as the wax compounds are semivolatile (Mukherjee *et al.*, 2014; Sarkar and Barik, 2014; Malik and Barik, 2015). Each arm of the olfactometer was connected to glass-made micro kit adapter fitted into a glass vial (1× 3 cm). One glass vial contained a 2 cm^2 Whatman No. 41 filter paper moistened with 1 ml of the tested concentration of wax, whilst the other glass vial contained a filter paper of the same size moistened with 1 ml of the control solvent (petroleum ether) which did not attract the test insect. Preliminary assays with 90 naïve insects were tested with petroleum ether solvent loaded air flow against clean air flow, and it was observed that the test insects did not indicate any positive response to the solvent control (petroleum ether) (χ^2=0.18; df =1; P > 0.05). Charcoal filtered air was pushed into the system at 150 ml min^{-1}. The stem of the olfactometer was connected to a porous glass vial (1 × 3 cm) in which test insect was released. The experiment was conducted at 27 ± 1ºC, 70 ± 3 % R.H. and 150 lux. One milliliter of tested wax concentrations / petroleum ether were applied to the filter paper pieces and allowed to evaporate and these filter papers were introduced into the glass vials before the first insect was released into the olfactometer, for each experiment. One adult female *C. maculatus* was introduced into the porous glass vial, which was then attached with the stem of the olfactometer. The behavior of each female was observed for 2 min and was considered to have made a choice if it reached at the end of either arm. The insect was removed from the Y-tube, and the choice made was recorded as a positive response or negative response by one unit, respectively. In contrast, a female was considered to have not made a choice if it remained in the common arm of the Y-tube during the observation period (Mukherjee *et al.*, 2013 and 2015; Adhikary *et al.*, 2015). Each dose was conducted until a total of 90 naïve female insects had responded (each insect was used once throughout olfactory bioassays). After testing five insects the olfactometer set-up was cleaned with petroleum ether followed by acetone, and the position of the two arms was changed in order to avoid positional bias.

Ovipositional assay

After a single copulation of the newly emerged male/ female, the female was placed in a sterilized glass jar (15 × 8 cm) containing 25 khesari seeds of the tested variety on a Petri dish (8 cm) in no choice test. The glass jars were lined with coarse grade emery paper to prevent oviposition on the wall of glass jars and bottom of the glass jars were covered by Whatman No. 41 filter paper. Twenty five inactive females were evaluated for each variety of khesari seed. The number of eggs per seed was counted daily and the seeds in each glass jar were replaced with uninfested seeds until death of the ovipositing female. Similar tests were conducted with wax removed khesari seeds of each variety to find out the role of surface waxes on egg laying.

In choice test, a specially designed square glass chamber (25 cm^2) was used. Inside the jars, emery paper/ Whatman No. 41 filter paper were used. Twenty five khesari seeds of each variety were placed separately in Petri dishes (8 cm) at the four corners of the square glass chamber. Newly emerged female, mated once was placed centrally in the glass chamber and covered with a glass lid to prevent outward movement of the insect from the glass chamber. The number of insects taken for the study and the observations on oviposition were similar to that mentioned in no choice test.

Statistical Analyses

The data obtained on responses of *C. maculatus* to surface waxes of khesari seeds were analyzed by Chi-square test (Sarkar and Barik, 2014 and 2015; Mukherjee *et al.*, 2015; Malik and Barik, 2015; Sarkar *et al.*, 2015). Insects that did not respond to any selection offered in the olfactometer were excluded from the analyses. The data on number of eggs laid by *C. maculatus* in no choice tests were subjected to *t*-test; whereas one-way ANOVA followed by Tukey test were adopted in choice tests (Zar, 1999).

RESULTS AND DISCUSSION

SEM study

The surface of Bio L 212 Ratan seed showed an obscure framework of cuticular hydrocarbon depositions surmounted by multiplied, flat, disc like asteroid configurations, and asteroids with a central flat disc and 7-12 radiating ribs which was diffused over seed surface (Fig. 1a). In Nirmal B-1 seed surface, a series of tent like (a framework of trabecular) configurations with fimbriated margin emanating from spermoderm was observed with numerous tertiary granular and a few large globular depositions, and the fimbria appear to be interlinked by trabecular strands (Fig. 1b). The surface of WBK-14-7 seed indicated a subtending trabecular framework of hydrocarbon depositions with asteroids both tent like as well as flat discoid, and tertiary depositions of different shapes (Fig. 1c); whereas in WBK-13-1, a trabecular framework with asteroids (disc part of asteroids very irregular and radiating area variable) and granular depositions were observed (Fig. 1d).

Surface waxes and olfactory assay

The *n*-hexane extracts from 100 g of Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds yielded 15.1 ± 0.6, 14.1 ± 0.9, 13.0 ± 1.0 and 10.6 ± 0.6 mg of surface waxes, respectively. Total amount of seed surface waxes differed significantly among the khesari seed varieties ($F = 5.68$; $df = 3, 16$; $P < 0.05$). The Tukey test revealed that total amount of surface waxes was lower in WBK-13-1 than Bio L 212 Ratan and Nirmal B-1, but the amount of surface waxes in WBK-14-7 seeds did not differ significantly between Bio L 212 Ratan or Nirmal B-1 or WBK-13-1 seeds.

The data on olfactory assay are presented in Table 1. Waxes from Bio L 212 Ratan seeds were attractive at 1μg ml^{-1} ($\chi^2 = 7.51$; $df = 1$; $P < 0.01$) and 2 μg ml^{-1} ($\chi^2 = 23.51$; $df = 1$; $P < 0.0001$). The surface waxes from Nirmal B-1 seeds elicited

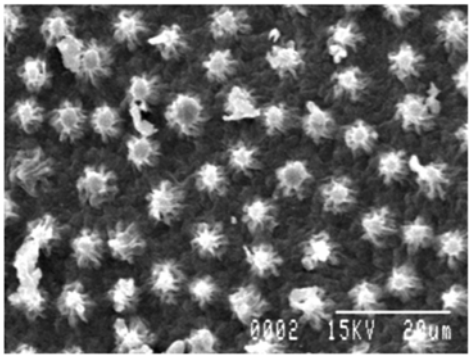

Fig. 1a.

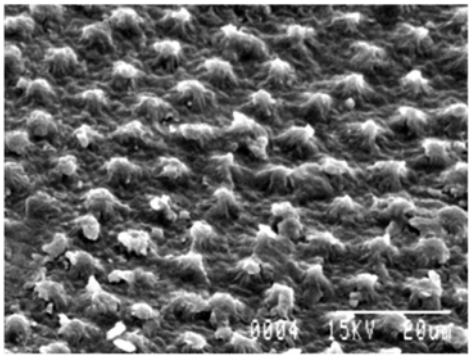

Fig. 1b.

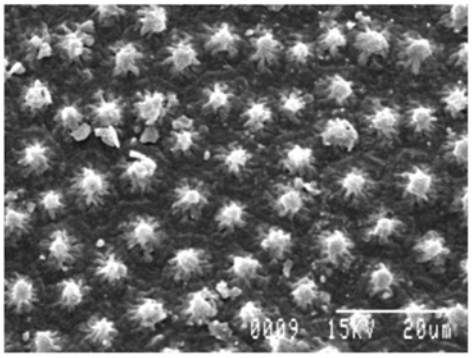

Fig. 1c.

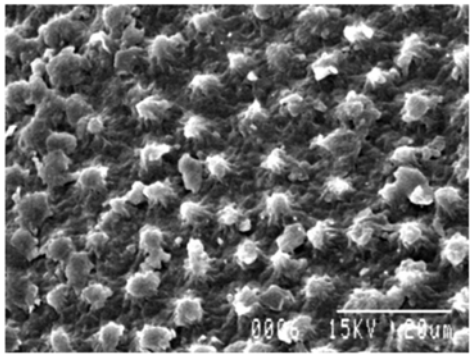

Fig. 1d

Fig. 1. Scanning electron micrograph of seed coat surface of Bio L 212 Ratan (a), Nirmal B-1 (b), WBK-14-7 (c), and WBK-13-1 (d) khesari seeds

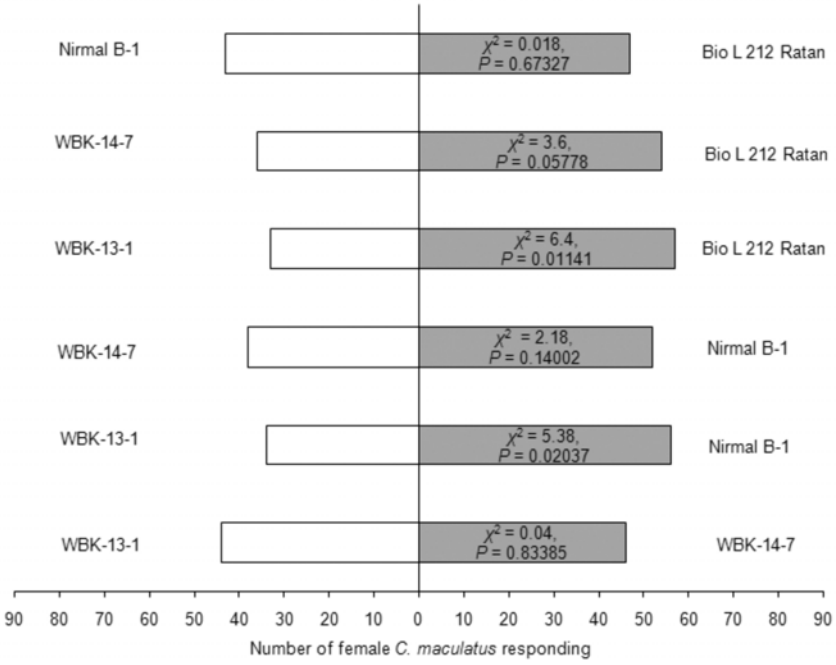

Fig. 2. Response of female *C. maculatus* to 2 µg ml^{-1} surface waxes from four varieties of khesari seeds tested against each other in olfactometer bioassay

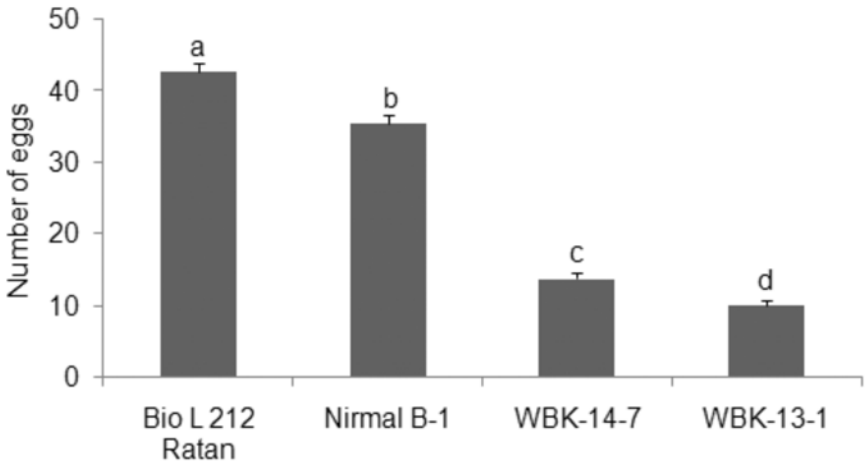

Fig. 3. Mean number of eggs laid by *C. maculatus* on different varieties of *Lathyrus sativus* seeds in choice test

Table 1. Response of female *C. maculatus* to surface waxes of different varieties of *Lathyrus sativus* seeds in olfactometer assay

T1	T2	Insects responded		χ^2 (df = 1)	P Values
		T1	T2		
Waxes	Control				
Bio L 212 Ratan (0.5 µg/ ml)	Solvent	52	38	2.18	0.67327
Bio L 212 Ratan (1 µg/ ml)	Solvent	58	32	7.51	0.00613
Bio L 212 Ratan (2 µg/ ml)	Solvent	68	22	23.51	< 0.0001
Nirmal B-1 (0.5 µg/ ml)	Solvent	50	40	1.11	0.29184
Nirmal B-1 (1 µg/ ml)	Solvent	55	35	4.44	0.03502
Nirmal B-1 (2 µg/ ml)	Solvent	67	23	21.51	< 0.0001
WBK-14-7 (1 µg/ ml)	Solvent	54	36	3.6	0.05778
WBK-14-7 (2 µg/ ml)	Solvent	59	31	8.71	0.00316
WBK-14-7 (4 µg/ ml)	Solvent	70	20	27.78	< 0.0001
WBK-13-1 (1 µg/ ml)	Solvent	52	38	2.18	0.14002
WBK-13-1 (2 µg/ ml)	Solvent	58	32	7.51	0.00613
WBK-13-1 (4 µg/ ml)	Solvent	68	22	23.51	< 0.0001

solvent – petroleum ether, N = 90

attraction at 1 µg ml^{-1} (χ^2= 4.44; df =1; P < 0.05) and 2 µg ml^{-1} (χ^2= 21.51; df =1; P < 0.0001). *C. maculatus* responded to the waxes from WBK-14-7 khesari seeds at 2 µg ml^{-1} (χ^2= 8.71; df =1; P < 0.05) and 4 µg ml^{-1} (χ^2= 27.78; df =1; P < 0.0001); whereas no clear positive or negative responses was observed at 1 µg ml^{-1} (χ^2= 3.6; df =1; P > 0.05). Waxes from WBK-13-1 seeds were attractive at 2 µg ml^{-1} (χ^2= 7.51; df =1; P < 0.05) and 4 µg ml^{-1} (χ^2= 23.51; df =1; P < 0.0001), but the insect did not elicit clear positive responses (χ^2= 2.18; df =1; P > 0.05) at 1 µg ml^{-1}. The insect showed preferences to 2 µg ml^{-1} concentration of surface waxes from Bio L 212 Ratan seeds (χ^2 = 6.4, *df* = 1, P < 0.05) vs. WBK-13-1 seeds, and Nirmal B-1 seeds (χ^2 = 5.38, *df* = 1, P < 0.05) vs. WBK-13-1 seeds; whereas the insect did not indicate clear positive or negative responses to surface waxes from Bio L 212 Ratan (χ^2= 0.18; df =1; P > 0.05) vs. Nirmal B-1, Bio L 212 Ratan (χ^2= 3.6; df =1; P > 0.05) vs. WBK-14-7, Nirmal B-1 (χ^2= 2.18; df =1; P > 0.05) vs. WBK-14-7

Table 2. Oviposition by *C. maculatus* on different varieties of *Lathyrus sativus* seeds with waxes and without waxes in no choice assay

Variety	Experiment 1		Experiment 2	
	Eggs laid on seeds with waxes	Eggs laid on filter paper	Eggs laid on seeds without waxes	Eggs laid on filter paper
Bio L 212 Ratan	105.24 ± 2.08	2.04 ± 0.23	54.6 ± 0.83	53.88 ± 0.67
Nirmal B-1	101.2 ± 3.02	6.96 ± 1.23	55.6 ± 0.52	54.84 ± 0.71
WBK-14-7	94.7 ± 2.9	11.8 ± 1.5	55.28 ± 0.62	53.64 ± 0.62
WBK-13-1	91.2 ± 3.01	15.1 ± 2.5	54.36 ± 0.49	53.56 ± 0.68

N = 25

and WBK-14-7 (χ^2= 0.04; df =1; P > 0.05) vs. WBK-13-1 (Fig. 2). This observation indicated that the test insect showed less attraction to surface waxes from WBK-13-1 khesari seeds than the other varieties of khesari seeds used in this study.

Ovipositional assay

Callosobruchus maculatus females showed significant oviposition in khesari seeds with waxes on Bio L 212 Ratan (t = 32.9, df = 48, P < 0.0001), Nirmal B-1 (t = 27.9, df = 48, P < 0.0001), WBK-14-7 (t = 25.7, df = 48, P < 0.0001) and WBK-13-1 (t = 19.6, df = 48, P < 0.0001) seeds in no choice assay (Table 2); whereas the insect did not indicate significant oviposition on khesari seeds without waxes on Bio L 212 Ratan (t = 0.678, df = 48, P > 0.05), Nirmal B-1 (t = 0.859, df = 48, P > 0.05), WBK-14-7 (t = 1.896, df = 48, P > 0.05) and WBK-13-1 (t = 0.959, df = 48, P > 0.05) seeds in no choice assay (Table 2). From this study, it is clear that waxes from four varieties of khesari seeds influenced oviposition of *C. maculatus*.

In choice tests, when *C. maculatus* were provided with four varieties of khesari seeds, and it was observed that *C. maculatus* displayed significant difference in egg laying through one-way ANOVA (F = 261.69; df = 3, 96; P < 0.0001), and the Tukey multiple pair wise comparisons test revealed that total number of eggs laid by the insects were significantly higher on Bio L 212 Ratan followed by Nirmal B-1, WBK-13-1 and WBK-14-7 (Fig. 3).

The cuticular wax serves many physiological functions. It also play an important role in seed-insect interaction (Schoonhoven *et al.*, 2005) and act as attractants for oviposition (Phelan *et al.,* 1991; Parr *et al.*, 1998; Nietupski *et al.*, 2005) and for feeding (Manosalva *et al.*, 2011; Mukherjee *et al.*, 2014; Mukherjee and Barik, 2014; Sarkar and Barik, 2014 and 2015; Malik and Barik, 2015). The amount of surface waxes was lower in WBK-13-1 seeds than Bio L 212 Ratan and Nirmal B-1 seeds, and the SEM study indicated considerable variations in their micro morphology, ranging from amorphous films to mixed arrays of wax tubes, rods, granules and plates (Sharma *et al.*, 1977; Murthy and Sanjappa, 2002; Mallick and Sawhney, 2003; Gohary and Mohammed, 2007; Al-Ghamdi and Al-Zahrani, 2010; Gandhi *et al.*, 2011; Tabaripour *et al.*, 2013). The results of the olfactory assay revealed clear olfactory responses to surface wax compounds, which are low-volatile substances that might act as close range allelochemicals after arrival of the insect to the seed. *C. maculatus* could detect wax compounds from Bio L 212 Ratan, Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds at 1, 1, 2 and 2 μg ml^{-1} respectively, and among the four varieties, the insect showed less attraction to surface waxes from WBK-13-1 than the rest three varieties indicating that surface wax compounds from WBK-13-1 are less attractive to *C. maculatus*.

In the present study, *C. maculatus* laid higher number of eggs on Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 seeds with surface waxes in no choice assay; whereas the insect did not indicate significant oviposition preference on wax removed seeds of any variety in no choice assay, implicating that surface wax compounds played an important role in oviposition of *C. maculatus*. Further, the insect preferred to lay higher number of eggs on Bio L 212 Ratan followed by Nirmal B-1, WBK-14-7 and WBK-13-1 seeds in choice assay, indicating that surface wax compounds from Bio L 212 Ratan and Nirmal B-1 might have influenced the insect for laying higher number of eggs on these two varieties which result in higher losses by the feeding *C. maculatus* larvae in these two varieties than in WBK-14-7 and WBK-13-1. Our previous study revealed that the amount of volatiles were highest in Bio L 212 Ratan, followed by Nirmal B-1, WBK-14-7 and WBK-13-1 khesari seeds, and the insect showed lowest attraction to the WBK-14-7 and WBK-13-1 (Adhikary *et al.*, 2015), and lower amounts of carbohydrates, proteins, lipids, nitrogen, water content and higher trypsin inhibitor activity of WBK-14-7 and WBK-13-1 caused higher developmental time and lower fecundity of *C. maculatus* on these varieties than Bio L 212 Ratan and Nirmal B-1. Further, WBK-14-7 and WBK-13-1 khesari seeds indicated lower amount of β-ODAP content than Bio L 212 Ratan and Nirmal B-1 (Adhikary *et al.*, 2016). It is well established that variation in seed size plays an important role in the oviposition of *C. maculatus* (Mitchell, 1983; Cope and Fox, 2003), but in this study since almost the same size for the different varieties khesari seeds were used it is seen that seed sizes did not play any role in the oviposition. On the basis of the present study and also on the basis of the previous investigations, it is suggested to promote the production of WBK-14-7 and WBK-13-1 khesari seeds than Bio L 212 Ratan and Nirmal B-1 seeds to reduce the loss caused by *C. maculatus*.

Long chain (>C16) free fatty acids constitute a large proportion of the surface waxes and these compounds have infrequently been shown to affect insect oviposition (Parr *et al.*, 1998). Therefore, it remains to be seen whether ovipositing female bruchids can similarly perceive differences between fatty acids in the four varieties of khesari seeds.

ACKNOWLEDGMENTS

We thank anonymous reviewer(s) for many helpful comments for an earlier version of the manuscript. The financial assistance from the Department of Science and Technology (DST) (SR/WOS-A/LS-106/2011), India as a Women Scientist Scheme, DST-FIST and DRS SAP for providing necessary instrumental facilities are gratefully acknowledged.

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(Received 23 November 2015; Accepted 05 January 2016; Published 31 March 2016)

Entomon 41 (1): 11-20 (2016)
Article No. ent. 41102

Morphology and biology of litter-inhabiting *Buchananiella indica* Muraleedharan (Hemiptera: Anthocoridae)

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ABSTRACT: The life history, biological parameters and predatory potential of litter- inhabiting *Buchananiella indica* Muraleedharan (Hemptera: Anthocoridae) have been investigated. The adult, egg and immature stages of this anthocorid are described with live images. *Buchananiella indica* is amenable to continuous rearing on eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and the anthocorid was continuously reared for 20 generations. Fertility studies indicated that the reproductive rate is 12.6 and the finite rate of increase 1.08.

KEYWORDS: Anthocorid, biology, *Buchananiella indica,* diagnostic characters, predatory potential, rearing

INTRODUCTION

Muraleedharan and Ananthakrishnan (1974) described some species of anthocorids including *Buchananiella* from India. *Buchananiella* Reuter 1884 is a relatively small taxon coming under the tribe Dufouriellini consisting of ten described species (Yamada and Yasunaga, 2009). The members of this taxon are distributed in the tropics and sub-tropics, except for *Buchananiella continua* (White), which is a pantropical species, quite widely reported in western Palaearctic zone (Kirby, 1999). *Buchananiella indica* Muraleedharan was described based on the material collected in Garo Hills, Meghalaya (Muraleedharan, 1977). In India, the known *Buchananiella* include *B. indica*, *B. garoensis* Muraleedharan, *B. crassicornis* Carayon, *B. carayoni* Muraleedharan and Ananthakrishnan, and *B. pseudococci pseudococci* Wagner. In general, little information is available on the biology and ecology of species of *Buchananiella.*

In the present paper, we present the morphological characteristics of the egg, immature and adult stages of *B. indica. Buchananiella* is sometimes confused with other taxa such as *Amphiareus. Buchananiella indica* and *Amphiareus constrictus* (Stål) were the two litter inhabiting anthocorids which were most commonly recorded and both were amenable to rearing. Hence, we have also reported some distinguishing characters which can be used to separate the two. Different species of *Buchananiella* generally inhabit either leaf litter or decaying plant material. However, Yamada *et al.* (2008) recorded *Buchananiella pseudococci pseudococci* (Wagner) (as *Cardiastethus pseudococci pseudococci* - transferred to *B. pseudococci pseudococci* by Ghahari *et al.,* 2009) from Kerala as a predator of coconut black-headed

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caterpillar *Opisina arenosella* Walker (Lepidoptera: Xylorictidae), a serious pest of coconut in Kerala and Karnataka, India. The authors could record *B. pseudococci pseudococci* from mango inflorescence associated with unidentified mites and thrips in Karnataka. The current study was undertaken to understand whether *B. indica* could be amenable to rearing so that its predatory role, if any, could further be ascertained. We succeeded in rearing *B. indica* continuously for more than 20 generations in the laboratory and through this paper information is provided on its biology, life stages, feeding potential, fertility table parameters and rearing protocol.

