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SHORT COMMUNICATION

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Identification and characterization of gut associated bacteria in *Epilachna vigintioctopunctata* Fab. (Coleoptera : Coccinellidae)

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ABSTRACT: *Epilachna vigintioctopunctata* Fab is a wide-spread key pest of the solanaceous and cucurbitaceous plants. *E. vigintioctopunctata* depends up on microorganisms that inhabit its intestinal tract. The diversity of the gut microbiota revealed 20 bacteria based on their morphological, biochemical, physiological and molecular characteristics. Out of twenty, six isolates were gram-negative bacteria. In total of 20 isolates 3 bacteria were sent to 16S rRNA partial gene sequencing and revealed the presence of *Bacillus subtilis* (EVI16), *B. vietnamensis* (EVI09) and *B. anthracis* (EVI07).

KEYWORDS: *Epilachna vigintioctopunctata*, gut associated bacteria, 16S rRNA sequencing, *Bacillus subtilis* (EVI16), *B. vietnamensis* (EVI09), *B. anthracis* (EVI07)

INTRODUCTION

Insects are extremely successful animals in view of their great adaptability to a wide range of terrestrial niches. Insect success is due to the collaboration with bacteria in term of symbiosis since bacteria play crucial roles in the biology and life cycle of most insect species, affecting nutrition, development, reproduction, immunity, defense against natural enemies and speciation (Moran and Baumann, 2000; Moran, 2001; 2006).

There has been growing interest in developing novel approaches to control insect pests through gut micro biota study. Most of the microorganisms found in nature have not yet been studied. Little is known about the composition and function of the insect gut microbiota. Moreover, most previous studies on diversity of gut microbiota of insects relied on culture-dependent methods using tra-ditional microbiological techniques to identify the gut microbiota (Dillon and Dillon, 2004). Traditional methods of bacterial isolation limit the species that can be grown and analyzed under laboratory conditions, although they can provide information about the biology and biochemical features of the isolated organism. Molecular approaches for characterization of microbes have been used in recent years. This approach based on nucleic acid sequence, par-ticularly the 16S rRNA gene, has enabled the definition of the microbial community of insects (Brauman, 2000). Knowledge of microorganisms' species can facilitate studies of the function of the gut microbiota and help to define

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interactions among members of the gut community that can lead to the development of insect control strategies (Broderick *et al.,* 2004).Moreover, the identification of microorganisms and their specific enzymatic activities can help in the understanding of the interactions between insects and provide information to control the pest.

The spotted leaf beetle or Hadda beetle, *Epilachna vigintioctopunctata* Fab. (syn. *Henosepilachna vigintioctopunctata* Fab.), is the key pest of the solanaceous and cucurbitaceous plants (Islam *et al*., 2011). The grub and adult feeds on the leaves, retarding the plant growth, which leads to loss of fruit production. Fruit reduction in yield up to 60% has been reported (Mall *et al*., 1992).Currently the overall knowledge of the bacterial communities in *E. vigintioctopunctata* and their associations with hosts is still limited and has to be studied extensively so that we may get many clues to control the pest.

MATERIALS AND METHODS

Adults of *E. vigintioctopunctata* were collected from brinjal farm, Bahour, Puducherry in the month of July 2016. The insects were surface sterilized with 70% ethanol for 1 min and rinsed in sterile water before dissection. The insect was dissected inside a sterile laminar flow using sterilized dissection scissors, needle and forceps. The head and last abdominal segment of insect were severed, and pressure was applied anterior to the crop to release the gut. The gut isolated and homogenized in 0.86% NaCl solution (Broderick *et al.,* 2004).

The stock solution was prepared by taking 1 ml of the suspension and was mixed with 9.0 ml saline. Thereby using serial dilution method seven dilutions were prepared. 1ml of each dilution was added to separate plate. Triplicates were made for each dilution. Then added 15 ml of nutrient agar medium and incubated for 24 hours at 37°C. Dominant colonies were picked out, purified three times by inoculating on the corresponding agar plates, and further transferred to agar slants (Huang, 1999).

The dominant frequently appearing gut associated bacteria were identified by bacteriological properties and16S rRNA gene sequencing. Morphological tests were done by standard procedures. The physiological-biochemical characteristics were determined on the basis of Gram stain, Catalase test, Lipolytic test, Gelatinase test and Cellulolytic test (Dong and Cai, 2001).