MATERIALS AND METHODS

Populations (4 adult females, 3 adult males) of *B. indica* were obtained from dry *Crossandra* (*C. infundibuliformis* Lamiales: Acanthaceae) flowers in Bangalore. These adults were released into ventilated plastic containers (adult containers, 7.5 cm height x 8 cm diameter) provided with UV irradiated *Corcyra cephalonica* Stainton eggs as feed at the rate of 8 to 10 eggs per adult per day and cotton fibres to prevent cannibalism. *C. cephalonica* eggs used as feed were UV irradiated to prevent hatching of the eggs. Mated females laid eggs either on the walls or at the base of the containers or in between cotton fibres, eggs were glued to the oviposition substrates. Eggs were collected and placed in hatching containers (ventilated plastic containers; 7.5 cm height x 8 cm diameter). When nymphs hatched, UV irradiated *C. cephalonica* eggs were provided at the rate of 5 to 8 eggs per nymph per day till they formed adults.

The experimental set up for biological studies was initiated with twenty pairs of adult *B. indica*, each pair maintained as a replication. *Corcyra cephalonica* eggs were provided as prey and data recorded on total longevity of the adults and number of eggs laid by the female in each replication. Ten sets of freshly laid eggs were placed in separate ventilated containers (6.5 cm h x 2.5 cm diameter) as replications and observations recorded on the developmental durations (incubation, nymphal and total developmental period). The number of nymphs hatching out of the eggs placed in each replication and number of nymphs surviving to adult stage was recorded, from which per cent hatching and per cent nymphal survival were calculated. For comparative studies, eggs and nymphs of the *A. constrictus* were collected from the live insect repository at NBAIR (National Accession No. NBAII-MP-ANT-11), where it is being continuously maintained (original culture was obtained from dry sugarcane leaves in Mandya, Karnataka). Morphometric measurements of all stages of *B. indica* and eggs and nymphs of *A. constrictus* were obtained using the images of live materials (n = 20 for each stage) using Olympus Microscope SZX 16 and Cell sens software. Descriptions of the nymphal and adult stages are based on the dorsal view. Scanning Electron Microscopic studies were also done to study the eggs of *B. indica* and *A. constrictus* using Zeiss EVOMA 10 Scanning Electron Microscope at 20.00 KV and 122 pa between 276 x and 600 x. Biological studies were carried out in the laboratory at 26±3°C; 60±10% RH and 13: 11 L: D.

Ten nymphs and ten adults were maintained as replications to study the predatory potential. Five UV irradiated eggs of *C. cephalonica* were provided daily for each nymph till five days of nymphal period, beyond which each day ten eggs were provided for each mature nymph till it moulted into an adult. Ten eggs were provided daily for each adult till its mortality. Preliminary studies were conducted to distinguish between the fed and unfed eggs, based on which the eggs which appeared flattened or crushed were considered as fed. The number of eggs fed by one nymph / one adult in one day was recorded throughout the nymphal and adult stages, respectively, from which the total predatory potential was calculated.

Fertility table studies were conducted based on the data obtained on the day-wise fecundity of *B. indica.* This experiment was conducted with four replications and with five pairs of adults in each replication. The adults utilised in this experiment were from the culture which was laboratory reared over 15 generations. Adults were provided with *C. cephalonica* eggs as food and the number of

eggs laid in each replication was recorded daily. Mortality of adult female in each replication was also recorded. The freshly hatched neonate nymphs were provided with *C. cephalonica* eggs as food and when they became adults, they were sexed to arrive at the female progeny produced by each female per day. The age specific survival (l_x) and age specific fecundity (m_x) at each pivotal age x were worked out for the entire reproductive period. The number of individuals alive at age x as a fraction of 1 was recorded as l_x and the number of female offspring produced per female at age interval x as m_x. Utilising these, the fertility table parameters were calculated based on the methods following Birch (1948) and Andrewartha and Birch (1954).

Net reproductive rate (R_0) = $\Sigma l_x\ m_{x;}$ approximate duration of a generation (T_c) = $\Sigma_x\ m_x/R_{0;}$ approximate intrinsic rate of increase (r_c) = $\log_e R_0/\ T_{c;}$ precise intrinsic rate of increase (r_m) = $e^{-r}{}_m$ x $l_x\ m_x$ = 1; net generation time (T) = $\log_e R_0/\ r_{m;}$ finite rate of increase (λ) = anti $\log_e r_{m;}$ weekly multiplication of the population (r_w) = ($_e$ rm)^7; hypothetical F_2 females = $(R_0)^2$; doubling Time (DT) = $\log_e 2/r_{m\ ;}$ weekly multiplication rate (WMR)= $(\lambda)^7$. All the experiments were conducted in the laboratory (temperature: 26±5°C; humidity: 60±10%).

RESULTS

Buchananiella indica could be reared continuously in the laboratory and UV irradiated *C. cephalonica* eggs were used as prey. The morphology, biological parameters, predatory potential and morphometrics of *B. indica* are provided in this paper (Table 1, Fig 1, 2 and 3).

Eggs: Eggs are laid loose by the adult females, eggs glued to the substrates, slightly bent at the opercular region (Fig 1B) The eggs measure 512-556 µm in length and 213-230 µm wide; operculum diameter 112 – 120 µm (n=20). Freshly laid eggs milky white with faint orange markings and mature eggs yellowish. Besides the bright red markings of the eye spots and abdominal scent glands of the embryo, concentrated red patches at the anterior and posterior ends of eggs with the central region appearing as a transparent transverse band (Fig 1B). Mean incubation period 4.2 days and mean per cent hatching 99.3. The egg surface covered with raised dots, evident in Electron Micrographs (Fig 1J). Operculum circular, central region slightly convex, covered with sharp edged engraved reticulations, the reticulations well defined in the central region in contrast to the faint reticulations in the periphery (Fig 3A).

First instar nymph: The neonate nymph is reddish, except the first tergite, which appears as a transparent white band in mid dorsal region of the nymph. The abdominal scent glands present as red spots (Fig 1C). The antennae and legs whitish. The length, greatest thoracic width and greatest abdominal widths 722 - 776, 178 – 205 and 205 - 242 µm, respectively. The first-instar nymphal duration 4 (range 2 to 5) days.

Second instar nymph: Reddish, abdomen orangish red, one pair of long bristles at the apex of the abdomen. The head and the pro, meso and metanotum reddish (Fig 1D). Whitish first two tergites prominent in contrast to the deep red thoracic region and posterior abdominal tergites. The abdominal scent glands present as red marks as in the first instar nymph. The mean length 918 - 1051 µm and the greatest thoracic and abdominal widths 200 - 239 and 289 - 380 µm, respectively. The mean duration of the second instar nymph 3.3 (range 2 to 5) days.

Third instar nymph: The mean duration of this instar 2 to 4 days. Wing buds evident at this stage. The femur and the apex of the first antennal segments darker. The spines on the antennae, legs, head, thorax and abdominal regions and one pair of long bristles at the apex of the abdomen clearly visible (Fig 1E). As in the previous instars, the initial tergites of the abdominal region whitish in clear contrast to the red colour of the remaining parts of the nymphal body. The red marks of the abdominal glands not distinct, appear to merge with the red colour of the posterior abdominal tergites. The mean length 1248 to 1482, thoracic width 240 to 326 and abdominal width 432 to 586 µm, respectively.

Fourth instar nymph: Head yellowish red, pronotum and the region beneath the wing buds

deep red, tergites other than the first two deep red. The other characters as in the previous instar. Wing pads more developed, with the scutellum clearly visible between the base of the wing pads (Fig 1F). Length, greatest thoracic width and greatest abdominal width 1611 to 1729, 340 to 396 and 547 to 642 µm, respectively. This instar lasts for 2 to 4 days (mean 2.6 days).

Fifth instar nymph: The mean duration of this instar is 5.1 days (range 4 to 6 days). The general colour and structure as in the previous instar. Wing pads fully developed, extend beyond the thoracic region (Fig 1G). Length 1731 to 2228, thoracic width 458 to 541 and abdominal width 640 to 783 µm, respectively.

The total nymphal duration was recorded as 16 days and total developmental duration 20 days. Percent nymphal survival was 93-100. The predatory potential of the nymphs on *C. cephalonica* eggs was 2.6 per day per nymph and 29.8 eggs throughout its nymphal period.

Adult: The adults blackish. Apical portions of first and second antennal segments deep blackish brown, base of the last antennal segment dark. Last segment of the rostrum curved and pointed (Fig 2E). Legs yellowish brown. The last abdominal sternites in male consists of asymmetrical pygophore with the evenly straight and short paramere visible (Fig 2D). The adult female larger than the male. In female, a tiny opening (omphalus) present in medioventral part of abdominal sternite VII. One-third of the second and third antennal segments from the apex darker in contrast to the pale yellow base in female; more than three quarter of the second and third antennal segments dark in male (Fig 2A and B). The length, thoracic and abdominal widths of male 1734 – 1889, 630 – 664 and 647 - 713 µm, respectively, the corresponding figures in female 2064 - 2297, 716 - 739 and 817 - 845 µm, respectively. Males lived for 10 - 89 days and females for 21 - 52 days. One female could lay 38 to 50 eggs and 32 to 55% were female progeny. The predatory potential of one adult male was 134 eggs and 137 eggs in female; while in one day an adult could feed on 2 to 3 eggs.

Comparison between *Buchananiella indica* and *Amphiareus constrictus*

The general shape and size of the egg of *B. indica* (Table 1) almost similar to that of *A. constrictus*. Operculum diameter, length and greatest width of *A. constrictus* egg - 111.3, 530.3 and 213.9 µm, respectively. The eggs and nymphs of *B. indica* differ from those of *A. constrictus* in the following combination of characters (Fig: 3). 1) In EM image of *B. indica* egg, the central region of operculum strongly convex, covered with well defined sharp angled reticulations in the central region and faint reticulations in the peripheral region, no furrow separating the operculum from the well defined narrow chorionic rim (*vs* in *A. constrictus,* central region slightly convex, uniform well defined follicular cells throughout the opercular region, furrow present separating the operculum from the broad chorionic rim) (Fig 3A and B). 3) In LM image, egg reddish with a transverse transparent band in the central region (corresponding to the first two whitish abdominal tergites of the embryo) (*vs* egg dark yellowish with the eyes and abdominal scent glands of the embryo appearing as red marks) (Fig 3C and D). 4) The thoracic region and the posterior abdominal tergites of the nymph dark red, the first two pale abdominal tergites appearing as a transverse transparent band (*vs* nymphal abdomen dark yellow with scent glands appearing as dark spots) (Fig 3E and F). 5) Last segment of rostrum curved (*vs* not curved) (Fig 3G and H).

Fertility table: The fertility parameters of *B. indica* are depicted in Table 2. With a generation time of 31 days, the reproductive rate of *B. indica* was 12.6 and the intrinsic and finite rates of increase were 0.08 and 1.08, respectively.

Fig 4 presents the age-specific survival and fecundity of *B. indica*. The immature stage occupied 20 days. The first mortality occurred on the 22nd day from adult emergence and 100% mortality was recorded on day 40. Egg laying was recorded two days after emergence and female

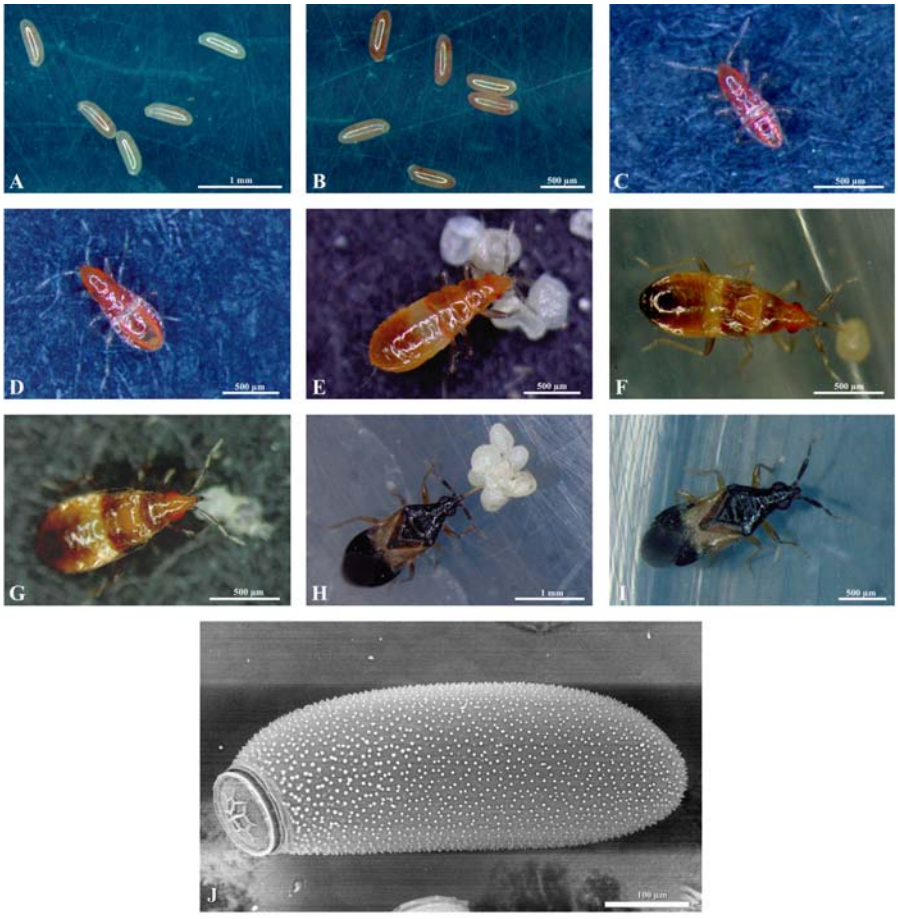

Fig 1. Life stages of *Buchananiella indica:* Fresh eggs (A), Mature eggs (B), First instar nymph (C), Second instar nymph (D), Third instar nymph (E), Fourth instar nymph (F), Fifth instar nymph (G), Adult female (H), Adult male (I), SEM image of egg (J)

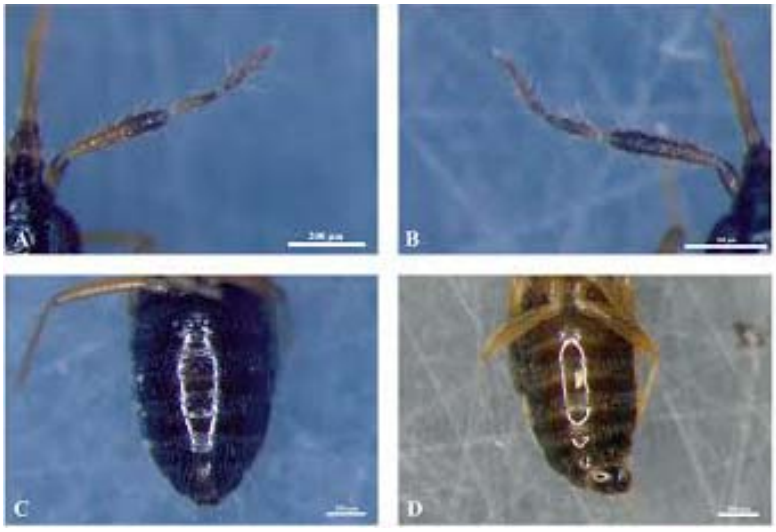

Fig 2. Antennal and abdominal characters of *Buchananiella indica :* Female antenna (A), Male antenna (B), Female abdomen (C), Male abdomen (D)

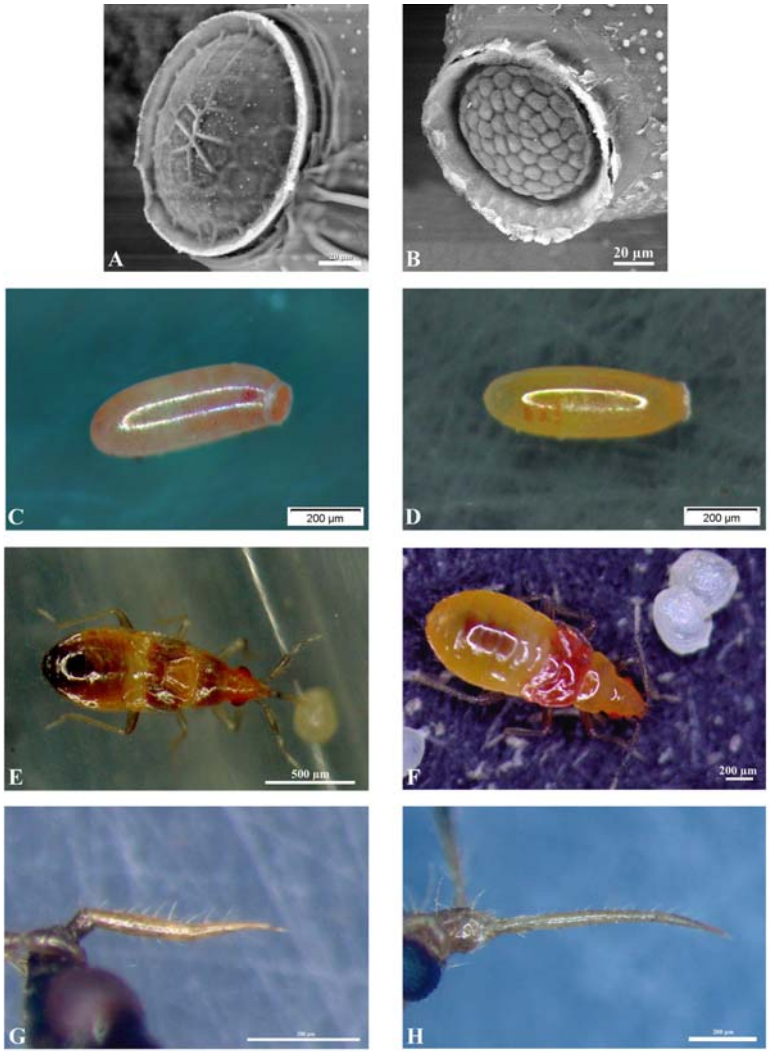

Fig 3. Characters to differentiate between *Buchananiella indica* and *Amphiareus constrictus* SEM image of egg operculum: *B. indica* (A), *A. constrictus* (B) LM image of mature egg: *B. indica* (C), *A. constrictus* (D) Mature nymph: *B. indica* (E), *A. constrictus* (F) Rostrum: *B. indica* (G), *A. constrictus* (H)

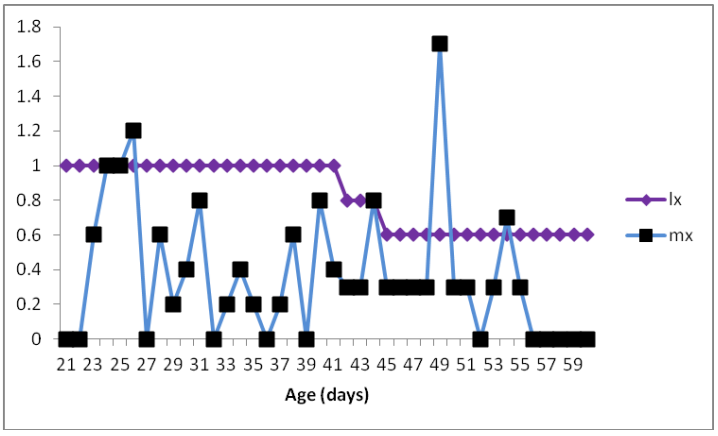

Fig 4. Age specific survival (lx) and age specific fecundity of *Buchananiella indica* (0 to 20 days – immature stages; 21 to 60 days - adult stage)

Table 1 Biological parameters and morphometrics of *Buchananiella indica*

Parameters		Duration (days) Mean±SE	Morphometrics Mean±SE		
Egg and incubation period		**Incubation period (days)**	**Operculum diameter (ìm)**	**Greatest length (ìm)**	**Greatest width (ìm)**
		4.2±0.1	116.4±0.4	523.1±8.6	218.9±1.5
Per cent hatching		99.3±0.7			
Nymphal instars		**Developmental period (days)**	**Total length (ìm)**	**Greatest Thoracic width(ìm)**	**Greatest Abdominal width (ìm)**
1st instar		4.0±0.4	736.7±8.8	188.0±4.2	226.6±5.2
2nd instar		3.3±0.4	976.2±31.3	220.2±10.6	331.9±23.7
3rd instar		3.0±0.3	1368.5±36.2	284.6±12.4	487.3±23.1
4th instar		2.6±0.4	1664.1±25.8	372.8±10.5	587.8±22.1
5th instar		5.1±0.3	1934.8±50.9	494.5±9.0	711.4±17.8
Total nymphal duration (days)	**Male**	15.9±0.3			
	Female	15.8±0.2			
Total developmental duration (days)	**Male**	20.1±0.3			
	Female	20.0±0.2			
Adult male			1830.4±18.1	642.8±3.9	676.1±7.3
Adult female			2171.0±28.3	724.7±2.5	827.7±3.0
Per cent nymphal survival to adult stage		98.2±1.8			
Male longevity (days)		35.9±10.5			
Female longevity (days)		34.6±5.6			
Mean Fecundity (number of eggs / female)		43.0±3.6			
Per cent females		42.8±4.7			
Predatory potential (on *C. cephalonica* eggs)					
Mean number of eggs consumed (Mean±SE)					
A) a nymph in one day **Throughout the nymphal duration**		2.6±0.3 29.8±3.0			
B) an adult male in one day **Throughout its life time**		2.4±0.2 133.8±29.6			
C) an adult female in one day **Throughout its life time**		2.8±1.0 136.7±53.8			

Table 2 Fertlity parameters of *Buchananiella indica*

R_0	T_c	r_c	r_m	T	λ	DT (days)	Hypo. F_2 ♀s	WMR
12.6	35.89	0.07	0.08	31.09	1.08	8.66	158.76	1.71

progeny produced by one female per day ranged from 0 to 1.7, peak female progeny production was recorded when the parent female was 49 days old and ceased when it was 56 days old (Fig 4).

R_0: Net reproductive rate; T_c: Approximate duration of a generation; r_c: Approximate intrinsic rate of increase; r_m: Precise intrinsic rate of increase; T: Net generation time; λ: Finite rate of increase; Hypo.: Hypothetical; DT: doubling time; WMR: Weekly multiplication rate

Rearing protocol

The nymphs and adults can be reared in plastic containers (7.5cm h x 8cm dia) with a ventilated lid. Based on the predatory potential studies, it was concluded that *C. cephalonica* eggs are to be provided as food for nymphs and adults at the rate of five eggs per nymph / adult per day. In a container holding 50 adults, every three days, 0.05 cc of eggs are to be provided as food. Cotton strands are to be placed in the containers in order prevent cannibalism, they also serve as oviposition substrates. Eggs can be collected on alternate days and placed in a separate ventilated container (7.5cm h x 8cm dia) for hatching. Based on the oviposition pattern, egg harvesting can be initiated two days after emergence and can be continued for one month. Following this procedure, *B. indica* could be reared continuously for 20 generations. In a production system for *B. indica,* utilising 1 cc of *C. cephalonica* eggs (approximately 15000 to 17000 eggs), 97 nymphs/adults (with 43% females) could be reared. From a container holding 50 adults, 1100 eggs / nymphs can be harvested.

DISCUSSION

In this study, we report sexual dimorphism not only in the abdominal characters (which is common in Anthocoridae), but also in the antennal characters of *B. indica*. *Buchananiella* is sometimes confused with *Amphiareus* and we generally collect *B. indica* and *A. constrictus* from leaf litter and decaying plant materials and both were observed to be amenable to rearing. The taxonomic characters of adult stages are reported by Muraleedharan (1977), Yamada (2008) and Yamada and Hirowatari (2003). However, the egg and nymphal characters reported in this paper can be used to differentiate the immature stages of *B. indica* and *A. constrictus* in the rearing units and thus to maintain pure cultures.

Male genitalia characters are generally used for identification in family Anthocoridae. However, the general shape and size of the eggs and the structure of the chorial surface, operculum and flange are considered as important characters in egg systematics (Cobben, 1968; Carpintero, 2002). Information on the structure of eggs and nymphs of a few Anthocoridae is available (Southwood, 1956; Sands, 1957; Muraleedharan and Ananthakrishnan, 1978; Schuldiner-Harpaz and Coll, 2012). Anthocoridae, such as species of *Orius* and *Anthocoris* and *Blaptostethus pallescens* Poppius, *Carayanocoris indicus* Muraleedharan and *Xylocoris afer* (Reuter) insert eggs into plant tissue, with only the opercular region visible. However, Anthocoridae such as *Xylocoris flavipes* (Reuter), *Amphiareus constrictus* (Stål), *B. indica, Cardiastethus exiguus* Poppius and *Cardiastethus affinis* Poppius lay their eggs in either exposed or concealed substrates (e.g. beneath bark, in leaf litter, among decaying plant materials). Such egg laying behaviour enables us to examine the whole egg structure. SEMicrographs of eggs of four species of *Orius* were used to

determine the species based on the structure of the operculum and the follicular cells along the outer ring of the operculum (Schuldiner-Harpaz and Coll, 2012). In the current study, the egg and nymphal characters of *B. indica* are described which can serve as additional characters for a non-destructive identification process, when either adult females or immature stages are collected from the field.

This study highlights an easy rearing protocol enabling the laboratory rearing of *B. indica* on *C. cephalonica* eggs. The fact that *B. indica* does not require a plant substrate for egg-laying, makes the rearing procedure simple and user-friendly.

No precise information is available on the prey range of *Buchananiella* In Thailand, *B. crassicornis* Carayon, *B. atrata* Yamada and Hirowatari and *B. pericarti* Yamada and Yasunaga were recorded from dry leaf materials (Yamada and Yasunaga, 2009). *B. crassicornis* and *B. leptocephala* Yamada and Hirowatari were recorded in Japan from dry banana leaves and dry leaves of broad-leaved trees, respectively (Yamada and Hirowatari, 2005). However, *B. pseudococci pseudococci* was recorded as a predator of *O. arenosella* (Yamada *et al.,* 2008). It is interesting to note that *A. constrictus*, a litter inhabiting species, was recently recorded as a predator of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Queiroz *et al.,* 2015).

The reproductive rate and intrinsic rate of increase can be used to estimate the growth rate of the population of a parasitoid / predator, which in turn can be used to optimise a mass rearing protocol. The fertility table parameters of *B. indica* is comparable to those of potential anthocorid predators *viz. Orius majusculus* (Reuter)*, Orius laevigatus* (Fieber) and *Orius tantillus* (Motsch.), which are also highly amenable to rearing on eggs of alternate laboratory hosts (Tommasini *et al.,* 2004; Ballal *et al.,* 2012). Based on the above and considering the fact that *B. indica* could be successfully reared for more than 20 generations, it would be worth investigating if this anthocorid has a functional role as a potential predator of field crop pests and storage pests. This study can form a prelude to future studies on prey preferences, dispersal and predatory attributes of this and other related species of anthocorids in different regions and agro-climatic conditions.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, ICAR-NBAIR for the facilities provided and to Dr V. V. Ramamurthy, National Co-ordinator, Network Project on Insect Bio-systematics, Division of Entomology, IARI, New Delhi for the EM facilities provided. We are also thankful to Mr. K. Raja and Mr. S. Jogesha for assistance in continuous maintenance of the anthocorid cultures and for technical assistance.

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(Received 18 December 2015; Accepted 19 January 2016; Published 31 March 2016)

Entomon 41 (1): 21-28 (2016)
Article No. ent. 41103

Cloning Folmer region of *mtCOI* gene diagnostic for sugarcane early shoot borer, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae)

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ABSTRACT: Early shoot borer (ESB), *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae) is an important pest of sugarcane and distributed across the Indian subcontinent. It was originally described as *Diatraea saccharalis* and after several revisions by many taxonomists, it was finally accepted as *C. infuscatellus*. The limitations associated with conventional alpha taxonomy such as phenotypic plasticity of key morphological traits, could be the possible reason for taxonomic uncertainty of the species. DNA barcoding has emerged as a complementary approach to conventional taxonomy and has also been proved as a powerful tool to identify cryptic species in the population. Hence, the 'Folmer region' of mitochondrial *cytochrome c oxidase I* (*mtCOI*) gene from early shoot borer has been cloned to serve as the barcode for the species. The DNA barcode developed in this study would address the anomalies that exist in the identification of early shoot borer. The barcode generated by us is the ideal one as it is exactly 658 bp in size and carries no stop codon in its amino acid sequence. KM453722 is the unique GenBank accession number for the DNA barcode of *C. infuscatellus.* The DNA barcode developed in this study would serve as an ideal molecular diagnostic kit for correct identification of *C. infuscatellus* irrespective of its sex, stage and polymorphism.

KEY WORDS: *Chilo infuscatellus, COI,* diagnostic kit, DNA barcode, sugarcane

INTRODUCTION

Early shoot borer (ESB) of sugarcane, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae) is a major pest of sugarcane and distributed across the Indian subcontinent. The annual yield loss due to this pest has been estimated to be 22-33% (Directorate of Sugarcane Development, 2016). It has become a major threat to sugarcane cultivation in the coastal cane belts of peninsular India and coastal Andhra Pradesh in particular, where it also behaves as internode borer (Bhavani, 2013). It is generally brought under check by the insecticides as the population often crosses the economic threshold level. The taxonomy of sugarcane pests including the ESB has been under constant revision over the years ever since they were originally described. ESB was originally described as *Diatraea saccharalis* Fab. by Cotes (1889) and after several revisions by many taxonomists (Lefroy, 1906; Fletcher, 1926; Kapur, 1950; Bleszynski, 1965) it was finally accepted as *Chilo infuscatellus* Snellen (Avasthy and Tiwari, 1986). It has been accepted worldwide that the conventional alpha

taxonomy suffers from its own limitations. Among the several constraints, phenotypic plasticity of key taxonomic traits employed for species discrimination might be one of the major constraints leading to wrong identification of pest insects. Besides, the conventional taxonomy relies on the morphological traits of adult insects such as the structure of genitalia and immature stages of pest insects are generally ignored for describing a species. Sometimes, the genitalia, which often distinguish the species, fails to delineate the cryptic species in the population (Hebert *et al.,* 2004).

Since Lepidoptera is the second largest order of the class Insecta with more than 1.6 lakhs described species, correct identification of Lepidopterans up to species level certainly requires unparalleled expertise in alpha taxonomy. The decreasing number of insect taxonomists worldwide and India in particular, also poses difficulty in getting the specimens identified in right time. The taxonomic identity of the pest must be ascertained to realize the success of any pest management programme irrespective of the crop under stress. The limitations associated with conventional alpha taxonomy are however, adequately addressed by the novel approach named DNA barcoding. Besides being a powerful tool to identify the cryptic species, DNA barcoding does not require the support of insect taxonomists. Once the barcode has been developed for a species in complementarity with conventional taxonomy, a non-insect taxonomist can identify the species without any ambiguity with the help of unique DNA barcode. As of now, ideal DNA barcodes are not available for many of the important cane pests including the ESB. Sugarcane entomology in India has long been dominated by the scientists specialized in the field of biological control. To the best of our knowledge, sugarcane entomologists having expertise in both insect taxonomy and insect molecular biology are very rare in the country. This could be the possible reason for lack of information on DNA barcodes for insects in sugarcane ecosystem. Hence, we have developed DNA barcode for ESB after ascertaining its taxonomic position through conventional taxonomy. The barcode developed in this study is ideal (658 bp) and best-ever DNA barcode for the species under investigation. This barcode would serve as an ideal diagnostic kit for unambiguous identification of ESB.

MATERIALS AND METHODS

DNA isolation, PCR and cloning

Larvae of ESB collected from the research farm at ICAR-Sugarcane Breeding Institute, Coimbatore were cultured in the laboratory till the emergence of adults. The adults (F_0) were allowed to lay eggs and the first generation adults (F_1) obtained from the egg mass of a pair were used for identification through conventional taxonomy and for developing the DNA barcodes. The taxonomic identity of *C. infuscatellus* was ascertained by running the keys developed by Sallam and Allsopp (2008). This was followed by the development of barcode.

DNA isolation, polymerase chain reaction (PCR) and cloning were performed by adopting the protocols as described by Ramasubramanian *et al.* (2015d). The protocol followed to isolate DNA from ESB is briefed here. The body tissues of a de-winged adult insect were homogenized in warm CTAB buffer (Tris 100 mM, EDTA 20 mM, NaCl 1.4 M, CTAB 2%, β-mercaptoethanol 0.2%). The well-homogenized sample was kept in water bath at 65°C for 1 hr. This was followed by centrifugation at 12,000 rpm for 10 min. at 4°C. The supernatant was pipetted out and equal volume of chloroform: isoamyl alcohol (24:1) mixture was added. The contents were shaken vigorously and again centrifuged at 12,000 rpm for 10 min at 4°C. The top layer was collected and 0.7 volume of ice-cold isopropanol was added to the recovered layer. The supernatant was drained after brief centrifugation and the DNA pellet settled at the bottom of the tube was washed with 70% ethanol. The dried DNA pellet was dissolved in 30-50 µL of sterile water. This was followed by RNaseA treatment. The resultant RNA-free DNA was stored at -80°C until further analysis. The quantity and quality of the DNA were determined in NanoDrop ND 1000 spectrophotometer (Thermo Scientific Inc., USA).

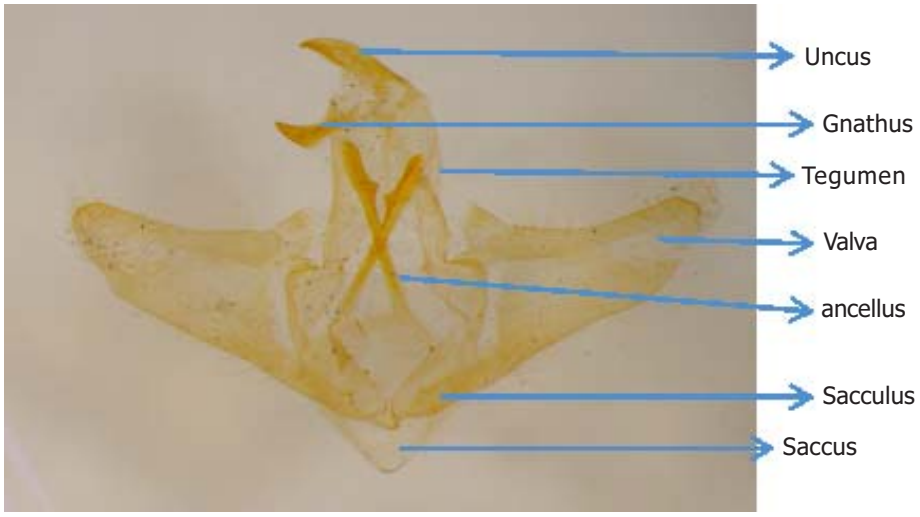

Fig 1. Male genetalia (Ventral view)

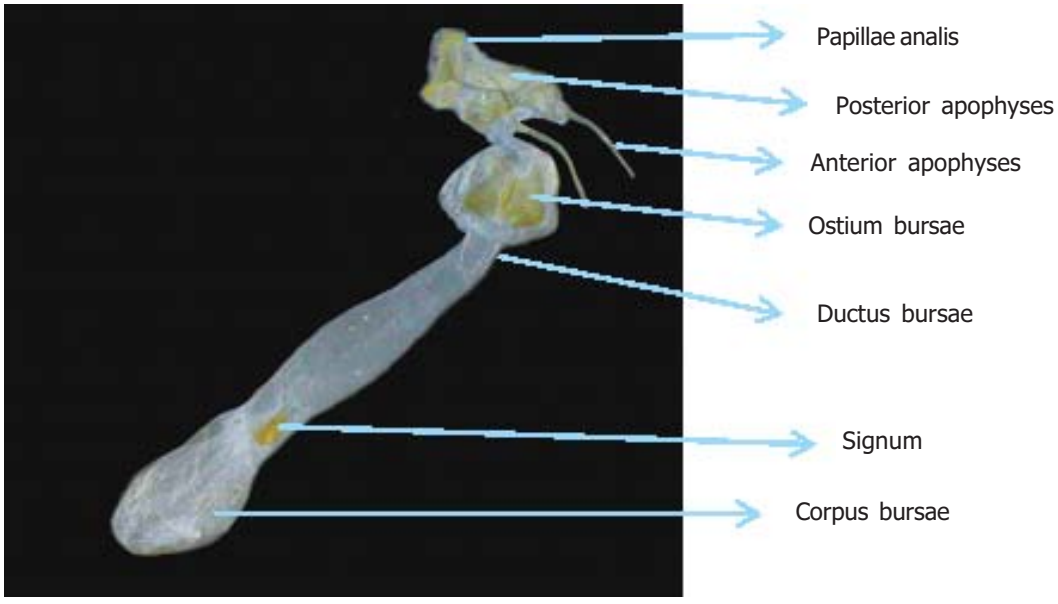

Fig 2. Female genetalia

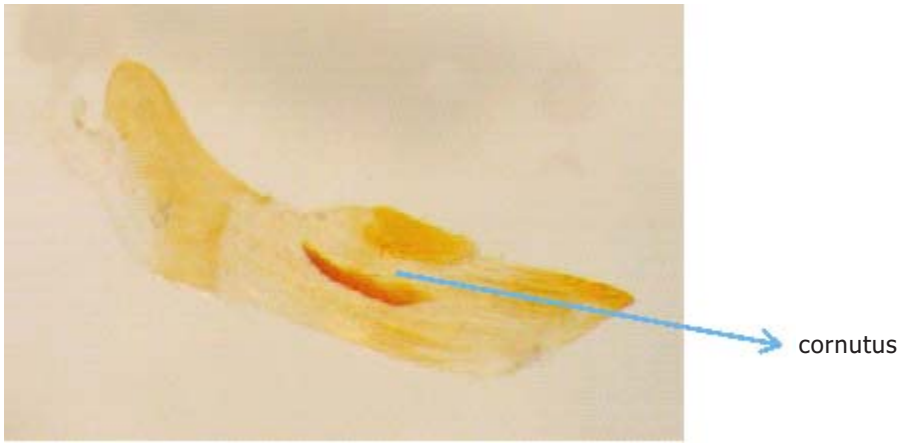

Fig 3. Aedeagus

AACTTTATATTTTATTTTTGGAATTTGAGCAGGAATAATTGGAACTTCCCTTAGACTTTTAATT
CGAGCTGAATTAGGGACTCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTT
ACAGCTCATGCATTTATTATAATTTTTTTTATAGTAATACCAATTATAATTGGGGGATTTGGA
AATTGATTAGTTCCTTTAATACTAGGGGCACCTGGTATAGCTTTTCCACGAATAAATAATATA
AGCTTTTGATTATTACCACCATCATTAACATTATTAATTTCTAGAAGAATTGTTGAAAATGGG
GCAGGGACTGGTTGAACTGTTTATCCCCCTTTATCCTCAAATATTGCTCATGGGGGAAGCTC
TGTAGATTTAGCAATTTTTTCCCTTCACTTAGCGGGTATTTCATCAATTTTAGGAGCTATTAA
TTTTATTACAACAATTATTAATATACGAGTTAATGGTCTATCATTTGATCAAATACCTTTATTT
GTTTGATCTGTAGGTATTACAGCACTTTTATTATTACTTTCTCTACCAGTATTAGCAGGTGCT
ATTACTATACTACTAACTGATCGAAATTTAAATACATCTTTTTTTGACCCTGCTGGAGGGGGG
GATCCAATCCTTTATCAACATTTATTT

Fig 4. DNA barcode of sugarcane early shoot borer, *C. infuscatellus*

TLYFIFGIWAGMIGTSLSLLIRAELGTPGSLIGDDQIYNTIVTAHAFIMIFFMVMPIMIGGFGNWLV
PLMLGAPGMAFPRMNNMSFWLLPPSLTLLISSSIVENGAGTGWTVYPPLSSNIAHGGSSVDLAIFS
LHLAGISSILGAINFITTIINMRVNGLSFDQMPLFVWSVGITALLLLLSLPVLAGAITMLLTDRNLN
TSFFDPAGGGDPILYQHLF

Fig 5. Uninterrupted ORF of *COI gene* fragment from *C. infuscatellus*

PCR was performed in S-1000 PCR Touch Cycler (BioRad, USA) and LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were the primers used to amplify the target fragment (Folmer *et al.* 1994). After amplification, the PCR products were purified using GenElute Gel Extraction Kit (Sigma-Aldrich India Private Ltd., Bengaluru) by following the manufacturer's instruction. The purified PCR products were cloned into pTZ57R/T plasmid vector as per the manufacturer's instruction (Thermo Scientific Inc., USA). This was followed by transformation of *Escherichia coli* (strain DH5α) competent cells. The recombinant clones were confirmed by colony PCR and also by restriction digestion of plasmids isolated from the recombinant colonies. Plasmid DNA was isolated from single recombinant colony using Plasmid DNA MiniPreps Kit (Bio Basic Canada Inc.) as per the instruction given by the manufacturer. Sequencing of purified recombinant plasmids was done by Chromous Biotech India Private Ltd., Bengaluru. The sequencing was done in both forward and reverse directions to find out mismatches if any, in the target sequence. Homology search was made in the National Centre for Biotechnology Information (NCBI) using blast algorithm. The nucleotide sequence was translated into amino acid sequence using ExPASy (Expert Protein Analysis System) translate tool of Swiss Institute of Bioinformatics and the open reading frame (ORF) was obtained using invertebrate mitochondrial genetic code. The well-characterized barcode was submitted in the GenBank of NCBI and unique accession number was obtained.

RESULTS AND DISCUSSION

The taxonomic identity of *C. infuscatellus* was ascertained by running the keys developed by Sallam and Allsopp (2008). The male and female genitalia were dissected and studied for the key characters specific to *C. infuscatellus* (Figs. 1-3). The voucher specimens were deposited at the Division of Crop Protection, ICAR-Sugarcane Breeding Institute, Coimbatore, India. DNA barcode was developed only after confirming the identity of the species by conventional alpha taxonomy.

In the present study, we could isolate DNA of better quality and yield from ESB of sugarcane. Quantity of the DNA extracted varied between 900.7 ng/µL and 1090.6 ng/µL across the individuals. The 260/280 ratio of the DNA was in the range of 1.77-1.83. The intact genomic DNA as visualized in 0.8% agarose gel also indicated its suitability for PCR. A 658 bp DNA fragment (Folmer region) that lies in the 5' end of *mtCOI* gene has been designated as the standard DNA barcode for identification of species in the animal kingdom (Hajibabaei *et al.,* 2005; Floyd *et al.,* 2009) including insects. The primers designed by Folmer *et al.* (1994) could amplify the target fragment from *mtCOI* gene of ESB. DNA fragment of ~700 bp in size was amplified from all the positive colonies after transformation. The recombinant clones were further confirmed by restriction-digestion of the recombinant plasmids. DNA barcode developed for *C. infuscatellus* is a complete barcode of 658 bp in size (Fig. 4). The amino acid sequence of the barcode does not have any stop codon (Fig. 5). The uninterrupted open reading frame (ORF) indicates the flawlessness of the *COI* sequence. The occurrence of mismatches was eliminated by performing the sequencing in both directions. The barcode generated in this study was submitted in the GenBank of NCBI with accession number KM453722. As of now, we could retrieve only one *COI* gene fragment of *C. infuscatellus* from NCBI. The barcode sequence retrieved from the public domain was 678 bp in size (Accession No. JQ066747) and may not be considered as an ideal one. Conversely, the barcode generated by us is the ideal one as it is exactly 658 bp in size. The ESB *COI* sequence cloned in the present study showed 96.16% identity with the one retrieved from the public domain. The *COI* sequence of *C. infuscatellus* developed in the study was aligned with the barcode fragment of *Chilo auricilius* Dudgeon (KR153874) developed by us earlier. The extent of identity between these two *COI* sequences was 89.21%. The inter-specific sequence variation of more than 10% would undoubtedly delineate these two species without any ambiguity, which were once considered as single species.

Lepidoptera is one of the taxonomically most diverse orders of the class Insecta with low sequence divergences. However, DNA barcoding with *COI* gene fragment has been shown to be highly successful in correct identification of closely related species of lepidoptera. The *COI* fragment could successfully delineate 196 out of 200 lepidopteran species (98%) prior to their morphological studies with minimal interspecific genetic divergence of 3%. The *COI* fragments of only four congeneric species of very recent in origin showed less than 3% (0.6-2.0%) interspecific genetic variation (Hebert *et al.,* 2003). Since the species involved in the present study (*C. infuscatellus*) is a member of lepidoptera, we have employed the most reliable *COI* gene fragment as DNA barcode. It is an indisputable fact that the barcode generated in the present study is the best one for the ESB of sugarcane. The 658 bp *COI* fragment was reported to be quite successful in identifying insects irrespective of the orders they belong to. The *COI* fragment could delineate most (96%) of the aphid species (335 species from 134 genera) belonging to the subfamily Aphidinae with intra-specific sequence divergence of 0.2% (Foottit *et al.,* 2008). Besides, the sequence divergence among the individuals of *Aphis gossypii,* which has several host-associated genotypic lineages, was less than 0.62% (Foottit *et al.,* 2008). Low level of intra-specific sequence divergence and significantly high level of inter-specific sequence divergence make this DNA fragment as an ideal DNA barcode (Savolainen *et al.,* 2005; Simon *et al.,* 1994) for delineating closely related species.

As many as 212 insects were recorded as pests of sugarcane (David and Nandagopal, 1986). Although sugarcane entomologists are competent enough in identifying the pests of regular occurrence, they are often approaching insect taxonomists for unambiguous identification of occasional pests and biocontrol agents of rare in occurrence. Being a major pest of sugarcane, ESB can be identified easily by observing key morphological traits. However, the existence of cryptic species among the diverse populations of ESB cannot be ignored

due to its pan-India distribution. The conventional alpha taxonomy often fails to identify the morphologically identical yet reproductively isolated cryptic species in the population. Some of the species-specific parasitoids will miserably fail to parasitize the host when the target species is wrongly identified. Hence, correct identification of cryptic species is inevitable to achieve desired level of control of target pests. This can be achieved only by DNA barcoding approach. Neotropical skipper butterfly *Astraptes fulgerator* (Lepidoptera: Hesperiidae), which was originally described in 1775, has long been considered as single species for more than two centuries until 2004. Hebert *et al.* (2004) were the first to identify ten cryptic species in the population of *A. fulgerator* with the help of DNA barcoding approach. Hence, an ideal DNA barcode was generated for *C. infuscatellus.* The barcodes to be developed for diverse populations of ESB will be compared with the barcode generated in the study to fish out the cryptic species if any, in the sugarcane ecosystem.

Worldwide development of DNA barcodes for insects is at its rapid pace. The development and use of DNA barcodes for correct identification of pests and beneficial insects in agro ecosystems need to be intensified in India. In India, *COI*-based DNA barcoding approach has been employed to differentiate closely related species form across the orders of class Insecta (Asokan *et al.,* 2012; Rebijith *et al.,* 2012; 2013; Ojha *et al.,* 2014; Tembe *et al.,* 2014; Jalali *et al.* 2015). Rebijith *et al.* (2013) could identify three cryptic aphid species for the first time from India with the help of *COI* gene sequences. Only few attempts were made to generate DNA barcodes for insects in sugarcane ecosystem. Rakshit *et al.* (unpublished) have submitted partial CDs of *COI* gene pertaining to sugarcane borers collected from Indian subcontinent. The *COI* sequences of internode borer *Chilo sacchariphagous indicus* Kapur (KC306951), stalk borer *C. auricilius* (KC306949) and top borer *Scirpophaga excerptalis* Walker (KC306948) were observed to be 611 bp in size. The *COI* gene fragment of *Sesamia inferens* Walker (KC911715) was found to be of 659 bp in size. However, Ramasubramanian and Ramaraju (2014) and Ramasubramanian *et al.,* (2014, 2015a, 2015b, 2015c, and 2015d) have been consistent in cloning ideal DNA barcodes (658/ 649 bp) for sugarcane pests. They have cloned and characterized the *mtCOI* fragments from *S. inferens* (KJ013410), *Melanaspis glomerata* Green (KR153875), *Melanaphis sacchari* Zehntner (KM453722),*Tetraneura javensis* van der Goot (KM453723), *Aleurolobus barodensis* Maskell (KF986269), *Neomaskellia bergii* Signoret (KF986270) and *Pyrilla perpusilla* Walker (KJ013412). The *COI* fragments of *M. glomerata* and *A. barodensis* are of 649 bp in sizes and the loss of 9 bases (3 amino acids) has been reported as real deletion from the COI enzyme of insects (Ramasubramanian et al., 2015b and 2015d). The barcodes developed for *P. perpusilla*, *M. glomerata*, *T. javensis* and *N. bergii* were the firsts and no other barcodes are available as on date for these species in the public domain. Since the Folmer region of *mtCOI* gene cloned from *C. infuscatellus* is exactly 658 bp in size, it would certainly serve as an ideal species diagnostic kit for unambiguous identification of ESB irrespective of its sex, stage and polymorphism.