Preparation of template DNA – Pure cultured bacterium was used for gene sequencing. Colonies were picked up with a sterilized toothpick, and suspended in 0.5 ml of sterilized saline in a 1.5 ml centrifuge tube and centrifuged at 10,000 rpm for 10 min. After removal of supernatant, the pellet was suspended in 0.5 ml of Insta Gene Matrix (Bio-Rad, USA). Incubated 56^{o}C for 30 min and then heated 100^{o}C for 10 min. After heating, supernatant was used for PCR.

PCR - 1µl of template DNA was added in 20 µl of PCR reaction mix. 518F/800R primers were used and then performed 35 amplification cycles at 94^{o}C for 45 sec, 55^{o}C for 60 sec, and 72^{o}C for 60 sec. DNA fragments were amplified about 1,400 bp in the case of bacteria including a positive control (*E.coli* genomic DNA) and a negative control in the PCR.

518F	5'CCAGCAGCCGCGGTAATACG 3'
800R	5' TACCAGGGTATCTAATCC 3'

Purification - Purification of PCR products of approximately 1,400 bp were sequenced by using the primers and dNTPs from PCR products by using Montage PCR clean up kit (Millipore).

Sequencing - The purified PCR products of approximately 1,400 bp were sequenced by using general primers (785 F 5' GGA TTA GAT ACC CTG GTA 3' and 907 R 5' CCG TCA ATT CCT TTR AGT TT 3'). Both this primers amplify the V5- V6 region of the 16S r RNA gene. Sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied BioSystems model 3730XXL automated DNA sequencing system (Applied Bio Systems, USA) (Weisburg *et al.,* 1991).

The culture sequence obtained were subjected to BLAST analysis, the phylogenetically similar type strains sequence and other phylogenetic related sequence were selected from the GeneBank and they were subjected to multiple sequence alignment and then align sequence were trimmed to similar length in nucleotides and were subjected to phylogenetic tree (neighbour joining) construction using MEGA 6. In the tree the numbers at the nodes indicates the levels of the bootstrap support [high bootstrap values (close to 100%) meaning uniform support] based on a neighbour joining analysis of 1,000 re-sampled data sets. The bootstrap values below 50% were not indicated. Bar 0.005 substitutions per site.

RESULTS AND DISCUSSION

Using the isolation procedure, a total of 20 dominant isolates were successfully collected from the gut of the adult and classified based on the colony color, size, and cellular morphology and biochemical activity. Among the 20 bacteria isolated from *E. vigintioctopunctata* adult, majority were gram-positive bacteria (14 isolates) and only six were gram-negative bacteria.

Three bacteria identified were isolated using pure culture method and subjected to 16S rRNA partial gene sequencing. The sequences obtained were analyzed using BLAST and other programs and placed on the phylogenetic tree. *B. subtilis* (EVI16), *B. vietnamensis* (EVI09) and *B. anthracis* (EVI07) species were identified based on their similarities with other sequences (Table 1).

Genbank accession numbers of bacterial isolates:

The GenBank accession numbers for the partial sequence of the 16S rRNA gene sequences for the isolates EVI09 (*B. vietnamensis)*, EVI07 (*B. anthracis*) and EVI16 (*B. subtilis*) were KY002646, KY002647 and KY002648 respectively.

The bacteria isolated from the gut of *E. vigintioctopunctata* were able to produce lipase, protease, cellulase and catalase enzymes in a similar

Table 1. Morphological characteristics of dominant bacteria in the gut of the adults of *E. vigintioctopunctata*

Colony	Shape	Colour
EVI01	Filamentous	Yellow
EVI02	Rhizoid	White
EVI03	Filamentous	White
EVI04	Lobate	White
EVI05	Lobate	White
EVI06	Filamentous	White
EVI07	Filamentous	White
EVI08	Filamentous	White
EVI09	Round	Cream
EVI10	Round	White
EVI11	Filamentous	White
EVI12	Filamentous	Cream
EVI13	Lobate	Cream
EVI14	Lobate	Cream
EVI15	Filamentous	White
EVI16	Lobate	White
EVI17	Filamentous	White
EVI18	Round	Yellow
EVI19	Irregular	Yellow
EVI20	Irregular	White