ACKNOWLEDGEMENTS

The first author is grateful to Dr. Bakshi Ram, Director, ICAR-Sugarcane Breeding Institute; Dr. R. Viswanathan, Head, Division of Crop Protection and Dr. R. Jayanthi, Principal Scientist for their constant encouragement and support in carrying out this research.

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(Received 11 January 2016; Accepted 19 February 2016; Published 31 March 2016)

ENTOMON 41(1): 29-38 (2016)
Article No. ent. 41104

Oxidative effects of tarragon (*Artemisia dracunculus* L.) on biostages stages of *Drosophila melanogaster* Meigen

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ABSTRACT: Tarragon (*Artemesia dracunculus* L.) is a traditional spice often used in local food dishes. This study was undertaken to determine the effects that nutritional tarragon has on oxidative stress in various developmental stages of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Larvae of *D. melanogaster* were reared to adulthood on artificial diets containing varying amounts of tarragon ranging from 10 to 2000 µg. The effects of the various concentrations of tarragon on major indicators of oxidative stress including lipid peroxidation products, the production of malondialdehyde (MDA) and detoxification enzyme, and glutathione-S-transferase (GST) activity were investigated in 3rd instar larvae, pupae and adult fruit flies. The results indicate that the effectiveness of tarragon as an oxidative stress agent in *D. melanogaster* is dependent on its concentration in the fly's diet.

KEYWORDS: *Drosophila*, *Artemisia dracunculus*, oxidative stress, malondialdehyde, glutathione-S-transferase.

INTRODUCTION

Foods serve for energy production by oxidative phosphorylation, and nutrition are essential for the oxidant-antioxidant network in many organisms (Sies *et al.,* 2005). Because nutritional oxidative stress shows a disturbance of the redox state resulting from excess oxidative load or from nutrient supply (proteins, fat, carbohydrates, minerals, vitamins) favoring prooxidant reactions (Sies *et al.,* 2005). Increased ingestion of natural products are associated with a diminished risk, but the organism is unable to mitigate the free radicals, damage to biological molecules may occur, formed by oxidative stress (Joanisse and Storey, 1996 a; 1996 b).

Artemesia dracunculus L. (Tarragon) is used in food and perfume industry, antiseptics, pharmaceutical (aperient, stomachic, stimulant, febrifuge), sanitary, cosmetic, antioxidant - prooxidant activity, food industries and as an appetizer in Central Anatolia. The tarragon essential oils or components have been studied in different concentration of many organisms such as bacteria, fungi, arthropods etc (Hatimi *et al.,* 2001; Lamiri *et al.,* 2001; Farzaneh *et al.,* 2006; Kordali *et al.,* 2005; Liu *et al.,* 2006; Saleh *et al.,* 2006; Van de Sande *et al.,* 2007; Bakkali *et al.,* 2008). Desiccated powder of tarragon is not genotoxic but consumed fresh it is harmful to humans (Insttitut Pasteur de lille, 2008-2010). Its essential oils have been cytotoxic capacities and damages for some tissues of various animals.

Drosophila melanogaster (Meigen) has been studied as a model organism for the research in cell and developmental biology (Adams *et al.,* 2000). Despite tarragon is used as insecticide (natural deterrent) in biological control system, there has been no report about the determination of its

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effects on developmental stages of *D. melanogaster* in oxidative stress including ROS-specific lipid damage products, the production of malondialdehyde (MDA) and detoxification enzyme, and glutathione-S-transferase (GST) activity .

Investigations were made to understand whether tarragon ingestion causes oxidative stress on insect development and what kind of effects Tarragon in peroxidation (indicate lipid damage; MDA)-detoxification (indicate antioxidant activity; GST) mechanisms created on non-target organisms.

MATERIALS AND METHODS

The experiments were maintained at 25°C, 60% humidity and 12 h light/dark photoperiodic cycle, at a density of 30-35 flies per vial. The mixed-age and mixed sex fly stocks (Wild type, W_{1118}) were cultured in glass vial (250 cc), with an artificial diet (Rogina *et al*., 2000; Lesch *et al*., 2007). Methyl 4-hydroxybenzoate (0.2%; 100 g nipagin, 700 mL 96% ethanol and 300 mL water) was added to the diet to inhibit mold growth (Dahmann, 2008). Newly eclosed flies (6 male: 18 female) were collected in separate vials, mated and fed for two days before becoming flies, after laying eggs for 18 h, flies were removed. Nutritive value of tarragon are presented in table 1, and total essential oils are presented in table 2. Tarragon seeds (Zengarden, 838H) were planted and grown in flower pots, and its fresh leaves were crushed with liquid nitrogen in sterile muller. 100 newly hatched larvae were collected and distributed (directly incorporated into freshly diets) to ~~either~~ bottles with varying concentrations of tarragon (10, 200, 600, 900, 1200, 2000 µg/mL). The control contained only water. These concentrations were used based on the results of our preliminary experiments (unpublished; Güne , 2014) within the tolerance range of. *D. melanogaster* and the results of previous studies on other insects exposed to tarragon (Azaizeh *et al.,* 2007; Bakkali *et al.,* 2008; Hifnawy *et al.,* 2001; Soliman, 2006; Tani *et al.,* 2008; Mihaljilov-Krstev *et al.,* 2014). The exposure schedule lasted until flies come to the 3rd instar larvae, puparium and adult (newly enclosed virgin female and male) stage. These samples (n=20, per concentration) were collected and frozen in the freezer (-18ºC) for 5 mins. They were transferred to a labeled micro centrifuge tubes and homogenized in 1 ml cold homogenization buffer (0.5 M potassium phosphate buffer pH 7.2) for three times using ultrasonic processor (Homogenizer, Branson) on ice. The supernatants were collected and used for biochemical analysis. All homogenates were centrifuged at 20,000g for 30 min, at 4ºC.

Biochemical analysis:

The MDA content and GST activity (EC 2.5.1.18) of each supernatants were assayed via measuring the absorbance of the samples in spectrophotometer (Biochrom Libra S22) as described previously (Jain and Levine, 1995; Habig *et al.,* 1974; Fig. 1). At the same time protein concentrations were determined according to the method of Lowry *et al.* (1951) by using bovine serum albumin (BSA) as a standard. Data graphics were calculated using the computer program (Microsoft Excel). All chemicals used in this experiment were analytically pure and obtained from Sigma-Aldrich.

Statistical analysis:

The experiments were performed four times. Experimental data were expressed as means ± S.E. The data (MDA and GST activity) were subjected to statistical analysis by one-way analysis of variance (ANOVA) was followed by lest significant difference (LSD) test to determine significant differences between means. A values of $p<0.05$ was considered significant (SPSS, 1997).

RESULTS

The MDA contents and GST activities obtained from larvae, pupae and adults stages were shown in Figure 2 and 3. The effective Tarragon concentration was determined to be 10 µg in larva. It was determined that the MDA content was found lower, and GST activity was found higher in 100 µg/L plant application, but these two parameters were increased and stabilized in higher concentrations. It is thought that while the lower

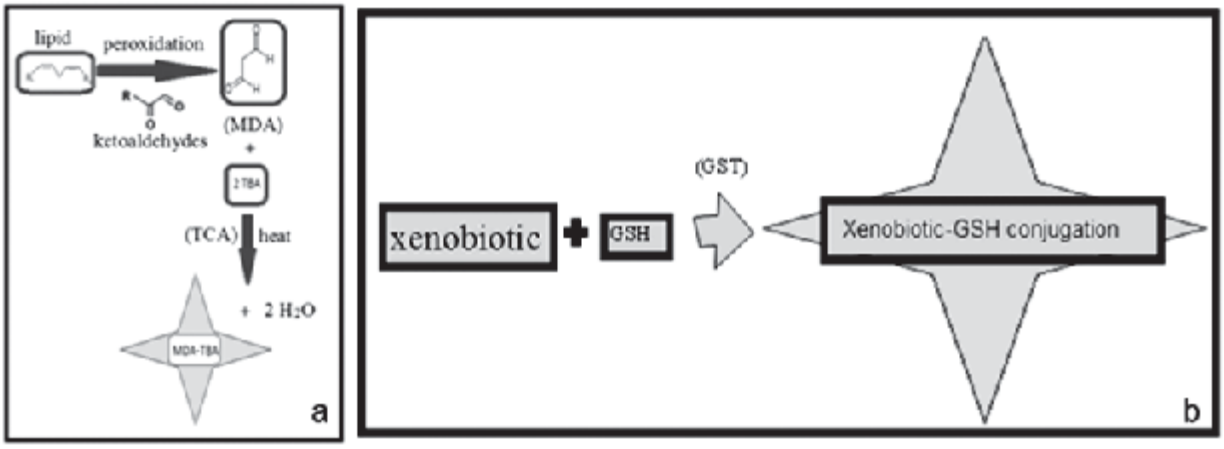

Figure 1. The principles of biochemical analysis (a: MDA content, b: GST activity)

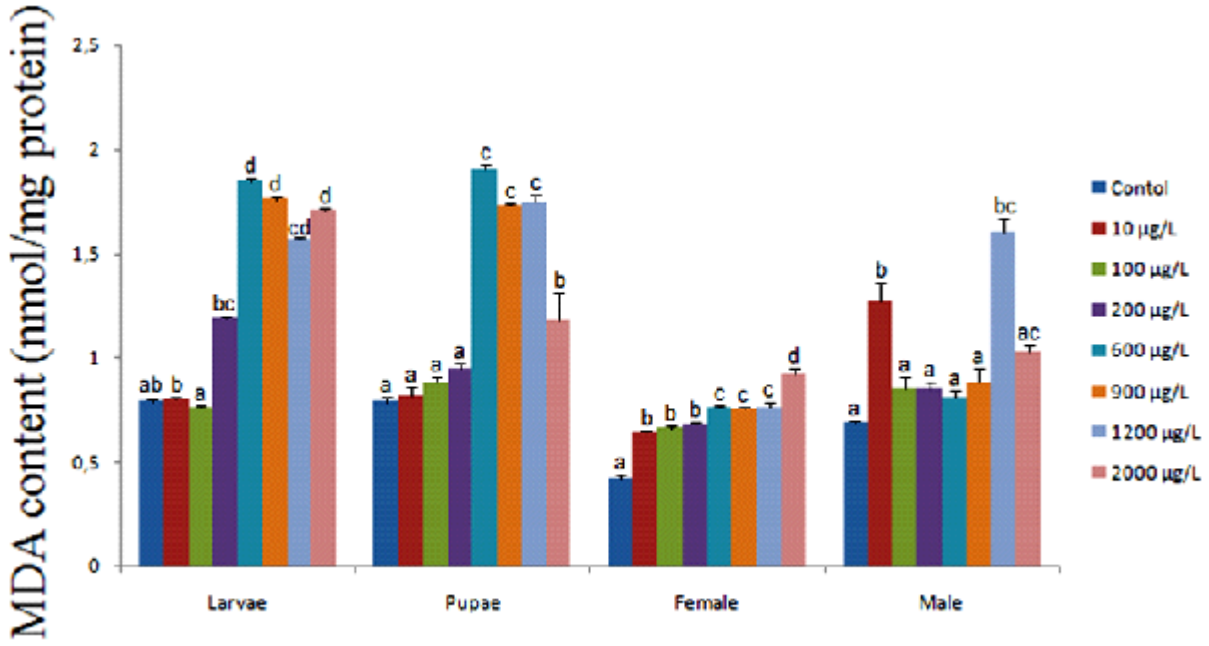

Figure 2. The Tarragon effects of MDA content were indicated on larvae, pupae and adults (female and male) of *D. melanogaster*. Samples with increasing concentration of Tarragon: control (0.00 mg/L); 10 µg/mL; 200 µg/mL; 600 µg/mL, 900 µg/mL, 1200 µg/mL and 2000 µg/mL. Each histogram bar represented the mean of four replicates (± S.E., n=20) in each of treatment groups.

concentrations of the plant can be tolerated, the toxic impact in higher concentration cannot be tolerated by the insect.

MDA contents were not significantly different from 0.0 to 200 µg/L tarragon concentration in pupae. GST activity increased sharply in pupae after 200 µg/L plant, but it decreased slightly from 600 to 2000 µg/L (Fig. 2). It was determined that the MDA contents were observed as gradual increases dependent on Tarragon concentration in female, and as well as the GST activities were increased with the activation of the detoxification mechanisms when we compared to control group.

In addition, the males' MDA contents were not significantly ($p>0.05$) different from 0.0 to 900 µg/L, but MDA content was significantly increased oxidative stress by feeding with 1200 µg/L tarragon, and there were parallel increase in these GST

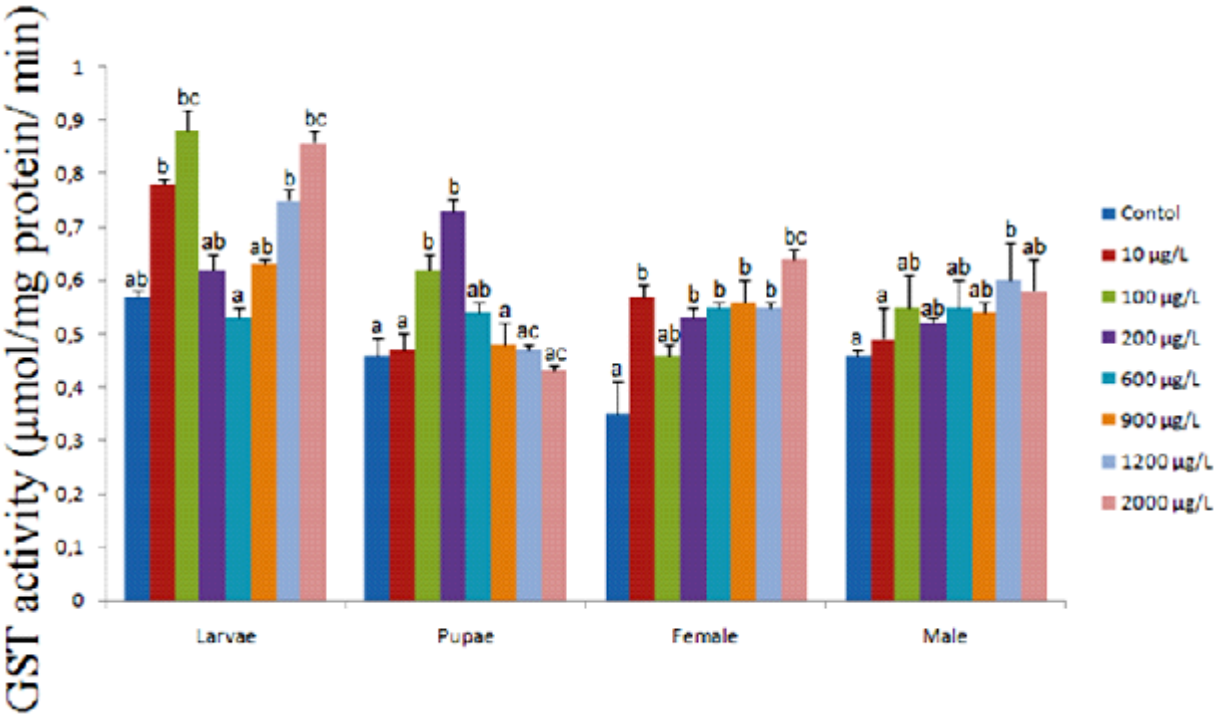

Figure 3. The Tarragon effects of GST activity were indicated on larvae, pupae and adults (female and male) of *D. melanogaster*. Samples with increasing concentration of Tarragon: control (0.00 mg/L); 10 µg/mL; 200 µg/mL; 600 µg/mL, 900 µg/mL, 1200 µg/mL and 2000 µg/mL. Each histogram bar represented the mean of four replicates (± S.E., n=20) in each of treatment groups.

Table 1. Approximate composition of Tarragon /100g of edible portion (Farrell, 1990)

Energy (kcal)	295	
Protein (g)	22.8	
Fat (g)	7.2	
Total carbohydrates (g)	50.2	
Minerals (mg)	Calcium	1139
	Fe	32
	Mg	347
	P	313
	K	3020
	Na	62
	Zn	4
Vitamins (mg)	Riboflavin	1
	Niacin	9
	Vitamin A (IU)	4200
Fibre (g)	7.2	

Table 2. The chemical constituents of the *A. dracunculus essential oil* (Sayyah *et al.,* 2004)

Component	Kovats' index	Content (%)
á-Pinene	922.7	5.1
â-Pinene	959.5	0.8
Limonene	1015.5	12.4
á-*trans*-Ocimene	1026.7	20.6
á-Terpinolene	1069.2	0.5
Allo ocimene	1113.4	4.8
trans-Anethole	1195.3	21.2
Bornyl acetate	1259.4	0.5
Methyl eugenol	1364.8	2.2
Bicyclogermacrene	1470.2	0.5

activities. As can be seen in Figure 2 and 3., minimum LPO levels and detoxificatin activities were observed in females, and these parameters maximum levels observed in the third instar larvae of *D. melanogaster*.

DISCUSSION

The experimental organisms are influenced by nutrition, genotype, age, and various aspects of the environment. Nutrition has influences on development, fertility, longevity, immune defense in variety of animals (Piper *et al.,* 2005; Unckless *et al.,* 2015). *D. melanogaster* is suitable for experimental design for detailed nutritional studies, and it provides an overview (Piper *et al.,* 2005). A great deal of literature has been published concerning the effects of nutritions, quantitative nutritional requirements, food or dietary restrictions, diet interaction drive phenotype and etc. on *Drosophila* (Sang, 1956; Piper *et al.,* 2005; Reed *et al.,* 2010; Sisodia and Singh, 2012; Wong *et al.,* 2014; Unckless *et al.,* 2015).

Many species of *Artemisia* plants (Compositae) have been identified and they are known to have pharmaceutical (treatment, drug, antioxidant, antitumor, antifungal) and industrial properties (Zani *et al.,* 1991; Meepagala et al., 2002; Ribnickya *et al.,* 2004; Sayyah *et al.,* 2004; Kordali *et al.,* 2005; Emami *et al.,* 2009; Shahriyary and Yazdanparast, 2009; Hatami *et al.,* 2014). Tarragon, also known as *A. dranculus*, has been safely and widely used as a food in Central Anatolia (seasoning, salads, vinegar etc.). Some studies have shown that it has a safe use as a dietary supplement or in functional foods (Ribnickya *et al.,* 2004; Kordali *et al.,* 2005). *Drosophila* needs of the salts such as K, O, Mg, Na (Sang, 1956), and these materials are available in sufficient amounts for tarragon-feeding. It has been shown that the LD_{50} for Tarragon is greater than 2000 µg/L on different developmental stages of *D. melanogaster*. Previous studies have indicated that toxic effect of Tarragon is started especially in higher concentrations (Bakkali *et al.,* 2008; Emami *et al.,* 2009; Güneş, 2014). Similar studies have been concluded that some *Artemisia* species (for example *A. absinthium*) are toxic for developing insect larvae such as *M. domestica* and *D. melanogaster* (Bezzi and Caden,1991; Mihaljilov-Krstev *et al.,* 2014). Because of this feature, it may be effective on insecticidal and radical scavenging activity (Saadali *et al.,* 2001; Parejo *et al.,* 2002; Sayyah *et al.,* 2004).

The amount of nutrients and supplements consumed by organisms a strong impact on stress and resistance (Sisodia and Singh, 2012). The crude plants were evaluated for pesticidal activity and used in pest management to adults, *Artemisia* essential oil was tested in larvacidal, insecticidal activities against house flies (Hifnawy *et al.,* 2001;

Soliman, 2006; Ebadollahi, 2008; Tani *et al.,* 2008). Several studies have demostrated that the tarragon toxic effects are dose (concentration) dependent (not linear) and diminishes rapidly at low exposures that levels can be detoxified by organisms. This is concentration depent effect in range of 10 through 2000 µg/mL. A similar effect observed for some other studies (Azaizeh *et al.,* 2007; Emami *et al.,* 2009). In previous studies, some monoterpens (The most abundant essential oil in Tarragon) have protective effects, cytotoxic (at 1.6 mg/mL) and genotoxic/antigenotoxic (Sayyah *et al.,* 2004; Fernandes *et al.,* 2013). Concentration-dependent of tarragon increase in GST activities may not be able to protect the organism beyond a particular limit. Therefore, it seems that the detoxified effects of *A. dracunculus* may be related dose–response relationship in developmental phases. In addition, *trans-anethole* is the main component of tarragon oil. Its esential oils have low toxicity (Sayyah *et al.,* 2004), and this finding may support the low toxicity of our feeding experiments.

A potential source of cellular damage associated with nutrient is through the production of reactive oxygen species (ROS) and respiration mechanism (under aerobic or anaerobic conditions) (Sies *et al.,* 2005). So, the physiological or biological condition of an organism under this stress factors (metabolic and environmental oxidative stress, photooxidative stress, drug-dependent oxidative stress, or nitrosative stress etc.) can be assessed using different biochemical (like antioxidant enzyme activities) and molecular markers (Sies, 2000; Siddique *et al.,* 2007). Some pathways (JNK) and enzyme systems (GST, SOD etc.) can protect fruit flies against oxidative damage. *Drosophila* possess both enzymatic and non enzymatic defenses to cope with reactive oxygen species (ROS) such as Catalase (CAT), Superoxide dismutase (SOD), Reduced glutathione (GSH), Glutathione reductase (GR), GST, Disulfide reductase, Methionine sulfoxide reductase (MSR) and Thioredoxin peroxidase (TRXP) (Moskovitz *et al.,* 1997; Missirlis *et al.,*2003; Valko *et al.,* 2006; Siddique *et al.,* 2007). Insects exploit a series of antioxidant and detoxification enzymes such as GST that may form a combined response to chemicals or food supplements (Felton and Summers, 1995; Krishnan *et al.,* 2007) and MDA is an indicator of cellular oxidation (Shahriyary and Yazdanparast, 2009). The determination of MDA content was often accompanied with a measurement of GST or SOD activity (Lei *et al.,* 2014). For example, Fennel was contributed to the daily antioxidant diet (Shahat *et al.,* 2011; Amkiss *et al.,* 2013). Inorganic insecticides, plant esential oils or food supplements lead to oxidative stress and altered GST activities and MDA content in virtual tissues (Hyrsl *et al.,* 2007; Ebadollahi, 2008). Some researchers have shown that 0.8 and 4 mg/mL of hawthorn extracts (increased CuZn-SOD, CAT enzyme activity but decreased MDA levels; Rosemary extract (1-5 mg/mL) can improve the antioxidant enzyme activity (SOD, CAT), inhibit the lipid peroxidation (MDA) in *Drosophila* (Zhang *et al.,* 2012; Zhang *et al.,* 2014). *Kunlun Chrysanthemum* flowers (China herb) have shown antioxidative effect (improved SOD activity and decreased MDA content) feeding with 0-0.6 % doses on *Drosophila* (Jing *et al.,* 2015). We infer from these findings that Tarragon influences life history parameters of *D. melanogaster*. The results indicate that the diet containing the highest tarragon concentration led to increased MDA content and GST activity but not of the pupal stages in whole body and the effect was dose dependent. MDA contents increased in pupal stages, probably caused by the use of the lipid storage as in Lepidoptera (Warbrick-Smith et al., 2006). Tarragon exhibited low toxicity to the adult stages and higher toxicity to the larval and pupal stages. Previous studies shown that oxidative effects of a dietary supplements on development depends on its interaction with feeding for instance *Artemisia ssp.* (49 mg/mL) is toxic for developing insect larvae after 15 days (Mihaljilov-Krstev *et al.,* 2014), because the flies are fed in adult and larval stages. Feeding can affect developmental stages such as growth and reproduction, and larval nutrition may affect a range of different stages as well as response to cellular stress in adult (Sisodia and Singh, 2012). Normal growth and development are suspended during stress (Tettweiler *et al.,* 2005), the dietary supplements such as essential oil also affected by the development of insect larvae and delayed achievement of the pupal stage

(Mihaljilov-Krstev *et al.,* 2014). In addition, the high Tarragon exposure demonstrated to induce an increase in oxidative stress, including an increase in MDA and decreases in GST activities. Because the level of MDA content and GST activity reflects the level of cells attacked by free radicals and oxygen free radical scavenging ability (Lei *et al.,* 2014).