gut environment. The insect gut microbiota is considered to be a complex ecosystem containing over a hundred bacterial species including anaerobes and facultative anaerobes (Brauman, 2000). Microbial colonization depends on the physicochemical conditions in the lumen of different gut compartments, and these can display extreme variation in both pH and oxygen availability (Appel and Martin, 1990; Harrison, 2001). The gut of the insect is anaerobic in nature. It is conducive for the aerobic bacteria to live in. Therefore bacteria produce catalase to generate oxygen so that the bacteria can use oxygen for respiration. These bacteria may be pure aerobes or facultative anaerobic organisms (Engel and Moran, 2012). In this study 50% of the bacteria are involved in catalase activity (Table 2).

Bacteria associate with insects in providing essential nutrients. Insects belonging to the order Hemiptera like aphids, white fly, mealy bug, plant hoppers feed

Table 2.Biochemical characteristics of dominant bacteria in the gut of the adults of *E. vigintioctopunctata*

Colony	Grams Stain	Catalase	Lipolytic	Gelatinase	Cellulolytic activity
EVI01	+	-	+	+	+
EVI02	+	+	+	+	-
EVI03	-	+	+	+	+
EVI04	+	-	+	-	-
EVI05	+	-	-	+	-
EVI06	+	-	-	+	-
EVI07	+	+	+	+	-
EVI08	+	+	+	+	-
EVI09	-	+	+	+	-
EVI10	-	-	+	+	+
EVI11	-	+	+	-	+
EVI12	+	-	+	+	+
EVI13	+	-	+	+	+
EVI14	+	-	-	+	-
EVI15	-	+	+	+	-
EVI16	+	+	-	-	-
EVI17	+	+	-	-	+
EVI18	+	+	+	+	+
EVI19	-	+	+	+	+
EVI20	+	+	+	+	+

exclusively on the plant sap – a freely available sugar rich diet. Since plant sap is very poor in nitrogen and amino acids, these insects have developed obligate symbiosis with bacteria like *Buchenra* (aphid) or *Portiera* (white fly), where in the bacteria supply all the essential amino acids required by the insect, while the insect accommodate these bacteria in specialized structures in their gut - mycetomes / bacteriocytes (Douglas, 2006). Among 20 bacteria isolated from gut of *E. vigintioctopunctata*, 16 isolates including *B. anthracis* and *B. vietnamensis* were involved in proteolytic activity (Table 2). A recent study reported that a part of velvet bean caterpillar gut protease was secreted by their gut bacteria (Visotto *et al*., 2009).

Several bacteria isolated from the soil have been reported as cellulase producer such as *F. johnsoniae, Pseudomonas mendocina* (Lednicka *et al.*, 2000) and also from the gut of scrab *H. parallela* (*P. nitroreducens*) (Huang *et al.*, 2012). Cellulase activity was also reported in *Acinetobacter anitratus* (Ekperigin, 2007). *Acinetobacter lwoffi* and *Microbacterium paraoxydans* were found in the gut of *Ostrinia nubilalis* (*Lepidoptera*). Subodh *et al.* (2012) isolated bacteria from the gut of termite showing cellulolytic activity. They were identified as *Citrobacter, Enterobacter* and *Cellulomonas*. Among 20 bacteria isolated from gut of *Epilachna vigintioctopunctata*, 10 isolates were involved in cellulolytic activity (Table 2). The bacterial strain EVI09 was identified as *B. vietnamensis* which had 98% of sequence similarity with said sequence *B. vietnamensis* is a common soil bacterium that belongs to *Firmicutes*. It has a thick cell wall, forms endospore, highly resistant to extreme environments. This bacterium (isolate EVI09) has much similarity and requires further verification.

This may act as endosymbiont that helps in digestion and assimilation of food by the insect.

The isolate EVI07 has been identified as *B. anthracis* (Fig.1). This bacterium is known to cause anthrax in mammals. The nucleotide similarity has 99% with *Bacillus anthracis.* In this study it has been noted that this bacterium is found in the insect gut. These bacteria are found in many environments, notably in the soil and on plants where they can be associated with invertebrates (Schuch *et al.*, 2010). The isolates EVI16 and EVI09 were identified as *B. subtilis* (Fig.2) and *B. vietnamensis*. In future studies, it may possible to analyse the presence of *B.anthracis* associated with insects. *B. subtilis* is aerobic, endospore-

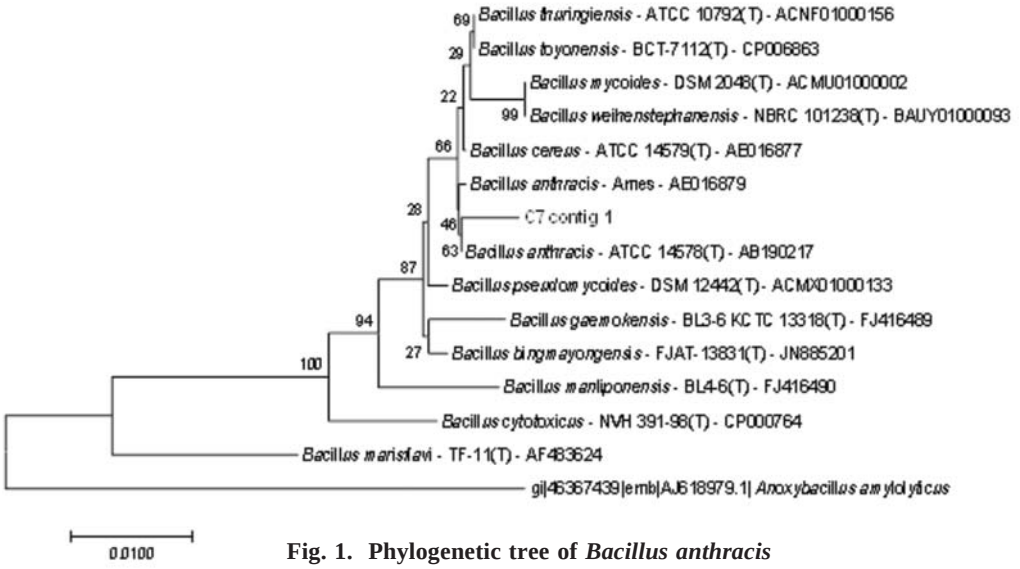

Fig. 1. Phylogenetic tree of *Bacillus anthracis*

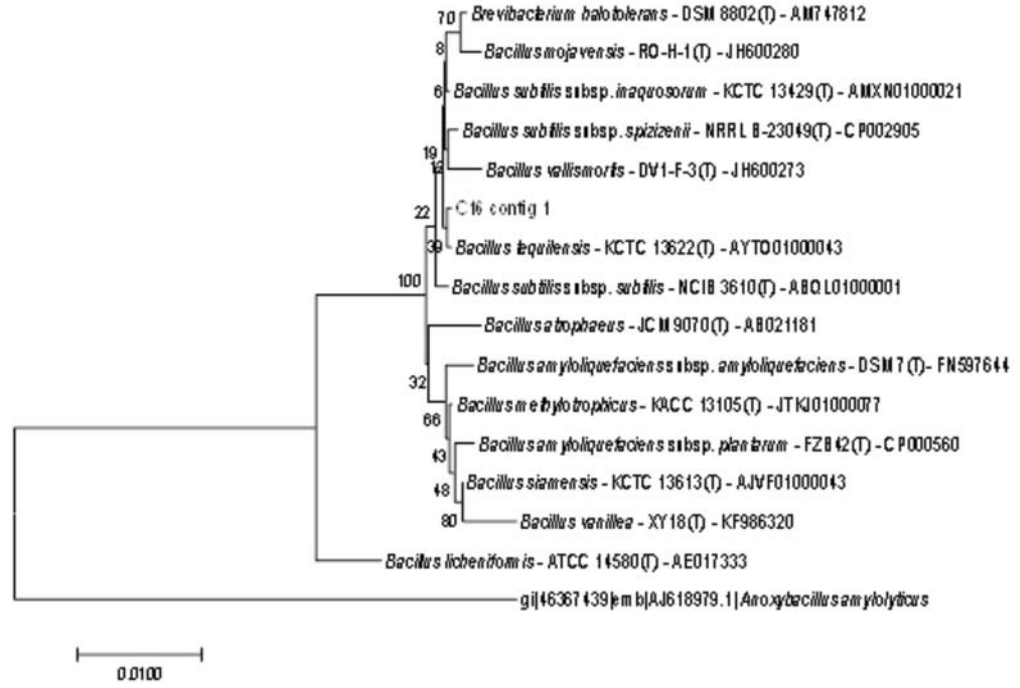

Fig. 2. Phylogenetic tree of *Bacillus subtilis* sub sp. *inaquosorum*

forming, gram positive bacteria and opportunistic pathogen. Mandla Rajashekhar *et al*., in 2017 studied the potential of *B. subtilis* as microbial insecticide for effective management of insect pests.