Tarragon was known with numerous polyphenols compounds such as phenyl carboxylic acids, flavonoids and coumarins (Obolskiy *et al.,* 2011; Pirvu *et al.,* 2014). It was also noted that the females MDA content and GST activity was concomitant increased compared to the control, as in similar studies (Navarro *et al.,* 2010). Polyphenols shows antioxidant features such as eugenol that is induced phase 2 antioxidant enzymes; *A. dracunculus* polyphenolic compounds used for preventing the diseases (Alma *et al.,* 2003; Miguel *et al.,* 2003; Scalbert *et al.,* 2005; Govorko *et al.,* 2007; Kim *et al.,* 2014). Some studies showed that plant compounds have antioxidant potential (El-Massry *et al.,*2002; Kim *et al.,* 2014). For example, females and males of *Drosophila* were fed either containing curcumin and supplemented at 0.5-1.0 mg/g of diet, MDA levels decreased and SOD activity increased in both diets (Shen *et al.,* 2013). It was highlighted in another study, black garlic extracts were possessed strong antioxidant capacity in vitro in a dose-dependent manner and the content of MDA was decreased by improving SOD and CAT activities (Lei *et al.,* 2014). Thus, it might have prevented the toxicity related disorders by feeding high concentration of tarragon. Furthermore, if the food contains a high concentration of plant, MDA contents will increase, and this is probably caused by starvation or malnourishment, because insects are changing their feeding behaviour in response to prevent oxidative damage (Povey et al., 2009). Also positive correlation has a ratio between lipid content and starvation resistance among individuals of *Drosophila* (Sisodia and Singh, 2010).

The results indicate that the effectiveness of tarragon as an oxidative stress agent in *D. melanogaster* is dependent on its concentration in the fly's diet. This data suggests that adverse effects at lower levels (antioxidant activities) of daily exposure would not be expected and it would be taken through food chains on directly non-target organisms. It is belived that these increases in lipid peroxides are probably due to an tarragon accumulation. This work will serve as a point for studies seeking to understand the usage of tarragon as a nutrition in *Drosophila* whose nutrient-related signalling pathways are known to be similar with mammalian.

ACKNOWLEDGMENTS

A part of this article was presented at the 62nd annual Meeting of the Entomological Society of America. We also thank Department of Food Engineering, Faculty of Engineering-Architecture, Necmettin Erbakan University, Konya, Turkey for their support in this collaborative research.

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(Received 17 October 2015; Accepted 08 February 2016; Published 31 March 2016)

ENTOMON 41 (1): 39-46 (2016)
Article No. ent. 41105

New record of *Erianthus deflorata* (Brunner von Wattenwyl) with notes on *Xenerianthus affinis* (Westwood) [Orthoptera: Eumastacoidea: Chorotypidae) from Meghalaya, India

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ABSTRACT: Morphological characterization of the two species of monkey grasshoppers, *Xenerianthus affinis* (Westwood, 1843) and *Erianthina deflorata* (Brunner von Wattenwyl, 1893) collected from the northeastern state of Meghalaya, India is given. *Erianthina deflorata* has been recorded for the first time from India. A key to separate the two genera is also provided.

KEY WORDS: Erianthinae, *Xenerianthus*, *Erianthina*. New record

INTRODUCTION

Grasshoppers of the family Chorotypidae (super family Eumasticoidea), also termed as monkey grasshoppers, are sub-aerial herbivores of angiosperms and differ from the Acridoidea morphologically in having a head raised above the level of thorax, very short antennae, absence of abdominal tympani, wings (when present) widened distally and a laterally spread posture of the hind legs at rest in a majority of species. The Eumastacoidea known to be worldwide in distribution are predominantly tropical. They are entirely absent from Europe, New Zealand and Antarctica and have been considered an early branch of the Caelifera, a view confirmed by molecular systematic investigations which place them after the Tridactyloidea and Tetrigoidea, but before the remaining superfamilies (Flook and Rowell, 1997, Rowell and Flook, 1998, Flook *et al*., 1999).

The genus *Erianthus* is distributed in Indo-Malaysia and Africa; the subfamily Erianthinae is restricted to the Indo-Malaysian region (Descamps, 1973). The genus *Erianthus* Stal, 1875 was considered a heterogenous assemblage of species (Bolivar, 1930). Descamps (1975) divided this genus into ten genera and recognized ten species in *Erianthus* (*s. str*.); since this revision, one additional species has been described (Descamps, 1981). Earlier, Kirby (1914) had recorded 9 species of *Erianthus* from the Indian sub-continent; Ingrisch and Willemse (1988) revised the genus *Erianthus* reporting 10 species from Thailand and 2 additional species from Malaysia. Erianthinae subfamily includes 12 genera and 42 species (Eades *et al.,* 2015).

MATERIAL AND METHODS

The monkey grasshoppers were collected from the northeastern state of Meghalaya, India during June, 2013 under the Indian Council of Agricultural

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Research (New Delhi) sponsored Network Project on Insect Biosystematics unit at Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan. All specimens were either collected with a sweep net or hand-picked during early hours of the day and late at night, under illumination. Digital photographs of specimens and their body parts were taken with the help of Stemi 2000 C Stereozoom binocular microscope of Carl Zeiss make; the software used for linear measurements was Axio Vision L.E. 4.5. Line drawings were made with the help of a drawing tube attachment of Nikon SMZ 1500 binocular microscope. The type specimens are deposited in the Department of Entomology, Rajasthan College of Agriculture, MPUAT, Udaipur, and the University of Agricultural Sciences, GKVK, Bangalore.

RESULTS

Tribe Erianthini

Subfamily ERIANTHINAE Karsch, 1889: 27.

Genus ***Butania*** Bolivar, I., 1903: 303.

Butania lugubris major Bolivar, C., 1930: 143.

Genus ***Khaserianthus*** Descamps, 1975: 92, 109.

Khaserianthus acutipennis (Saussure, 1903: 78)

Genus ***Xenerianthus*** Descamps, 1975: 92, 94.

Xenerianthus affinis (Westwood, 1843: 54)

Key to the genera of Erianthini from India

Fastigium of vertex raised; vertex appears bifid in frontal view; head compressed, straight, pronotum lobate …….. *Xenerianthus*

Fastigium of vertex raised; vertex acuminate in frontal view; slant headed, compressed; pronotum not lobate ……...…….*Erianthina*

Genus *Xenerianthus* Descamps, 1975

Erianthus, Stål, Bih. Svensk. Akad. Handl. iii (14), 1875, p. 36; Brunner, Abh. Senckenb. Ges. Xxiv, 1898, p. 221; Burr, Gen. Ins., ortho. Eumast. 1903, pp.6, 7., Saussure, Rev. Suisse Zool. Xi, 1903, pp. 75,77. Type species: *Mastix affinis* Westwood, by original designation.

Fastigium of the vertex erect, tapering, with the tip straight or slightly reflexed, and obtuse; front flattened, rugose, with a smooth dilated ridge between the antennae. Pronotum smooth, slightly raised, truncated in front, obtusely produced behind, and longitudinally carinated. Tegmina narrow, broader towards the extremity, with a few veins, and more or less sub-hyaline. Wings triangular, sub-hyaline, not longer than the tegmina. Femora slightly compressed, carinated above, and produced into a tooth behind; hind femora slender, serrated above; hind tibia with 20-25 equal spines on the inner carina, and 25 on the outer; first joint of hind tarsi sulcated above, and dentated on the outer carina. Abdomen with the 8th segment expanded in the male, and the anal appendages very large; in the female bifid at the extremity, and grooved on each side; lower valves with the basal plates smooth and punctured, and the upper border dilated.

***Xenerianthus affinis* (Westwood, 1843)**

[**Plates: I; Fig. 1 - 7**]

Mastax affinis, Westwood, Arcana Ent. Ii, 1834, p. 54, note.

Erianthus acutecarinatus, Brunner, Ann. Mus. Genova, xxxiii, 1893, p. 117, pl. v, fig. 48; Saussure, Rev. Suisse Zool. Xi, 1903, pp. 78, 80, pl. iii, fig. 11.

Xenerianthus affinis : Descamps 1975 : 95.

Xeneriathus affinis (Westwood, 1843)

Material examined: (15 Specimens, 6 ♂ & 9 ♀) Meghalaya: North Khasi Hills, East Khasi Hills: 9.VI.2013, Coll. Jhabar Mal (Boirymbong); 9. VI.2013, Coll. Rajendra Nagar (Boirymbong); 3.VI.2013, Coll. Jhabar Mal (Umiam); 9.VI.2013, Coll. R. Swaminathan (Boirymbong); 3.VI.2013, Coll. R. Swaminathan (Ri-bhoi); 8.VI.2013, Coll. Rajendra Nagar (Upper Shillong); 9.VI.2013, Rajendra Nagar (Boirymbong); 5.VI.2013, Coll.

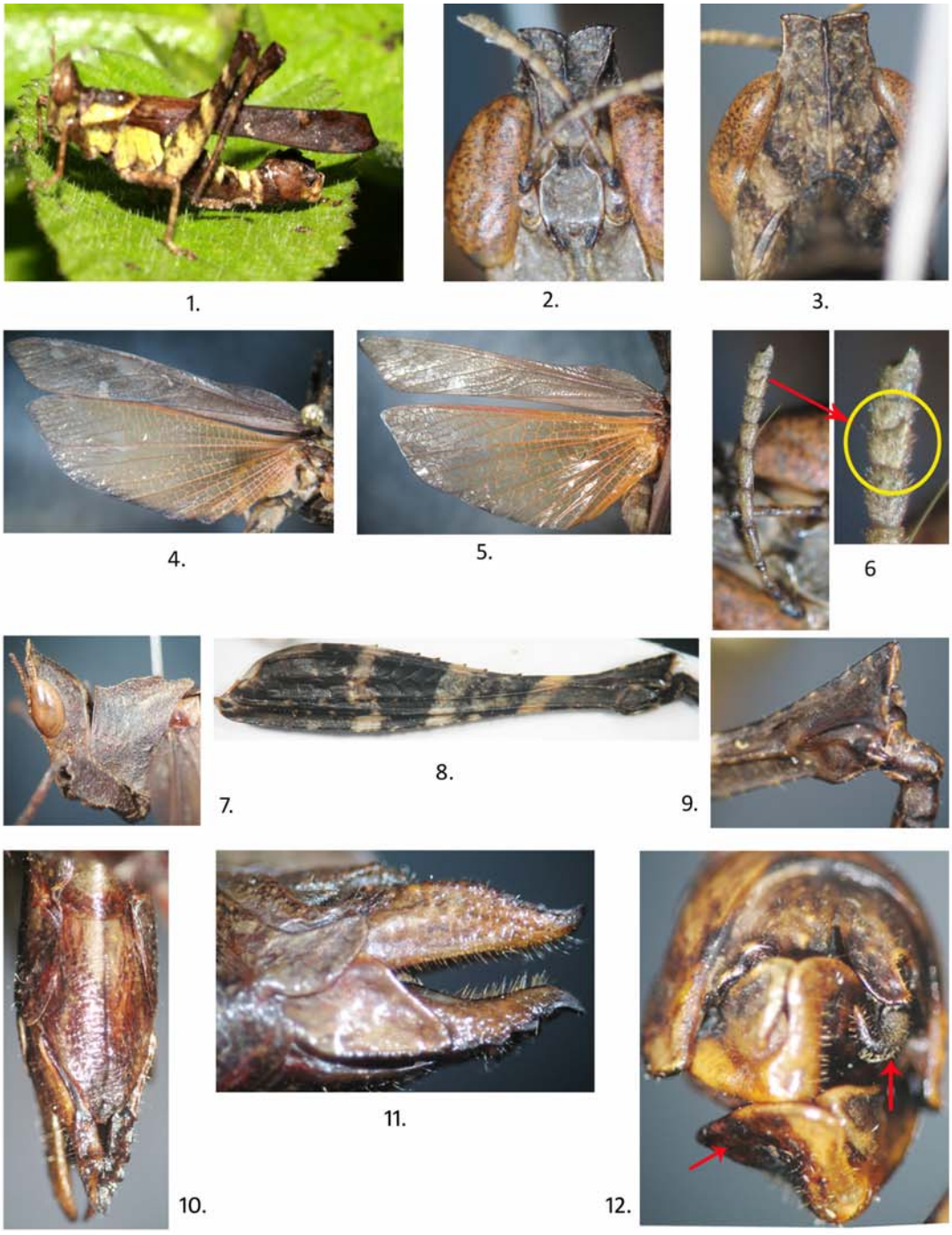

Plate I: *Xenerianthus affinis* (Westwood)

1 -1 2: 1, Male habitus; 2, Head frontal view; 3, Head rear view; 4, Wings male; 5, Wings female; 6, Antennae; 7, Pronotum; 8, Hind femur banded; 9, Hind knee; 10, Subgenital plate female; 11, Female last abdominal segment bifid at the extremity, grooved on each side; lower valves with basal plates smooth and punctured; 12, Male abdomen with the 8th segment expanded and the anal appendages large

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Plate II: *Erianthina deflorata* (Brunn.), male

1 - 9: 1, Head frontal view; 2, Head rear view; 3, Antenna; 4, Pronotum; 5, Wings 6, Tegmina apex; 7, Hind knee; 8, Hind femur; 9, Male tenth tergite split, sub-genital plate large and curved, reaching the tenth tergite.

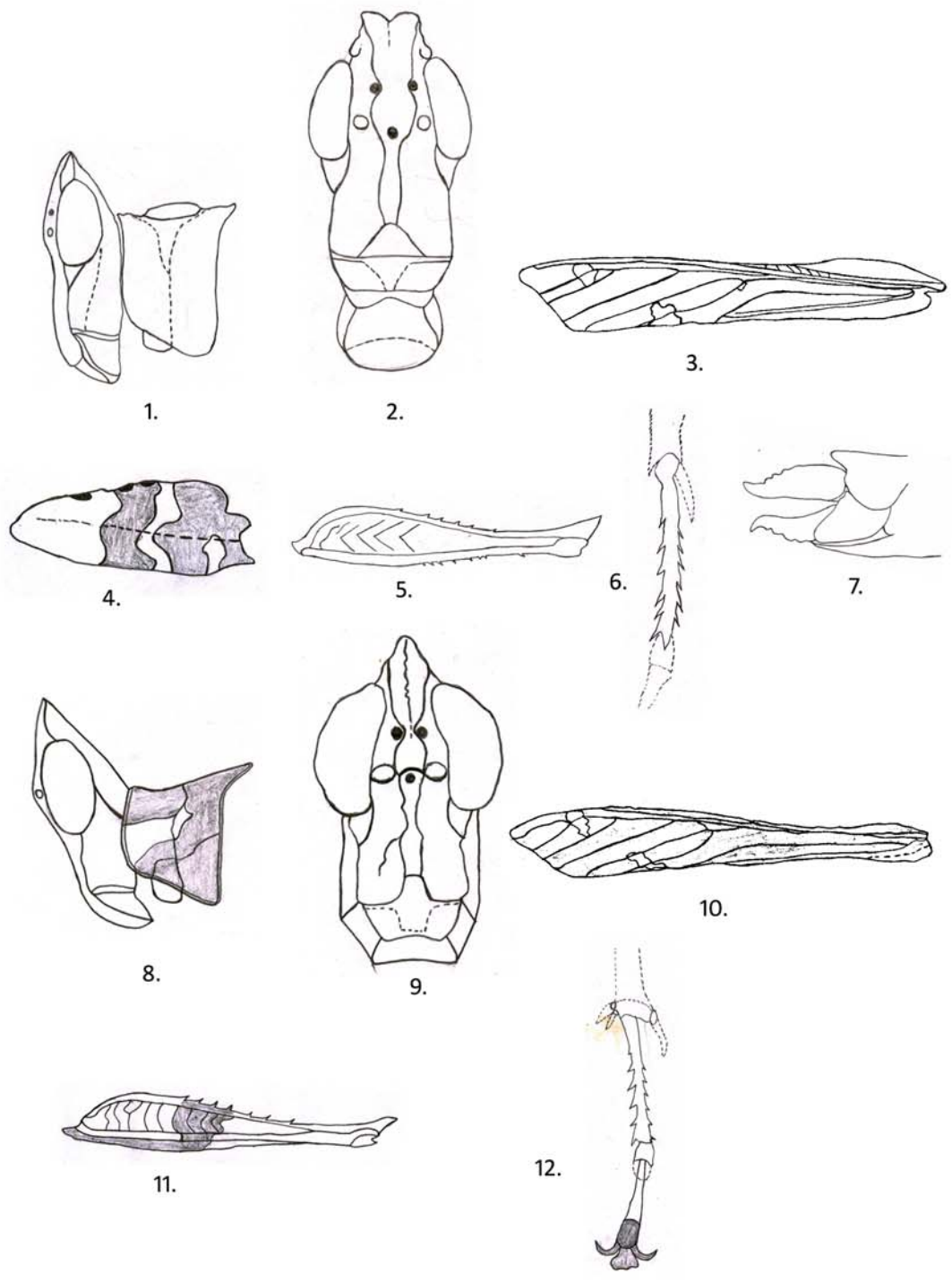

Fig.: 1-7 *Xenerianthus affinis* (Westwood): 1. Head with pronotum lateral view 2. Head frontal view 3. Tegmina 4. Anterior femur 5. Hind femur 6. Hind tarsus 7. Ovipositor

Fig.: 8-12 *Erianthina deflorata* (Brunn.): 8. Head with pronotum lateral view 9. Head frontal view 10. Tegmina 11. Hind femur 12. Hind tarsus

R. Swaminathan (Jowai); 7.VI.2013, Coll. Rajendra Nagar (East Khasi Hills); 8.VI.2013, Coll. Jhabar Mal (Mawflang); 9.VI. 2013, Coll. Yeshwanth (Ribhoi); 8.VI.2013, Coll. Yeshwant (Umiam).

Brown, inclining to rufous, fastigium of the vertex erect, very broad, and more or less bifid at the extremity. Pronotum rugose with a high irregular median carina. Tegmina with ferrugenous network, the spaces between sub-hyaline, especially above the principal nervure, and an oblique whitish stripe at about four fifths of the inner margin, running towards the tip. Wings fulvo-hyaline, with ferrugenous nervures, and a narrow brown hind margin. Abdomen ferrugenous brown, especially at the extremity. The upper appendages of the female are finely serrated and the lower appendages have three small teeth before the extremity. The male has a small white spot towards the apex of the tegmina. Femora blackish, strongly compressed and laminate- carinate above and below; hind femora with three white bands, and the upper carina terminating in a sharp triangular tooth.

Genus *Erianthina* Descamps, 1975

Descamps. 1975. Ann. Soc. ent. Fr. Nouvelle série 11(1):130 >> Note: Erianthinae > *Erianthina* urn : lsid : Orthoptera.speciesfile.org:TaxonName:41888

Otte, D. 1994. Orthoptera Species File 2:9 >> *Erianthina*

Yin, X.-C., J. Shi & Z. Yin. 1996. Synonymic Catalogue of Grasshoppers and their Allies of the World (Orthoptera: Caelifera) 791 >> *Erianthina*

Type species: *Erianthina kalawensis* Descamps, by original designation

***Erianthina deflorata,* Brunn.**
[Plate: II; Fig. 8 - 12].

Erianthus defloratus, Brunn. Ann. Mus. Genova, xxxiii, 1893, p. 116; id., Abb. Senckenb. Ges. Xxiv, 1898, pp. 222, 224; Saussure, Rev. Suisse Zool. Xi, 1903, pp. 78, 81.

Erianthus birmanicus Saussure 1903: 82. Synonymised by Descamps 1975: 132.*Erianthina deflorata*: Descamps 1975: 134.

Material examined: (5 Specimens, 2 ♂ & 3 ♀) Meghalaya: North Khasi Hills: 5.VI.2013, Coll. Jhabar Mal (Jowai); 4.VI.2013, Coll. Rajendra

Table 1: Measurements of different body parts of male monkey grasshoppers

Linear measurements (mm)	*Xenerianthus affinis*	*Erianthina deflorata*
Antennae	3.12	2.76
Tegmina length	16.58	19.71
Tegmina width	2.03	2.25
Wing length	18.27	18.96
Body up to genitalia tip	21.62	24.15
Body up to wing tip	29.52	18.31
Pronotum	3.10	2.95
I Femur	3.44	3.77
II Femur	3.70	3.80
III Femur	11.97	12.58
I Leg	9.61	10.14
II Leg	10.23	11.16
III Leg	28.15	29.23

Nagar (East Khasi Hills); 5.VI.2013, Yeshwanth (Umiam); 6.VI.2013, Yeshwanth (Mawflang); 8.VI.2013, Yeshwanth (Upper Shillong); 9.VI.2013, Yeshwanth (Ri-Bhoi).

Chestnut – brown, face olive, sides of pronotum often yellowish. Fastigium of the vertex obtuse, carinated. Median carina of pronotum acute, but not lobate. Tegmina brown, more or less sub-hyaline towards the base, and with or without a sub-hyaline spot at three- quarters of the inner margin; wings yellowish. Hind femora unspotted.

The measurements of different body parts in the males of the two species are given in table 1.

ACKNOWLEDGMENTS

The authors gratefully thank Dr. V. V. Ramamurthy, Principal Scientist, Entomology and National Coordinator, ICAR Network Project on Insect Biosystematics, IARI, New Delhi for inspiration, guidance and funds; the Director Research, MPUAT, Udaipur, Rajasthan for providing all facilities and encouragement to carry out the work. We sincerely thank Dr. C.A. Viraktamath, Professor Emeritus and Principal Investigator of Network Project, GKVV, Bangalore for loaning us the monkey grasshopper specimens collected by Dr. Yeshwanth for study.

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(Received 22 September 2015; Accepted 19 December 2015; Published 31 March 2016)

ENTOMON 41 (1): 47-60 (2016)
Article No. ent. 41106

Many-fold less than the field recommended concentrations of neonicotinoids and malathion affect foraging of honeybee in three important crops in India

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ABSTRACT: Although insecticides effectively control the insect pests in different agro-ecosystems, they also reportedly affect the non-target insects including bee pollinators at the sub-lethal concentrations. A series of field experiments were conducted to evaluate the effects of two neonicotinoids and one organophosphate insecticide on the foraging activity of honeybee in three test crops at the sub-lethal concentration during flowering. The mean number of the dwarf honeybee (DHB), *Apis florea* (F.) (Hymenoptera: Apidae) recorded during the pre-spraying did not differ between treatments on each of the three crops. However, it differed significantly during the post-spraying except for malathion on inflorescences of the pearl millet. The DHB foraging time remained generally constant during the pre-spraying and varied greatly during the post-spraying on the three test crops and both groups of insecticides. The neonicotinoids and-malathion significantly reduced visits of the DHB on the inflorescences of the test crops, their foraging activities and time spent on the inflorescences at the concentration many-fold (5-50 fold) less than the field recommended concentration of the insecticide.

KEY WORDS: Neonicotinoids, imidacloprid, thiamethoxam, malathion, honeybee, *Apis florea*

INTRODUCTION

Insect pollination accounts for about 75 per cent of cultivated crops (Klein *et al.*, 2007) and 80 per cent of wild plant species (Potts *et al*., 2010) that help in production of seeds and fruits. Of the insects, honey bees are the important pollinators. Despite potential role of honey bees in maintaining agro-ecosystems, the insecticides which are needed for the control of harmful pests to enhance crop productivity threaten their role as pollinators even at sublethal concentrations (Desneux *et al.,* 2007, Feltham *et al.,* 2014). Insecticides are globally used for crop protection to the extent of about two million tons per year, of which 24 per cent is in the USA alone, 45 per cent in Europe and 25 per cent in the rest of the world including India (De *et al.,* 2014). Although their adverse effects on insect pollinators are suspected, the newly discovered neonicotinoids became a chief target in view of their high contact toxicity to bees and persistence in agro-ecosystems. In India, neonicotinoids such as imidacloprid, thiamethoxam, nitenpyram and the sulfoximine, sulfoxaflor are registered for pest control. Neonicotinoids are systemic insecticides. These are extensively applied by seed dressing (Halm *et al.*

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2006). Besides, these are also sprayed during crop growth. These are specifically used for the control of various sucking insect pests in India (Jeyalakshmi *et al.,* 2011, Mandal *et al.,* 2012, Gavkare *et al.,* 2013). It is reported that their residues are translocated to nectar and pollen, thereby affecting the pollinators coming in contact with the inflorescence. The neonicotinoids have the same target as neuron transmitter, acetycholine, and acting as agonist at nicotinic acetycholine receptor in the post-synapse during impulse transmission in an insect nerve, thus influencing neural behaviour (Schmuck *et al.,* 2003, Elbert *et al.,* 2008, Yang *et al.,* 2008).

Adverse effects of neonicotinoids reported as early 2001 are alleged to have caused bee decline (Jones *et al.,* 2006). This has led to either ban or restriction on their uses in some European countries (Kindemba 2009). For example, neonicotinoids affected waggle dance in the forage bees (Eiri and Nieh, 2012) and also decreased bee avoidance of predators (Tan *et al.,* 2014). Imidacloprid at the field-realistic concentrations decreased bee foraging activity (Decourtye *et al.,* 2004, Schneider *et al.,* 2012) and the ability of bees to successfully return to the nests (Desneux *et al.,* 2007, Henry *et al.,* 2012). Repellency of pollinators to the neonicotinoids at the field-realistic concentration was shown in the baited yellow pan traps tests (Easton and Goulson 2013). Contrary to these adverse reports, there are studies that contradict decline in honeybee population due to neonicotinoid use in the field crops (APVMA, 2014, Fairbrother *et al*., 2014).

Although studies have reported lethal and sublethal effects of neonicotinoids on different aspects of bees, there is little information on the effects of the neonicotinoids and organophosphate, malathion, on honeybee behavior exclusively at the sublethal concentration in the important crops under the field conditions in India. The present study therefore reports the effects of the two neonicotinoids *viz*., imidacloprid and thiamethoxam and organophosphate, malathion on three parameters, i) number of the dwarf honeybee (DHB) (*Apis florea* (F.)) (Hymenoptera: Apidae) visiting the test inflorescences, ii) foraging activities and iii) time spent on foraging during the observation period.

MATERIALS AND METHODS

(i) Experimental Sites

Experiments were conducted in the fields planted with pearl millet (*Pennisetum typhoides*), Indian plum (*ber*) (*Ziziphus mauritiana*) and mustard (*Brassica juncea*) at Indian Agricultural Research Institute (IARI), New Delhi, India. The pearl millet field was located between latitude N 26° 37.9' and longitude E 77° 09.3' and about 224.94 m.a.s.l and the Indian plum field between latitude N 28° 38.8' and longitude E 77° 09.2' and 198.12 m.a.s.l and the mustard field between 28° 38.8' and longitude E 77° 08.2' and 207 m.a.s.l. The mean temperature recorded at the IARI weather station ranged between minimum of 6.7-24.0 ºC and the maximum of 20.4-34.4ÚC from September to December, 2014. Sunrise time varied from 6:05 to 7:05 h Indian standard time (IST) and sunset varied from 17:30 to 18:26 h IST.

The neonicotinoid insecticides tested were imidacloprid17.8% SL, Confidor® (Bayer CropScience Limited, manufactured by Saraswati Agro Chemicals Pvt. Ltd, Jammu and Kashmir, India) and thiamethoxam25% WG, Tagxone™ (Tropical Agrosystem Pvt. Ltd, Chennai, India). Organophosphate insecticide tested was malathion50% EC, Suthion (manufactured by Super Ford Insecticide Limited, Secunderabad, India).

(ii) Evaluation of imidacloprid and malathion in pearl millet

The study was conducted during flowering of the pearl millet (Pusa composite 612) between the 27th September and the 10th October, 2014. The Pearl millet flowering is protogynous, stigmas mature first and its emergence begins near the tip and progress to the base. Its flowering period has been observed to coincide with high incidence of bees visiting inflorescences. Imidacloprid was applied at the selected inflorescences of the pearl millet in a

randomized block design. The effect of the neonicotinoid was determined on three parameters stated previously. The experimental site was divided into five plots, having pearl millet inflorescences of similar in size and flowering intensity. In each plot, five pearl millet inflorescences were selected randomly for the experimentation. One inflorescence at the middle was treated with 5 ppm of imidacloprid at a rate of 100 ml per inflorescence and four neighboring inflorescences were untreated and acted as control treatments (control one to four). The four neighbouring inflorescences were sprayed with 1% emulsifier solution (Triton X-100) and covered by polythene plastic bags to avoid drift of insecticide treatment. The spraying was done by using a 1.5 l Pneumatic Hand Sprayer (ASPEE Agro Equipments Pvt. Ltd, Mumbai, India). The spraying of imidacloprid was done late in the evening from 05:45 h IST. At this time the bee foraging activities were greatly reduced thereby avoiding direct contact with the imidacloprid during the spraying.

Abundance of bees visiting the inflorescences was determined twice per day between 08:00-10:00 and 16:00-18:00 h IST for six consecutive days, three days pre-spraying and three days post-spraying. Five minute observation period was made for each replicate and three evaluation parameters were monitored during the observation period. Six foraging activities were frequently exhibited by the bees. These included i) tasting inflorescence ii) picking nectar from flowers, iii) rasping legs on flowers, iv) transferring pollen to a basket leg (pumping legs), v) collecting pollen by abdomen hairs and vi) rasping flower with mouth parts.

(iii) Evaluation of imidacloprid and malathion in Indian plum

The study was also conducted during flowering of the Indian plum (variety, Umran) between the 26th October and the 8th November, 2014. Unlike the pearl millet, the Indian plum flowering is protandrous, having the anthers come to maturity before the stigmas. The spraying of imidacloprid and malathion was done at the randomly selected branches of Indian plum trees. Thirty branches of Indian plum tree similar in size and flowering intensity were selected randomly. In each treatment, 10 branches of similar size, one from each tree were treated with 5 ppm of imidacloprid at a rate of 200 ml/ branch; another 10 branches were treated with 5 ppm of malathion at the rate of 200 ml/ branch. Further, 10 branches were sprayed with 1% emulsifier solution which acted as control. The spraying time and techniques was similar to the previous study conducted in the pearl millet.

The effect of imidacloprid and malathion was also determined on the three parameters by using the same experimental protocol as before for the pearl millet except for reduction in the observation period to three minutes for each replicate.

(iv) Evaluation of malathion and thiamethoxam in mustard

The study was conducted during the mass flowering of mustard (Pusa mustard-28, 2012) from the 18th November to the 5th December, 2014. Like the pearl millet, the mustard flowering is protogynous, having the stigmas come to maturity before the anthers. The experiment protocol was similar to the previous study carried out in the Indian plum. However, in this field experiment, imidacloprid was replaced by thiamethoxam. The application of malathion and thiamethoxam was done at the floral parts of randomly selected mustard crop plants using the same field spraying protocol used in the previous experiment.

The effect of malathion and thiamethoxam was also determined in the mustard field twice a day for ten consecutive days, three days pre-treatment and seven days post-treatment. Similar to the Indian plum field experiment, three minute observation period was made for each replicate following three evaluation parameters used in the previous experiment.

(v) Determination of potential bee pollinators

To assess the specificity of bee pollinators, sweep net (with lightweight aluminium frame, approx.30 cm diameter and a 0.6 m handle) was used to

sample flower visitors on each crop. A total of 10 sweepings were made in the randomly selected inflorescences in each field studied. The sampled bees were identified to species level and their relative abundance was quantified to determine the most frequent bee species in each field. Identification of bees was done in the Division of Entomology, IARI, New Delhi and voucher specimens were also kept at the same institute.

(vi) Data Analysis

Data were analyzed using SAS 9.3 software. Generalized linear model procedure (GLM) was used for the analysis. Analysis of variance (ANOVA) was used to compare the mean difference between treatments, day and time and their interactions. The parameters of the study were tested separately for each insecticide applied in the pearl millet field and their means were compared between treated inflorescences against each of the untreated inflorescences. The parameters were also tested separately for the three treatments in the Indian plum field (*i.e.* control, imidacloprid and malathion) and in the mustard field (control, malathion and thiamethoxam). Bonferroni correction was used to adjust for multiple mean comparisons. It is the most common way to control the family-wise error rate (SAS Institute Inc. 2008).

RESULTS

(i) Flower visitors

The DHB, *A. florea* was the most abundant flower visitors in all three crops tested. The mean percentage of this species accounts for about 82.67 per cent of all pollinators sampled. It has outnumbered other three species of the family Apidae *viz., Apis dorsata* (F.), *Apis mellifera* (L.) and *Tetragonula iridipennis* (Smith) (Table 1). Beside bees, there were other pollinators visiting the inflorescences of the test crops.

(ii) Abundance of bees on the inflorescences of the test crops

Pearl millet

The frequencies of DHB which visiting the

Table 1: Abundance (%) of the four species of bees sampled from three different experimental sites in IARI, New Delhi

Test crop	Species of bees	No. of individuals	% per crop
Pearl millet	*Apis florea* (F.)	53	77.94
	Apis dorsata (F.)	6	8.82
	Tetragonula iridipennis (Smith)	7	10.29
	Apis mellifera (L.)	2	2.94
Indian plum	*Apis florea* (F.)	50	83.33
	Apis dorsata (F.)	5	8.33
	Tetragonula iridipennis (Smith)	1	1.67
	Apis mellifera (L.)	4	6.67
Mustard	*Apis florea* (F.)	72	86.75
	Apis dorsata (F.)	5	6.02
	Tetragonula iridipennis (Smith)	2	2.41
	Apis mellifera (L.)	4	4.82

inflorescences were not significantly different between treatments prior to the spraying of the two insecticides, *viz.*, imidacloprid and malathion at the test inflorescences (Table 2 and 3). Following the spraying of these insecticides, the mean numbers of DHB did not differ significantly between malathion treated and untreated inflorescences neither morning nor evening (Table 2). The only significance difference in the mean numbers of DHB was between imidacloprid treated and untreated inflorescences ($F_{(1,59)} = 22.26$; $P < 0.0001$). However, there was no significant difference in their interactions: treatment, day and time during the pre-spraying of malathion (Table 2) and imidacloprid (Table 3). A similar trend was also recorded during the post-spraying of malathion and imidacloprid as indicated in the respective tables 2 and 3.

Indian plum

The mean numbers of DHB visiting the *ber* inflorescences was not significantly different between treatments and time during the pre-spraying of both imidacloprid and malathion (Table 4). Following the spraying of these insecticides, the numbers of DHB generally remained similar in the untreated inflorescences throughout the experimental period, but declined significantly in all the treated inflorescences ($F_{(2,179)} = 30.33$; $P<0.0001$) (Table 4). Similar to the Pearl millet experiment, the mean numbers of DHB did not differ significantly in the overall interactions during the pre-spraying and post-spraying of these insecticides (Table 4).

Mustard

There was also no significant difference in the mean numbers of DHB between treatment during the pre-spraying of both malathion and thiamethoxam at the mustard inflorescence. However, the mean numbers of DHB differed significantly between time (Table 5). Following the spraying of these insecticides, the mean numbers of DHB generally remained similar in the untreated inflorescences, but declined significantly in all the treated inflorescences ($F_{(2,179)} = 70.59$; $P<0.0001$). The mean numbers of DHB did not differ significantly in the overall interactions during the pre-spraying and the post-spraying (Table 5).

(iii) Bee foraging activities at the inflorescences of the test crops

Pearl millet

The mean numbers of DHB foraging activities during the pre-spraying was not significantly different between treatments and time at the test inflorescences (Table 2 and 3). The only significant difference in the mean numbers of bee foraging activities between the treated and untreated inflorescences was for imidacloprid, ($F_{(1,59)} = 31.87$; $P<0.0001$). The mean numbers of DHB foraging activities did not differ significantly in the overall interactions during the pre-spraying and the post-spraying of malathion and imidacloprid (Table 2 and 3).

Indian plum

The mean numbers of DHB foraging activities during the pre-spraying of Indian plum inflorescence with imidacloprid and malathion was not significantly different. The high mean numbers of DHB foraging activities was recorded in the untreated inflorescences than in treated inflorescences during the post-spraying experimental period. It varied significantly between the treated and untreated inflorescences ($F_{(2,179)} = 23.81$; $P<0.0001$) and the treatments (*i.e.* control, imidacloprid and malathion) as shown in table 4b. The overall interactions of the number of DHB foraging activities were not significant both during the pre-spraying and the post-spraying (Table 4).

Mustard

Although the mean numbers of DHB foraging activities was significantly different between morning and evening, it was insignificant between treatments during the pre-spraying of mustard with malathion and thiamethoxam (Table 5). The high mean numbers of DHB foraging activities was recorded in the untreated inflorescences than in treated inflorescences during the post-spraying

Table 2: Abundance of bees, foraging activities, time spent (X±SE) and inferential statistics (F and P-values) in pearl millet

	X±SE bees visiting each inflorescence for *5 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control 1	2.6±0.5a	1.6±0.3a	2.6±0.8a	2.0±0.3a	2.6±0.9a	1.6±0.5a	F(1,59)=1.59;P=0.21
Malathion	3.2±0.4a	2.0±0.3a	3.0±0.3a	2.6±0.3a	2.4±0.5a	1.8±0.4a	
F&P-values: Treat*day*time	F(2,59)=0.11;P=0.90						
Post-treatment							
Control 1	1.2±0.4a	1.6±0.7a	1.6±0.4a	2.0±0.5a	1.8±0.2a	1.0±0.3a	F(1,59)=0.07; P=0.79
Malathion	1.4±0.4a	1.4±0.4a	1.6±0.5a	1.6±0.5a	1.6±0.4a	1.2±0.5a	
F&P-values: Treat*day*time	F(2,59)=0.28; P=0.76						
Treatment	X±SE bees' activities recorded in each inflorescence for *5 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	4.8±.04a	3.8±0.4a	4.6±0.5a	5.0±0.3a	3.6±0.9a	3.6±1.0a	F(1,59)=1.59;P=0.21
Malathion	5.2±0.3a	5.0±0.3a	5.0±0.2a	4.0±0.6a	4.2±0.4a	4.6±0.3a	
F&P-values: Treat*day*time	F(2,59)=1.29;P=0.28						
Post-treatment							
Control 1	2.2±1.0a	2.8±0.8a	3.0±0.9a	3.8±0.5a	4.2±0.2a	3.6±0.9a	F(1,59)=1.34;P=0.25
Malathion	4.0±0.8a	3.6±0.9a	4.2±1.1a	4.0±1.1a	4.0±0.3a	3.2±0.9a	
F&P-values: Treat*day*time	F(2,59)=0.07;P=0.93						
Treatment	X±SE time spent by bees visiting each inflorescence during 5 *minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	236.6±38.1a	146.0±19.4a	194.6±48.2a	212.2±44.9a	169.4±54.9a	96.2±29.9a	F(1,59)=2.26;P=0.14
Malathion	234.4±11.1a	161.8±17.9a	221.8±29.4a	207.0±29.6a	213.0±21.1a	180.2±26.4a	
F&P-values: Treat*day*time	F(2,59)=0.35;P=0.70						
Post-treatment							
Control 1	160.4±49.9a	123.4±33.4a	136.2±38.9a	198.8±14.1a	191.4±10.2a	135.4±35.4a	F(1,59)=7.63;P=0.01
Malathion	105.4±37.0b	97.2±31.7b	107.6±29.0b	117.6±33.4b	120.2±34.1b	84.2±33.1b	
F&P-values: Treat*day*time	F(2,59)=0.47;P=0.63						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05 inflorescences pre-and post-malathion treatment at the division of Agronomy, IARI, New Delhi.

Table 3: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting pearl millet inflorescences pre-and post-imidacloprid treatment at the Division of Agronomy, IARI

	X±SE bees visiting each inflorescence for *5 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control 1	2.8±0.4a	1.6±0.7a	2.2±0.5a	2.2±0.05a	2.0±0.6a	2.2±0.6a	F(1,59)=0.95;P=0.34
Imidacloprid	3.0±0.7a	2.0±0.6a	2.4±0.4a	2.4±0.5a	3.2±0.4a	1.8±0.6a	
F&P-values: Treat*day*time	F(2,59)=0.85;P=0.43						
Post-treatment							
Control 1	2.6±0.4a	2.0±0.3a	2.4±0.3a	1.6±0.2a	2.4±0.5a	1.8±0.4a	F(1,59)=22.26;P<0.0001
Imidacloprid	1.0±0.5b	1.0±0.4b	1.6±0.3a	0.8±0.2b	1.4±0.4b	1.2±0.2b	
F&P-values: Treat*day*time	F(2,59)=0.19;P=0.83						
Treatment	X±SE bees' activities recorded in each inflorescence for *5 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	4.2±0.6a	2.2±0.9a	4.2±1.1a	4.2±0.7a	4.0±1.0a	4.0±0.7a	F(1,59)=0.66;P=0.42
Imidacloprid	4.4±0.4a	3.8±0.6a	5.0±0.5a	3.0±0.9a	4.4±0.3a	4.2±0.8a	
F&P-values: Treat*day*time	F(2,59)=1.42;P=0.25						
Post-treatment							
Control 1	4.4±0.3a	3.8±0.5a	3.8±0.4a	3.4±0.5a	3.6±0.9a	3.4±0.9a	F(1,59)=31.87;P<0.0001
Imidacloprid	1.6±0.8b	1.0±0.5b	1.8±06b	1.6±0.4b	2.2±0.7b	2.0±0.6b	
F&P-values: Treat*day*time	F(2,59)=0.01;P=0.99						
Treatment	X±SE time spent by bees visiting each inflorescence during 5 *minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control 1	216.6±28.5a	109.6±55.7a	179.8±18.6a	174.8±39.0a	180.2±47.2a	185.2±22.6a	F(1,59)=0.08;P=0.78
Imidacloprid	197.6±27.0a	166±33.1a	210.6±19.1a	148.4±37.4a	207.2±19.4a	149.0±24.6a	
F&P-values: Treat*day*time	F(2,59)=1.41;P=0.26						
Post-treatment							
Control 1	202.8±6.8a	169.6±13.9a	170.6±24.9a	129.4±16.3a	178.8±15.2a	111.0±18.5a	F(1,59)=94.05;P<0.0001
Imidacloprid	42.0±19.6b	39.6±16.8b	89.6±11.1b	37.2±11.1b	89.6±28.0b	59.0±19.4b	
F&P-values: Treat*day*time	F(2,59)=0.53;P=0.59						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

Table 4: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting *Indian plum* inflorescences pre-and post-imidacloprid and malathion treatments at the Division of Horticulture, IARI

	X±SE bees visiting each inflorescence for *3 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control	3.9±0.3a	3.4±0.2a	4.0±0.3a	3.0±0.3a	3.9±0.2a	2.9±0.2a	
Imidacloprid	3.7±0.3a	3.5±0.2a	3.6±0.3a	3.2±0.3a	4.0±0.3a	3.0±0.2a	F(2,179)=0.02;P=0.98
Malathion	3.8±0.3a	3.3±0.3a	3.5±0.2a	3.4±0.4a	4.2±0.3a	3.0±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.59;P=0.67						
Post-treatment							
Control	3.6±0.2a	3.0±0.3a	3.3±0.3a	3.2±0.2a	2.9±0.3a	3.2±0.3a	
Imidacloprid	2.3±0.3b	1.9±0.2b	2.4±0.3b	2.0±0.2b	1.7±0.3b	1.9±0.3b	F(2,179)=30.33;P<0.0001
Malathion	2.4±0.3b	2.1±0.2b	2.3±0.3b	2.5±0.3b	2.0±0.2b	2.1±0.2b	
F&P-values: Treat*day*time	F(4,179)=0.30;P=0.88						
Treatment	X±SE bees' activities recorded in each inflorescence for *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	4.2±0.2a	3.8±0.3a	3.9±0.2a	3.8±0.3a	4.0±0.2a	3.9±0.2a	
Imidacloprid	4.1±0.2a	3.5±0.3a	3.8±0.2a	3.6±0.3a	3.8±0.2a	3.6±0.2a	F(2,179)=1.12;P=0.33
Malathion	4.2±0.2a	3.7±0.2a	3.9±0.2a	3.7±0.2a	4.1±0.2a	3.5±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.19;P=0.94						
Post-treatment							
Control	3.9±0.3a	3.9±0.3a	4.4±0.3a	3.8±0.1a	4.1±0.3a	3.8±0.3a	
Imidacloprid	2.8±0.3b	2.9±0.2b	3.0±0.2b	3.0±0.3b	2.7±0.4b	3.3±0.4b	F(2,179)=23.81;P<0.0001
Malathion	3.1±0.3c	3.5±0.2c	3.1±0.2b	3.4±0.2c	3.3±0.2c	3.5±0.2c	
F&P-values: Treat*day*time	F(4,179)=0.48;P=0.75						
Treatment	X±SE time spent by bees visiting each inflorescence during *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	83.7±5.3a	78.5±3.5a	79.1±7.6a	84.5±4.0a	81.6±6.5a	78.2±3.2a	
Imidacloprid	84.2±5.0a	79.3±5.9a	83.6±2.8a	83.4±2.7a	82.0±3.5a	80.2±2.9a	F(2,179)=0.48;P=0.62
Malathion	84.8±4.1a	81.3±5.2a	85.0±3.2a	80.9±3.0a	86.0±4.6a	82.7±4.1a	
F&P-values: Treat*day*time	F(4,179)=0.25;P=0.91						
Post-treatment							
Control	84.3±2.2a	76.3±3.0a	79.4±2.5a	78.7±3.9a	76.8±2.5a	76.7±2.3a	
Imidacloprid	60.4±4.4b	53.9±3.0b	63.5±2.4b	51.1±3.2b	51.2±6.6b	55.0±7.0b	F(2,179)=55.12;P<0.0001
Malathion	70.4±5.4c	65.2±2.5c	74.8±4.1c	76.5±4.6c	62.4±1.6c	63.7±2.4c	
F&P-values: Treat*day*time	F(4,179)=0.86;P=0.49						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

Table 5: Abundance of bees, foraging activities and time spent (X±SE) and inferential statistics (F and P-values) visiting mustard inflorescences pre-and post-malathion and thiamethoxam treatments at the Division of Genetics, IARI

	X±SE bees visiting each inflorescence for *3 minutes* duration						
Treatment	Day1[1]	Day1[2]	Day2[1]	Day2[2]	Day3[1]	Day3[2]	F&P-values: Treatment
	Pre-treatment						
Control	5.7±0.2a	1.5±0.3a	5.5±0.3a	1.6±0.2a	5.6±0.2a	1.5±0.3a	F(2,179)=0.68;P=0.51
Malathion	5.4±0.2a	1.7±0.3a	5.1±0.3a	1.5±0.3a	5.3±0.3a	1.6±0.2a	
Thiamethoxam	5.6±0.2a	1.3±0.3a	5.3±0.2a	1.4±0.2a	5.3±0.3a	1.5±0.3a	
F&P-values: Treat*day*time	F(4,179)=0.14;P=0.97						
Post-treatment							
Control	5.4±0.3a	1.6±0.2a	5.4±0.2a	1.7±0.2a	5.2±0.2a	1.1±0.2a	F(2,179)=70.59;P<0.0001
Malathion	3.3±0.2b	0.9±0.2b	3.5±0.2b	1.3±0.2b	4.4±0.3b	0.8±0.2a	
Thiamethoxam	2.7±0.2c	1.0±0.2b	3.1±0.2b	1.2±0.3b	3.5±0.2c	0.9±0.2a	
F&P-values: Treat*day*time	F(4,179)=1.04;P=0.39						
Treatment	X±SE bees' activities recorded in each inflorescence for *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	4.2±0.2a	2.5±0.5a	4.3±0.2a	3.3±0.2a	4.5±0.2a	2.8±0.5a	F(2,179)=0.08;P=0.93
Malathion	4.1±0.2a	3.1±0.4a	4.1±0.2a	3.2±0.4a	4.2±0.2a	3.2±0.2a	
Thiamethoxam	4.5±0.2a	2.5±0.5a	4.5±0.2a	3.2±0.4a	4.5±0.2a	2.8±0.5a	
F&P-values: Treat*day*time	F(4,179)=0.21;P=0.93						
Post-treatment							
Control	4.6±0.2a	3.9±0.2a	4.6±0.2a	4.2±0.2a	4.4±0.2a	3.1±0.6a	F(2,179)=35.09;P<0.0001
Malathion	3.1±0.2b	2.7±0.5b	3.6±0.2b	3.6±0.2b	3.5±0.2b	2.3±0.5b	
Thiamethoxam	2.5±0.2c	2.2±0.3c	3.4±0.2b	2.5±0.5c	3.4±0.2b	2.4±0.5b	
F&P-values: Treat*day*time	F(4,179)=0.60;P=0.67						
Treatment	X±SE time (sec) spent by bees visiting each inflorescence during *3 minutes* duration						F&P-values: Treatment
	Pre-treatment						
Control	79.8±4.1a	44.7±7.5a	76.8±3.6a	54.8±1.3a	80.7±3.8a	45.3±7.7a	F(2,179)=0.26;P=0.77
Malathion	77.8±2.6a	53.8±6.3a	77.9±2.6a	50.7±5.8a	75.0±2.9a	54.8±2.1a	
Thiamethoxam	79.0±4.2a	46.0±7.8a	78.2±4.1a	50.2±5.7a	78.9±4.3a	46.5±8.0a	
F&P-values: Treat*day*time	F(4,179)=0.69;P=0.60						
Post-treatment							
Control	79.1±3.0a	52.9±2.9a	77.0±3.6a	53.5±3.1a	78.5±3.5a	40.6±6.9a	F(2,179)=87.59;P<0.0001
Malathion	48.3±2.5b	35.2±6.0b	54.4±3.2b	42.0±2.8b	56.9±2.9b	30.4±6.7b	
Thiamethoxam	36.3±2.8c	32.6±4.0b	43.2±2.9c	29.9±5.1c	46.9±2.7c	26.9±5.9b	
F&P-values: Treat*day*time	F(4,179)=0.41;P=0.80						

[1&2] Denotes morning and evening, respectively; figures followed by the different letters in a column show significant difference at P<0.05

period ($F_{(2,179)}$ = 35.09; P<0.0001). There was also significant difference in the foraging activities between the treatments (Table 5). The mean numbers of DHB foraging activities did not differ significantly in the overall interactions both during the pre-spraying and the post-spraying (Table 5).

(iv) Bee foraging time recorded in the test crops

Pearl millet

Apart from the DHB foraging activities, their mean foraging time/ observation period was also recorded to determine time spent on the test inflorescences. There was no significant difference in the mean foraging time (seconds) spent by DHB on the Pearl millet inflorescences prior to the spraying of the imidacloprid and malathion (Table 2 and 3). Following the spraying of these insecticides, the mean DHB foraging time varied significantly between malathion treated and untreated inflorescences ($F_{(1,59)}$ = 7.63; P = 0.01). A similar trend was also recorded between imidacloprid treated and untreated inflorescences ($F_{(1,59)}$ = 94.05; P < 0.0001). The overall interactions of the mean time spent foraging were also not significantly different both during the pre-spraying and the post-spraying trial (Table 2 and 3).

Indian plum

There were also no significant differences in the mean foraging time spent by bees on the Indian plum inflorescences prior to the spraying of both imidacloprid and malathion (Table 4). Following the spraying of these insecticides, the mean foraging time declined significantly in the treated inflorescences ($F_{(2,179)}$ = 55.12; P < 0.0001). The mean foraging time was also significant different between the three treatments as indicated in table 4c. However, the overall interactions of the time foraging were also not significantly different both during the pre-spraying and the post-spraying (Table 4).

Mustard

Similar to the mean numbers of DHB foraging activities, the mean time foraging was also significantly different between morning and evening. It was significant between treatments during the pre-spraying of the malathion and thiamethoxam (Table 5). Much more time was spent on the untreated than in treated inflorescences of mustard during the post-spraying period ($F_{(2,179)}$ = 35.09; P<0.0001). There was also a significant difference in the mean foraging time between the treatments (Table 5). Similarly, the mean foraging time did not differ significantly in the overall interactions both during the pre-spraying and the post-spraying (Table 5).

DISCUSSION

The three test crops used in the present study are predominantly cultivated in the States of Rajasthan, Gujarat, Uttar Pradesh and Haryana. Pearl millet is cultivated in about 9.3 m ha and mustard in about 6 m ha. The estimated country-wide area for Indian plum is about 22,000 ha (Radha and Mathew, 2007). The neonicotinoids are highly recommended for the control of insect sucking pests in different crops in India. But these are also found effective against other insect pests. For example, imidacloprid is widely used against shootfly and termites in pearl millet and Indian plum fruitfly, while thiamethoxam is used for the control of mustard aphids (Gavkare *et al.,* 2013, www.cibrc.nic.in). Further, malathion is frequently used for the control of aphid and sawfly in mustard, Indian plum fruitfly and earhead midge in sorghum, which is closely related to pearl millet on the basis of crops group concept (Mandal *et al.,* 2012, www.cibrc.nic.in).

Besides their insecticidal activity, no adverse effects were reported in field studies (Blacquiére *et al.,* 2012, APVMA, 2014). Yet, these are also implicated for adverse effects on the pollinators including honeybees. Neonicotinoids have high contact toxicity to honey bees (Suchail *et al.,* 2001, Iwasa *et al.,* 2004, Bonmatin *et al.,* 2005). Hence, it is expected that these will also affect foraging activity as neonicotinoids are persistent. Decourtye *et al*. (2003) reported chronic and sublethal concentrations of neonicotinoids impairing foraging and learning activities of bees. Similar studies were

also carried out by Aliouane *et. al*. (2008). Similarly, field studies have also reported low visitation rate of bumblebee on the inflorescences of an ornamental shrub, *Rhododendron catawbiense* (L.) (Ericales: Ericaceae) treated with imidacloprid than in the untreated shrub (Maus *et al.,* 2006). Even, dead bees were seen in treated plots but not untreated plots (Maus *et al.,* 2007). Lethal toxicity to honeybee in hive treated with imidacloprid, at dosages reflecting residue levels in the environment was also reported during the *in situ* study conducted in the central Massachusetts, USA (Lu *et al.,* 2012). Easton and Goulson (2013) also reported adverse effects of neonicotinoids on attraction of pollinators towards water using the baited pan traps.

The concern over the adverse effect of neonicotinoids on the pollinators including honey bees was also raised by the Government of India through Department of Agriculture and Cooperation (No. 13001/2013-PP-I, 8th July, 2013) to constitute an expert committee to examine the use of the registered neonicotinoids in the country (Anonymous 2013). These were deliberated and the expert committee recommended studies on the toxicity and foraging activities of the native bees in the different crops which are approved for the application of neonicotinoid insecticides (Anonymous 2014).

The present study was carried out on the adverse effects of imidacloprid and that of malathion at the concentration which was eight-time less than recommended foliar concentration of 40 ppm on pearl millet and 50-time less than the recommended concentration of 250 ppm on Indian plum. Similarly, the adverse effect of thiamethoxam on DHB was studied at the concentration which was five times less than the foliar concentration of 25 ppm recommended on mustard plants (www.cibrc.nic.in). Our study showed decline in visits of DHB to the treated flowers/inflorescences, less foraging activity and time spent on all the treated crops. Creswell (2010) also reported reduced honey bee behaviour between 6 and 20 per cent on sunflower and canola flowers containing low levels of 0.7 and 10 ppb of imidacloprid, respectively for the crops treated at the time of seed sowing. Our results are also in agreement with Feltham *et al.* (2014) who found that foraging ability of bumblebee workers was substantially affected at low residual levels (6 ppb in pollen) in flowers of domestic gardens in Central-belt, Scotland.

Although, present studies indicate adverse effects of neonicotinoids, results strengthen our knowledge base concerning the on-going debate of overall utility of these pesticides. The neonicotinoids are invariably used for seed coating of hybrids of some crops, notably Bt cotton cultivated over the large area of about 11 m ha annually. Similarly, seed coating with neonicotinoids is recommended for other crops like soybean, mustard. However, extent of seed coating in the test crops is limited. Foliar sprays of neonicotinoids and other insecticides are more common in mustard than other two crops. Hence, it appears that foliar/floral sprays of insecticides may have adverse impact on bee pollinators which are found in abundance during flowering in mustard than in any other two test crops. These short-term effects of neonicotinoids on DHB may not impair their long-term ability to pollinate the crops in the agro-ecosystems, as these crops are often cultivated on small farms, with wide temporal distribution of flowering and with infrequent use of insecticides as per needs. Hence, foliar or floral sprays may also provide ample scope for bees to visit nearby fields. At the same time, a precaution is needed that foliar sprays of insecticides may be avoided during intense foraging activities, provided at the same time, harmful pests do not cause substantial loss of productivity. It is essential to conduct a large scale ecosystem-wise analysis of these neonicotinoids on pollination services before arriving at a final conclusion, especially in the Indian context.

ACKNOWLEDGEMENTS

This work was funded by CV Raman International Fellowship under Department of Science and Technology and Ministry of External Affairs (MEA), Government of India, through the Federal of Indian Chambers of Commerce and Industry for the senior author.

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(Received 27 December 2015; Accepted 23 February 2016; Published 31 March 2016)

Entomon 41 (1): 61-66 (2016)
Article No. ent. 41107

First record of South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Tamil Nadu, India

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ABSTRACT: The South American tomato moth (SATM), *Tuta absoluta* is a quarantine pest, native to South America which was detected first time in Maharastra, India in late 2014 and then in Karnataka.The border district of Tamil Nadu was under vigil to monitor the activity through regular surveillance from March 2015 to know the presence of SATM in Dharmapuri district and the occurrence of *T. absoluta* was first noticed in Karimangalam block in the tomato hybrid Sivam. The widely cultivated tomato hybrids Sivam and Sagar were equally susceptible to the SATM with 20-32 per cent leaf damage and 28 - 53 per cent fruit damage. The sex pheromone traps attracted more number of adults per day. The damage was mostly found in the middle and lower leaves and half ripened and ripened fruits. In a single fruit 8-12 holes were noticed during the survey. This is the first report of this pest in Tamil Nadu. Main characteristics of the species are briefly reviewed, with notes on biology, distribution and damage. © 2016 Association for Advancement of Entomology

KEY WORDS: *Tuta absoluta*; Tomato; Quarantine pest; Tamil Nadu

Non-native invasive insect species are a significant threat to biodiversity and their ecological impacts are difficult to reverse. They also affect economic interests particularly within agriculture, horticulture and forestry (Mace and Kunin, 1994). The South American tomato moth (SATM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one such pest, originating from South America, that devastates tomato and closely related solanaceous crops in the world since, 1960's (EPPO, 2005). In 2004, *T. Absoluta* was added by the European and Mediterranean Plant Protection Organization (EPPO) to the A1 List of pests recommended for regulation (pests absent from the EPPO region), and in 2009 was transferred to the A2 list (pests locally present in the EPPO region), 3 years after its arrival in Spain (Urbaneja *et al*., 2007). During 2006–2012, the pest spread rapidly throughout the Mediterranean basin. *Tuta absoluta* is considered a typical invasive species, due to its capacity to develop very quickly in suitable agro- ecological conditions, spreading rapidly in new areas and causing economical damage (Desneux *et al*., 2010).

In India, it was first reported from Pune, Maharashtra during October 2014 (ICAR, 2014) and has rapidly moved across the states and later detected in Karnataka during the *rabi* (November) season of 2014, where, it has become a serious threat to tomato production in both greenhouse and outdoor crops (Sridhar *et al.,* 2014). Since then alert notice was issued by the Indian Council of Agricultural Research to keep vigil on the incidence of *T. absoluta* in different states. As a district

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adjoining to Karnataka, regular surveillance was conducted by scientists of Tamil Nadu Agricultural University in Dharmapuri district particularly in the border blocks. In Dharmapuri district tomato has been cultivated in 4000 hectares in which 40% area is under precision farming.

Tomato plants can be infested from seedlings to mature plants. *T. absoluta* reduces yield and fruit quality, causing up to 100% yield losses in severely infested crops (Arturo *et al*., 2012). The main damage is produced on the leaves and fruits, but inflorescences and stems can also be affected. Larvae of *T. absoluta* feed on the mesophyll of the leaf leaving only the epidermis intact. The galleries produced by young larvae may be confused with those produced by leafminers (*Liriomyza* spp.), but the gallery produced by *T. absoluta* subsequently widens and the damaged tissue dries. In the gallery, the larvae of the moth and its black frass can be seen. The economic impact is reflected by an increase in the cost of tomato production (additional costs for crop protection) and yield loss (lower marketable fruits production), as well as potential loss of markets if it were to become established. It is also very challenging to manage and limit the spread of the pest. Hence, there is an urgent need for domestic quarantine measures to curtail the pest from spreading further to other tomato growing regions of Tamil Nadu. The nature of spread, occurrence, damage potential and management options of *T. absoluta* in Tamil Nadu are discussed in this paper.

Explorative surveys were conducted in the border blocks of Dharmapuri district *viz*., Karimangalam and Palacode where tomato has been grown throughout the year in the district. Subsequently, during June 2015 a survey was conducted in five major tomato growing districts of Tamil Nadu *viz*., Dharmapuri, Krishnagiri, Salem, Coimbatore and Dindigul to assess the extent of spread and damage. Varieties *viz.,* Sivam and Sagar, US800, US1036 and Ruchi are widely cultivated. The 25-30 days old portray seedlings from shade net nurseries were procured and planted in the main field. Periodical surveys were conducted to monitor the incidence of SATM damage in the leaves and fruits. After noticing the initial damage in the field, the leaf damage was recorded at weekly intervals in randomly selected 25 plants. The fruit damage was calculated in the selected plants and also during harvest. The pin hole damaged fruits were sorted out during the harvest and percentage of affected fruits calculated to the total harvest during each harvest. To monitor the adult movement sex pheromone lure of *T. absoluta* from Pest control India Ltd., was installed in the field. Ten traps were installed for 0.4 ha. field. The number of adults collected and counted each day in these traps.

In March 2015, *T. absoluta* incidence was first noticed in the Kollupatti village of Karimangalam block in 45 days old crop. Followed by the detection of *T. absoluta,* 100 per cent damage was recorded in Jittandahalli, Palacode (Block), Dharmapuri District (Table 1). The per cent damage was low (43 %) in Velampatti, Nallampalli (Block), Dharmapuri District. As the leaf miner *Liriomyza trifolii* (Burgess) and *T. absoluta* incidence occurred simultaneously in the field the farmers were unable to distinguish between the damage symptoms. The coalescing of mines in the *T. absoluta* damage was different from that of leaf miner *L. trifolii* damage symptom. Initially *T. absoluta* larvae mines the leaves, later the mines coalesce to become necrotic lesions. The damage was noticed in 30-40 days old crop. The young and unripened fruits are not infested by *T. absoluta*. The damage was noticed mostly in the ripened and semi ripened fruits. The adult movement was noticed during the evening hours. In a single fruit a maximum 8 -12 holes were noticed during the survey. In the half ripened fruits the damage was noticed in the inter lobe and soft regions of the fruit. Recent survey on major tomato growing districts of Tamil Nadu also revealed that the pest has spread rapidly into neighbouring districts and the extent of damage ranged from 20-38 and 30-48 per cent on leaf and fruits, respectively. Maximum leaf (38%) and fruit damage (48%) was recorded in Dharmapuri district (Table 2).

In Sivam hybrid, leaf and fruit damage was 20 – 32 and 28 – 50 per cent, respectively whereas in Sagar it was 20 – 24 and 40 – 53 per cent (Table 3) and

Table 1. Incidence of *T. absoluta* in different villages in two Districts

Villages	Per cent damage*
Kollupatti, Karimangalam (Block), Dharmapuri District	70
Jittandahalli, Palacode (Block), Dharmapuri District	100
Mallupatti, Palacode (Block), Dharmapuri Dt	85
Kottur, Palacode (Block), Dharmapuri Dt	56
Kariappanahalli, Nallampalli (Block) , Dharmapuri Dt	50
Velampatti, Nallampalli (Block), Dharmapuri Dt	43
Jekkari, Kelamangalam (Block), Krishnagiri Dt.	66
Uthanapalli, Shoolagir (Block), Krishnagiri Dt.	70
Haleseepam, Kelamangalam (Block), Krishnagiri Dt.	72
Mettrai, Kelamangalam (Block), Krishnagiri Dt.	72

* Mean of ten fields

Table 2. Incidence of *T. absoluta* in five major tomato growing districts of Tamil Nadu

Districts	Leaf damage (%)	Fruit damage (%)
Dharmapuri	38	48
Krishnagiri	24	32
Salem	20	30
Coimbatore	30	47
Dindigul	35	48

* Mean of ten fields

the damage was almost equal in all the hybrids (Table 4). As the damage is more than 50% during the survey the further spread of this pest in Tamil Nadu will hamper the tomato cultivation. *T. absoluta*, originating from South America, has become one of the key pests of tomato in many South American countries since the 1960s (Garcia and Espul, 1982). It has been listed in the A2 quarantine list of the European Plant Protection Organization (EPPO, 2010). Feeding of the pest on other host plants of the Solanaceae family was also recorded (Pereyra and Sanchez, 2006). The moth can develop very quickly under suitable agro-ecological conditions and can breed 10 to 12 generations a year depending on environmental conditions. Control is extremely difficult once the pest is well established because the larvae are internal feeders. Hence, suitable integrated pest management strategies should be developed to manage *T. absoluta* effectively.

The pheromone traps kept in the field are able to trap 40 – 50 adults on the first day itself with the maximum of 102 adults per trap. The adults are silvery grey to brown with brown to black scales on the forewings. Adults are 6 -7 mm long with a wing span of about 8 -10 mm (Kilic, 2010). Taha *et al*., (2013) revealed that the pheromone baited traps alone recorded 37.44 per cent incidence of *T. absoluta* and concluded that the pheromone traps

Table 3. Damage potential of *T. absoluta* in different tomato hybrids

Tomato hybrids	Leaf damage (%)*				Fruit damage (%)*			
	L1	L2	L3	L4	L1	L2	L3	L4
Sivam	20	22	30	32	33	42	50	28
Sagar	21	24	22	20	40	52	50	53

*Mean of ten replications L – Location

Table 4. Incidence of *T. absoluta* in different tomato hybrids

Tomato hybrids	Per cent damage*
Sivam	82
Sagar	80
US 800	84
US 1036	85
Ruchi	82

* Mean of ten fields

are ideal management option for *T. absoluta* in integrated pest management programmes. In the present survey also the pheromone traps able to trap 40 moths per day. This study also confirms that *T. absoluta* also found in brinjal in Dharmapuri district, Tamil Nadu.

The SATM, *T. absoluta*, is a micro lepidopteran moth recently introduced to India. It has a high reproductive potential. Its main host is tomato, but it also infests other Solanaceae crops. Tomato plants can be attacked from seedlings to mature plants and in severely infested tomato crops it may cause yield losses of up to 100%. To avoid potential damage it is very important to detect symptoms early and especially during the egg to small gallery forming stage. Though the pathway for intensive spreading and dissemination of SATM is not known fully, it is considered that fruit importation might have carried the pest into Tamil Nadu from adjacent states. To manage the pest effectively combine all available control measures including cultural methods such as summer ploughing, removal and destruction of affected portion and installation of pheromone traps @ 6-10/ha may be encouraged and the correct use of registered/ recommended insecticides. As the insect has several other Solanaceae host plants chances are high for its occurrence on other crops, weeds, and wild plants also. Hence, continuous monitoring/ surveillance are required to contain the spread and timely adoption of management practices can further reduce the yield loss.

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(Received 23 November 2015; Accepted 05 January 2016; Published 31 March 2016)

Entomon 41 (1):67-70 (2016)
Short Communication No. ent. 41108

A new species of *Amblyseius* Berlese (Acari: Phytoseiidae) from Kerala, India

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ABSTRACT: During the course of investigation of phytoseiid mites inhabiting medicinal plants in North Kerala, a new species *viz*., *Amblyseius velayudhani* sp.nov. was identified and is described with appropriate illustrations. © 2016 Association for Advancement of Entomology

KEYWORDS: Predatory mite, Phytoseiidae, *Amblyseius,* New species.

Predatory mites are a significant beneficial group on account of their role in the maintenance of pest mite population below economic injury level. Predatory mites of the family Phytoseiidae are recognized as one of the most valuable groups of predators on plant feeding mites, especially spider mites.

The erection of the genus, *Amblyseius* was done by Berlese in 1914. The status of genus *Amblyseius* was made by Chant (1959). Wainstein (1962) again recognized *Amblyseius* and erected 7 subgenera and 8 sections. Genus *Amblyseius* is the largest group under the sub family Amblyseiinae with 400 species described from the world. Out of the 2436 phytoseiids from world, 195 species have been reported from India (Demite *et. al.*, 2014, Mallik *et. al.,* 2010).

The specimens under study were collected from infested leaves of medicinal plants and examined under steriozoom microscope. Mites were picked up with camel hair brush and permanent slides were prepared in Hoyer's medium (Haderson, 2001). The setal nomenclature follows that of Rowell *et.al.,*(1978), Chant and Yoshida-Shaul (1989, 1991) and leg chaetotaxy of Evans (1963). Classification of Phytoseiidae followed is that of Chant and McMurtry (2007). Measurements are in microns showing means and ranges.

The specimens are kept in the P.G. & Research Department of Zoology, Malabar Christian College, Calicut and will be deposited to the National Zoological Collection of the Zoological Survey of India, Calicut, Kerala.

***Amblyseius velayudhani* sp. nov. (Fig 1)**

urn:lsid:zoobank.org:act:9ABEAB36-2CA5-4320-9A01-274CD014789B

Female:

Dorsum: Dorsal shield **370** (350-400) long and **280** (250-290) wide, smooth with 17 pairs of setae and 2 small pores. Measurement of setae: j1-**30** (28-32), j3-**40** (38-42), Z4- **90** (80-95), Z5- **250** (220-255) s4-**100** (92-104), other setae like j4, j5, j6, J2, J5, z2, z4, z5, S2, S4, S5, r3 and R1 are minute. Distance between j1-**10** (9-11), j3-**40** (35-45).

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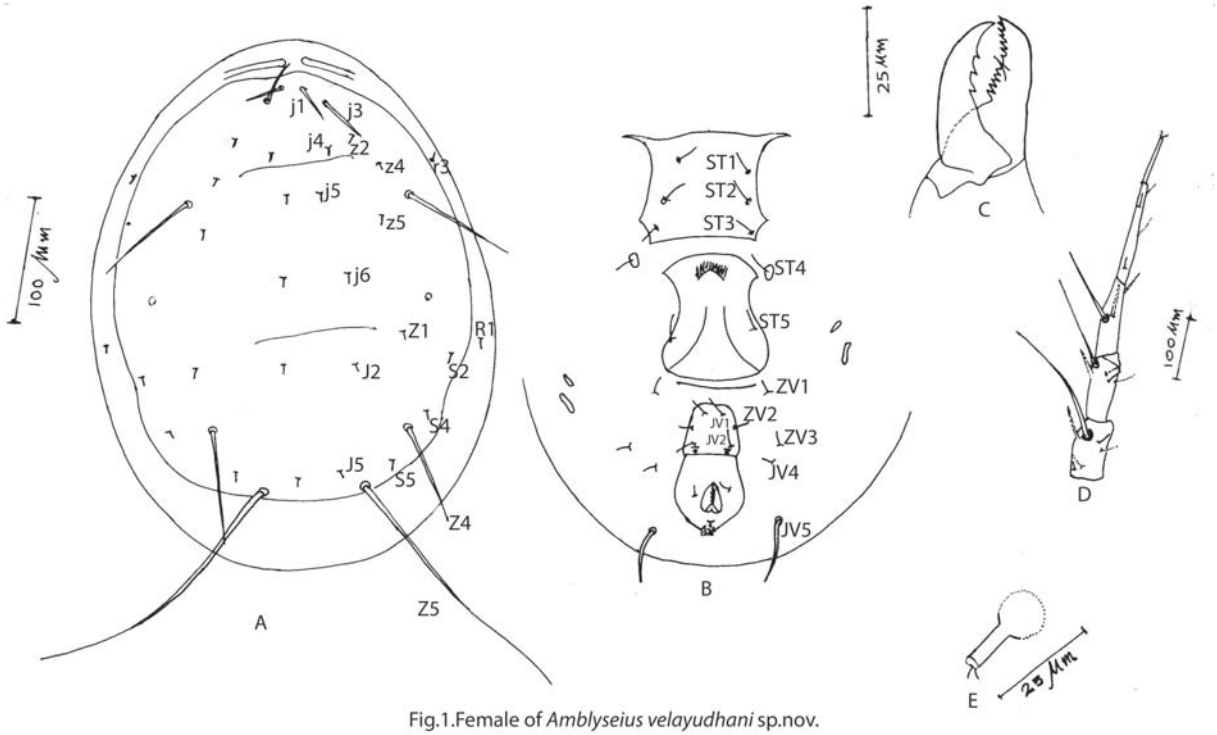

Fig.1.Female of *Amblyseius velayudhani* sp.nov.
A -Dorsal view .B-Ventral view .C-Chelicera .D-Leg.IV .E-Spermatheca

Venter: Sternal shield **90** (75-95) long, **100** (90-110) wide with 3 pairs of setae. Lateral margin of sternal shield slightly concave. ST1, ST2 and ST3-**20** (18-22), ST4-**15** (13-16), ST5-**14** (13-15). Distance between ST1-**50** (45-53), ST2 and ST3.

60 (58-63), ST5-**80** (76-83). Genital shield is **90** (85-96) wide. A clear septum is present between the ventrianal shield and genital shield. Ventrianal shield has a division below the elliptical pore but the shield is not separated each other. Ventrianal shield **100** (95-105) long and **50** (45-55) wide with 3 pairs of preanal setae measuring JV1-**10** (8-12), ZV2- **9** (8-10), JV2-**5** (6-7). Four pairs of setae present on the area around the ventrianal shield. Setae JV4- **4** (3-5), JV5-**50** (48-55), ZV1-**8** (7-9), ZV3-**6** (5-7). Paranal setae **6** (5-6.5), post anal setae -**7** (6-8). Two pairs of metapodal plate present. Primary one 10 long and accessory one 7 long. Peritreme extends anteriorly up to j1. Spermatheca with tubular cervix (**10**) and short atrium. Fixed digit of chelicera with 6 teeth anterior to a short

Leg chaetotaxy: genu II 2 1 / 1 --- ---- 1, / 2 0 ; tibia II 2 2 / 1 --- -- 1; / 1 0

genu III 2 1 / 1 --- --- 1, / 2 0 ; tibia III 2 2 / 1 -- ---- 1. / 0 0

pilus dentilis, 5 teeth posterior to it; movable digit with 3 teeth. Macrosetae present on leg IV- genu-**110** (100-120), tibia-**70** (68-78), basitarsus **50** (45-60)

Male: Unknown

Habitat: *Ocimum sanctum* (L.); *Cucurbita maxima* (Duch.)

Material examined: HOLOTYPE:Female, INDIA:KERALA: Botanical Garden, Calicut University, (Malappuram District), 18.iv.2014, ex:*Ocimum sanctum* (L.), coll. Santhosh (No.M 30/1). Five paratype slides with three females from Vengeri (Kozhikode district), 25.vi.2015, ex. *Cucurbita maxima* (Duch.), coll.Santhosh (No.K 30/2, 30/3, 30/4) and two female paratype, collection details same as holotype (No.M 30/5, 30/6).

Remarks: This new species closely resembles *A. cucurbitae* (Rather, 1985) in dorsal chaetotaxy but differs from it by the possession of the following features:

1. Dorsal shield longer and wider (400, 290) than that of *A. cucurbitae* (365, 197).
2. The ventrianal scutum shows division below the elliptical pore which is absent in *A.cucurbitae*.
3. In the new species Z4 (90) is shorter and Z5 (250) is longer, without serration whereas in *A.cucurbitae* it is serrated (Z4-109, Z5-235).
4. Fixed digit of chelicera with 11 teeth with short *pilus dentilis* whereas in *A.cucurbitae* it has 9 teeth without a *pilus dentilis.*
5. Movable digit of chelicera with 3 teeth, instead of 1 in *A.cucurbitae.*
6. Macro setae on leg IV genu-110, tibia-70, and tarsus- 50 long, whereas in *A. cucurbitae* genu -137, tibia -74, and basitarsus- 74 long.
7. Spermatheca with tubular cervix and short atrium instead of corniform cervix and bifid atrium in *A. cucurbitae*.

This new species also resembles *A. perditus* (Chant and Baker, 1965) in dorsal chaetotaxy, structure of ventrianal shield and chelicerae but differs from it by the following features:

1. Dorsal shield longer and wider (400, 290) than in *A. perditus* (351,212).
2. Spermatheca with tubular cervix and short atrium instead of tubular-pocular cervix with nodular atrium in *A. perditus*.
3. Length of macrosetae: genu-110, tibia-70, tarsus-50 compared to that of *A. perditus*, 78, 58, 64 respectively.

Etymology: The nomenclature of this new species is dedicated to the memory of late Mr. Velayudhan, who is the father of first Author.

AKNOWLEDGEMENTS

We wish to express our gratitude to the Principal and Manager, Malabar Christian College, Calicut, for the facilities provided. We are indebted to Dr. S.K. Gupta, Emeritus Scientist (MoEF), Colleges under Calcutta University, West Bengal for the confirmation of the new species. The First Author is also thankful to U.G.C, New Delhi for the financial assistance extended under FDP.

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(Received 13 October 2015; Accepted 05 December 2015; Published 31 March 2016)

ENTOMON 41(1): 71-72 (2016)
Short Communication No. ent. 41109

Occurrence of Cyperus root borer, *Athesapeuta cyperi* Marshall (Curculionidae: Coleoptera: Baridinae) as a minor pest of banana

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ABSTRACT: During the course of field investigation in North-eastern region, Guwahati, Assam the banana plants were found infested with the weevil *Athesapeuta cyperi* and it was a new report.

KEYWORDS: Cyperus root borer, *Athesapeuta cyperi*, banana, Assam.

Banana and plantains are infested more than dozen pests in banana growing areas of India (Padmanaban and Mustaffa, 2010) and more than twenty eight borer pests has been reported in banana worldwide of which stem weevil and corm weevils are economically important (Seshu Reddy et.al., 1993). Two minor coleopterans such as small banana weevil, *Polytes mellerborghii* Boheman and banana beetle, *Sybra praeusta* Pascoe were reported on banana from India (Padmanaban *et.al.* 2001). Cyperus root borer, *Athesapeuta cyperi* Marshall has been reported by many authors infesting on sugarcane and Cyperus (William, 1931; Ramesh and Ramamoorthy, 2012; Poinar, 1964; Marshall, 1928; Gosh, 1921). Biology and other details of Cyperus root borer has been reported by Kadam and Ibrahim (2003) but incidence of this pest on banana has not been reported.

During the field visit to North-eastern region, Guwahati Assam on banana plants a small curculionid weevil was recorded on cv.Malbhog (Silk –Rasthali AB) (Fig 1.a) and these small active weevils were harbouring in between the outer and inner leaf sheath situated below the leaf base area and little below. These weevils were found in aggregation of about 14 weevils per colony and up to 36-56 weevils per plant in four leaf sheaths were recorded. The Weevil makes small holes on the leaf sheath and feeding on the leaf sheath and feeding results in jelly exudation. This weevil was recorded in banana plants Kahikuchi and Bijoy Nagar area of Kamrup district. The weevil is very

Fig.1 a. *Athesapeuta cyperi* Marshall

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active and fast moving when compared to corm weevil and small banana weevil which has been already recorded.

In the field the weevil also found infected with a white cottony fungal growth (Fig. 1.b) and the same was identified as *Beauveria bassiana.* Out of the weevils collected the incidence of fungi was 4.25% (ranging from 1.78 to 3.57).

Fig1. b. *Beauveria bassiana* infected weevil

ACKNOWELDGEMENTS

Thanks are due to Dr V.V.Ramamurthy , Head, NPIB, Indian Agricultural Research Institute, New Delhi for identifying the weevil (RRS No.554-563/13) and Indian Type Culture Collection (ITCC) , IARI , New Delhi for identifying the fungus.

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(Received 06 January 2016; Accepted 29 February 2016; Published 31 March 2016)

Entomon 41 (1): 73-76 (2016)
Short Communication No. ent. 41110

First record of the pest termite *Coptotermes beckeri* Mathur and Chhottani (Isoptera: Rhinotermitidae) from Kerala

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ABSTRACT: *Coptotermes beckeri* Mathur and Chhotani, a subterranean termite species under Rhinotermitidae family is reported for the first time from Kerala. Subterranean termites are polyphagous and consume all available cellulose materials- paper, dead wood in structural frames, live wood in plantations and forests alike, hence gain a significant pest status. © 2016 Association for Advancement of Entomology

KEYWORDS: Subterranean, Polyphagous, *Coptotermes beckeri*, Kerala

The invasion of termites has always been a big problem for humans, since the damage caused by them lead to huge economic loss. They are one of the most destructive insect pests (Cheng and Cheung, 2014) all over the world. Among termites, *Coptotermes* spp. (Isoptera: Rhinotermitidae) are widely distributed and highly destructive in nature (Maiti, 2006). Most of the species of this genus are subterranean and are among the major house and structure infesting termites (Scheffrahn and Su, 2000). From India, seven species of *Coptotermes* are reported, *Coptotermes ceylonicus* Holmgren, *Coptotermes gaurii* Roonwal and Krishna, *Coptotermes gestroi* (Wasmann), *Coptotermes heimi* (Wasmann), *Coptotermes kishori* Roonwal and Chhotani, *Coptotermes premrasmii* Ahmad and *Coptotermes beckeri* Mathur and Chhotani, of these, the last three are endemic to India (Krishna *et al.,* 2013). Of the above *C. heimi* is a global invasive and widespread species.

A termite attack was noticed on wooden doors (Fig.5) and some concrete areas in an apartment (located on the third floor), in Chalapuram (lat-11.24°N long- 75.79°E), Kozhikode Dist, Kerala, on 27.xi.2014. A few samples were collected and taxonomically analysed. Based on Roonwal and Chhotani, 1989 and also Maiti, 2006, the termite was identified to be *Coptotermes beckeri* (Fig.1-2) a subterranean species, formerly reported only from Tamil Nadu (Krishna *et al.,* 2013). This report forms the first record of the species from Kerala. Three species of *Coptotermes* known hitherto from Kerala are *C. heimi*, *C. ceylonicus* and *C. kishori* (Amina and Rajmohana, 2014; Mathew, 2015). Sixty species were listed in the recent checklist on termites of Kerala by Amina and Rajmohana, 2014, and the present study updates the total number to 61. The checklist of termites of Kerala, by Mathew, 2015, listed only 58 species, missed to include *Heterotermes indicola* (Wasmann) (Amina and Rajmohana, 2013a) and *Ceylonitermellus periyarensis* Amina and Rajmohana (Amina and Rajmohana, 2013b).

C. beckeri is one of the smallest species among *Coptotermes* (Table-1). Their soldiers can easily be distinguished from other species by their small

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Table-1: **Measurements (in mm) of soldiers of *Coptotermes* spp**

Body parts	*C. beckeri*	*C. ceylonicus*	*C. heimi*	*C. kishori*
Head length to base of mandible	1.08-1.13	1.25-1.50	1.20-1.45	1.13-1.25
Max. head width	0.90-0.98	1.00-1.20	1.00-1.35	0.95-1.08
Head width index (width/length)	0.80-0.87	0.82	0.77-1.04	0.84-0.86
Mandible length	0.70-0.78	0.80-0.90	0.70-1.00	0.73-0.83
Head mandibular index (mandible length/head length	0.65-0.69	0.63	0.50-0.65	0.58-0.66
Length of postmentum	0.75-0.83	0.85-1.00	0.75-1.05	0.77-0.80
Maximum width of postmentum	0.31-0.34	0.35-0.40	0.38-0.45	0.35-0.38
Width of postmentum at waist	0.21-0.23	0.20-0.25	0.25-0.34	0.18-0.20

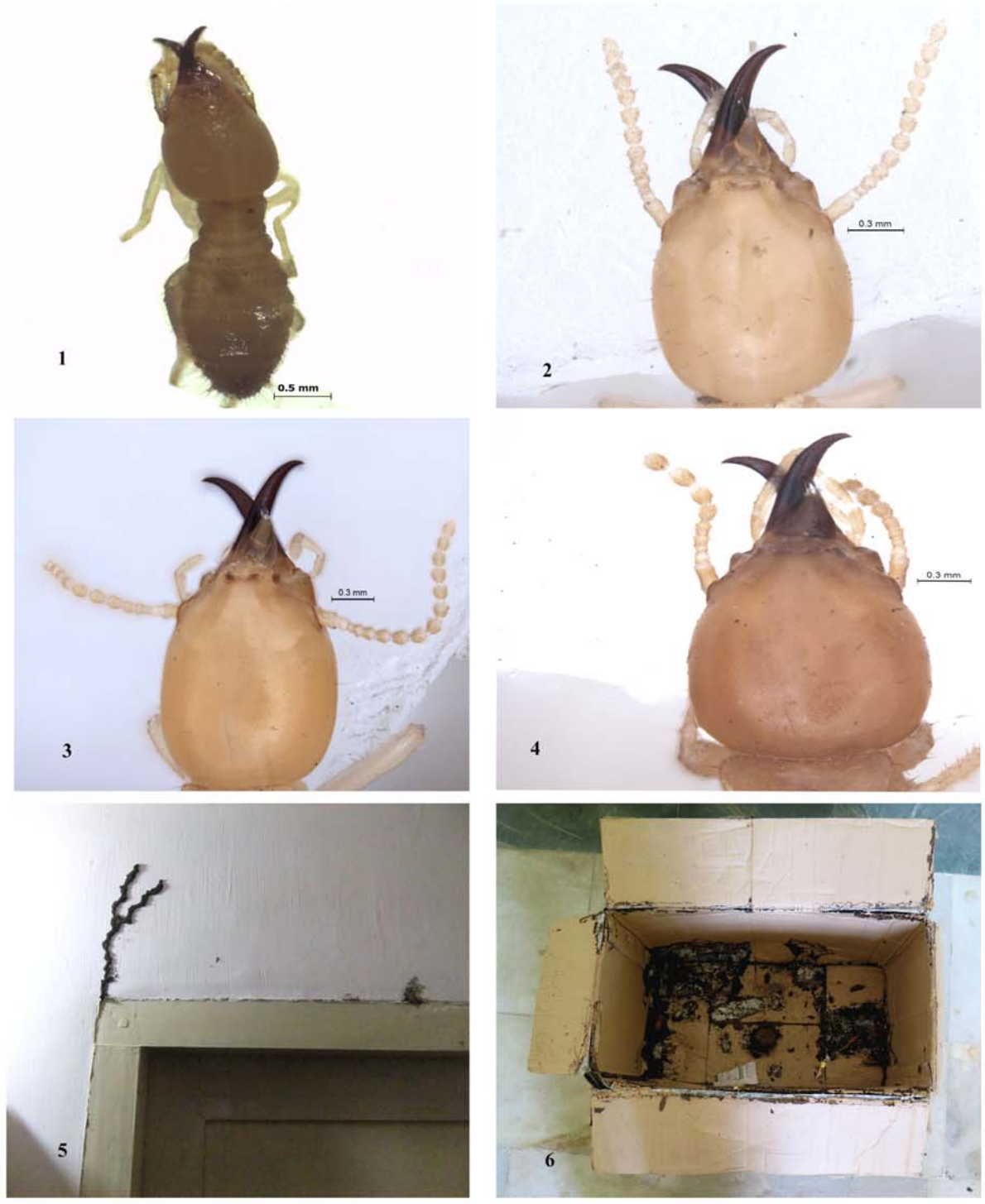
ovoid head, labrum subtriangular, sides strongly converging and terminating in a hyaline pointed apex. Antennae have 13 segments, segment 3 varying in size. Mandibles comparatively smaller in proportion to head length. Postmentum club-shaped and wide at waist. In worker caste, head capsule is subcircular and antennae are with 13 segments, of which segment 3 is sometimes subdivided and the postclypeus is slightly swollen.

In the present observation, the termite attack was manifested outwardly by a brownish-black colored tunnel on the wooden frames of kitchen door. When the tunnel building activity of *C. beckeri* was regularly monitored for a week, it was observed that at the onset, the tunnel length recorded an increase of 2cm after 24 hours since its first appearance on the wooden frames. On the second day, the tunnel had grown by 4cm and the following day, it had branched with a further addition of 2cm each along the branch and also the main course of the tunnel. By the seventh day, the main branch of the tunnel had grown further, till it touched the roof of the kitchen and diverged variably. It was observed that on a day to day basis, an active termite colony added atleast a minimum of 2cm to the original length of their tunnel.

Another instance of attack by *C. beckeri* was noticed on 16.v.2015, in a cardboard carton, kept inside the store-room in the same apartment mentioned earlier. A clear gape was made on the bottom of the carton (Fig.6). The two instances above reinforce the fact that *C. beckeri* is a polyphagous species with immense pest potential-attacking wood, concrete and paper, alike. Attack of this species on wood works of buildings was previously reported by Sundararaj *et al.,* 2013. The present study reports the attack of the species on paper too.

Other subterranean species like *C. heimi* (Fig.4), *C. ceylonicus* (Fig.3), *H.indicola* and *Heterotermes malabaricus* Snyder (Amina and Rajmohana, 2013a; Maiti, 2006; Roonwal and Chhotani, 1989; Sundararaj *et al.*, 2013), are similar to *C. beckeri* in their attacks. All of them attack both live and dead wood in agricultural/ plantation crops and forests alike. It can be concluded that the subterranean termites are mostly polyphagous and since they attack all the available cellulose materials, they gain a significant pest status.

Any cellulose material that can hold sufficient moisture can sustain small colonies of subterranean termites. Hence human transportation of plant parts and also wood for structural works, for constructing railway sleepers, as wooden posts, as wood packaging, as firewood, etc have largely promoted the spread of such termite pests. Though quarantine regulations that prohibit the transportation of materials infested with termites exist in some countries, such quarantine have been found virtually unenforceable (Henderson, 2014).



Figures: 1. Soldier of *Coptotermes beckeri* Mathur and Chhotani
2. Head of *C. beckeri* in dorsal view
3. Head of *C. ceylonicus*
4. Head of *C. heimi*
5. *C. beckeri* attack on wooden door
6. Attack on cardboard carton

In cases of extensive infestation by subterranean termites, tenting and fumigation of the entire building is advised (Oi *et al*., 1993). Oil- based chlorinated chemicals like lindane, chloroprin etc. as wood protectors when applied, prevent such attacks to some extent.

Material examined: INDIA: Kerala: Chalappuram, Kozhikode, 27.xi.2014, M. Shweta, 7 soldiers, 8 workers, colony code: ZSI/WGRC/IR/INV/5191 (deposited at ZSI, Kozhikode)

INDIA: Kerala: Chalappuram, Kozhikode, 16.v.2015, M. Shweta, 4 soldiers, 6 workers, colony code: ZSI/WGRC/IR/INV/5192 (deposited at ZSI, Kozhikode)

ACKNOWLEDGEMENT

The authors are grateful to the Director, Zoological Survey of India (ZSI), Kolkata and the Officer-in-Charge, ZSI, Calicut, Kerala, for support and encouragement. The first author is thankful to UGC for the award of Moulana Azad National Fellowship, towards the study and the second author thanks KSCSTE for the award of a Senior Research Fellowship.

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(Received 29 October 2015; Accepted 30 January 2016; Published 31 March 2016)

ENTOMON 41 (1): 77-78 (2016)
Short Communication No. ent. 41111

Occurrence of large spine- footed bug, *Physomerus grossipes* Fabricius (Coreidae: Hemiptera) on banana in India

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ABSTRACT: A new coreid bug identified as *Physomerus grossipes* Fabricius was recorded infesting banana as a pest. The coreid bug sucks the pulp from the fingers and the feeding damage results in the development of sunken black spots on the peel.

KEYWORDS: large spine- footed bug, *Physomerus grossipes* Fabricius, Banana, India

Banana and plantains are infested by more than a dozen pests (Padmanaban and Mustaffa, 2010). During our recent survey undertaken in certain banana growing districts of Maharashtra viz., Pune, Raver, Jalgoan, Ahmednagar, Aurangabad and Bhusawal, a new coreid bug was recorded infesting on banana fingers, the adults and nymphs were aggregated on banana stem and bunches (Fig. 1-3). The coreid bug sucks on the fingers and feeding damage results in the development of sunken black spots on the peel and the removal of peel indicated the damage on pulp also. The adults and nymphs were found on the stem and bunch. This appears to be a minor pest and causing damage to very few hands on the bunch and the affected fingers were found unfit for sale. The pest has been identified as large spine footed bug, *Physomerus grossipes* Fabricius (Coreidae: Hemiptera). The pest has been recorded on cv. Grand Naine in Raver, Jalgoan and Narayangoan in Jumner, Jalgoan and Pune districts of Maharashtra, India. The pest incidence was first noticed during March 2010 where as severe incidence was reported in August 2016.

Review of literature indicated that the bug is native to South East Asia and the species has spread to Pacific Islands. The pest has been reported to feed on a variety of plants, considered as a minor pest of sweet potato. The nymph and attacks on stem and petioles of sweet potato causing stunted growth and wilting. The other host plants reported include *Ipomoea aquatica, I. triloba* and *Bacilla rubra* (Broddley, 1991; Ronato, 1984 and Swaine *et al.,* 1991). In Australia, a similar fruit spotting bug, *Amblypelta lutescens* has been reported on banana in southern Queensland (Astridge *et al.,* 2004) the bug damage has been reported on avocados, bananas, custard apple, macademia nuts, pecans and citrus. Eulophid wasp parasitization on the eggs of this coreid bug has been reported under field conditions (Amalin *et al*., 1993).

The coreid bug damage is noticed only few plants in a garden and the feeding damage is very severe affecting the cosmetic value and pulp quality and not fit for sale. The bug damage in endemic areas can be prevented easily by the use of polypropylene bunch sleeves; the sleeve has to be tied on bunches immediately after opening of all hands. Bunch sleeving prevents the bug infestation and gets

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Fig.1 Coreid bug on banana finger

Fig.2 Feeding damage on banana fingers cv. Grand Naine

Fig.3 Adults and nymphs aggregation

blemishes free golden yellow colour banana fingers of good quality.

ACKNOWLEDGEMENTS

Thanks are due to Natural History Museum, London for identifying the insect. Thanks are also due to Dr.S.S.Ranade M/s Ranade Chemicals, Pune for providing necessary assistance for undertaking the survey in certain banana growing districts of Maharashtra. Also thanks to Shri.Shripad Jangale, Bushawal for his assistance in field visits.

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(Received 06 January 2016; Accepted 29 February 2016; Published 31 March 2016)

AUTHOR INDEX

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(Form IV, Rule 8 of Registration of Newspapers (Central) Rules 1956)

1.	Place of publication	:	Trivandrum
2.	Periodicity of publication		Quarterly
3.	Printer's name, nationality and address	:	Dr K D Prathapan, Indian, Secretary, Association for Advancement of Entomology, Department of Entomology, College of Agriculture, Kerala Agricultural University, Vellayani PO, Thiruvananthapuram 695522, Kerala, India
4.	Publisher's name, nationality and address	:	- do-
5.	Editor's name, nationality and address	:	Dr M S Palaniswami, Indian, Chief Editor, ENTOMON, Association for Advancement of Entomology, Department of Entomology, College of Agriculture, Kerala Agricultural University, Vellayani PO, Thiruvananthapuram 695522, Kerala, India
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31 March 2016

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