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Evaluation of entomopathogenic fungi *Metarhizium anisopliae* against dengue virus mosquitoes *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT: In this study, the bio-potential of the entomopathogenic fungus *Metarhizium anisopliae* was tested against *Aedes aegypti* under laboratory conditions. The study includes the analysis of the attractive response, survival and fecundity rate of non-blood and blood fed female mosquitoes exposed to the volatiles of two *M. anisopliae* strains. The attractive response was analysed using a two-choice behavioural bioassay, with three different sizes of dry spore plates (full, 1/4 and 1/16 plates). The survival and fecundity bioassay was conducted simultaneously in plastic pots. Log-rank survival curve analysis was used for statistical comparisons of the attractive response, survival and fecundity. Non-blood and blood fed mosquitoes were highly attracted to *M. anisopliae*-30 volatiles compared with that of the *M. anisopliae*-131 strain. Moreover, attraction was dependent on the size of the dry spore plate. Survival was completely abolished in unfed mosquitoes 5 and 6 days after treatment with 10^9 spores/mL of *M. anisopliae*-30 and *M. anisopliae* -131, respectively, whereas almost 80% of untreated unfed females survived more than 28 days. Survival in blood fed mosquitoes treated with same dose of *M. anisopliae*-30 and *M. anisopliae*-131was abolished after 6 and 7 days, respectively, while over 80% of untreated blood fed females survived more than 28 days in the controls. Mean number of eggs laid by blood fed mosquitoes treated with 10^9 spores/mL of *M. anisopliae*-131 was 26 ± 3 compared to control (67 ± 4). However for *M. anisopliae*-30, 19 ± 3 eggs were laid compared to control 72 ± 5 eggs. This study concludes that both the strains of *M. anisopliae* reduce egg laying capacity and survival rate in *Ae. aegypti*. As such, these strains can be useful for the development of mycoinsecticides for the control of the dengue fever vector mosquito, *Ae. aegypti*.

KEY WORDS: *Aedes aegypti*, attractive response, fecundity rate, *Metarhizium anisopliae*, survival rate, vector control

INTRODUCTION

Mosquito born-diseases are a major tropical health challenge, world-wide. Anthropogenic activities in tropical and subtropical countries play a significant role in increasing number of mosquito breeding sites (Scott *et al.*, 1997; Chareonsook *et al.*, 1996). Consequently, a high proportion of people suffer from viral transmission, including Japanese encephalitis, dengue fever and yellow fever

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(Heddini *et al.,* 2007; Nagi *et al.,* 2011; Chakravarti *et al.,* 2012) as well as the transmission of other pathogens causing diseases such as malaria and filariasis, (Ghosh *et al.,* 2012; Kundu *et al.,* 2013). Chemical pesticides are extensively used to control adult mosquitoes as well as immature stages in their breeding sites. However, due to their negative impact on the environment, non-target organisms, and development of resistance by target species, alternative measures are needed to replace chemical pesticides. As an alternative, the utilization of potential entomopathogenic fungi in pest control is considered an eco-friendly approach.

Entomopathogenic fungi are safe for human and other non-target organisms, do not have residual problem through food chain, enhance biodiversity, reduce the development of resistance (Scholte *et al.,* 2007), attract and kill the target organisms (George *et al.,* 2013), and reduce the fecundity and survival rate of mosquitoes (Paula, 2008; Scholte *et al.,* 2005; Paula *et al.,* 2011; Darbro *et al.,* 2011; Blanford *et al.,* 2012). The bio-potential of entomopathogenic fungi is variable and dependent on the developmental stage of the mosquito species. Earlier report highlight that the application of fungal pathogens, such as *Lagenidium*, *Coelomomyces* and *Culicinomyces*, are effective against the larval stages, whereas *Hypomycetes*, *Beauveria bassiana* and *Metarhizium anisopliae* are highly pathogenic to adult mosquitoes (Scholte *et al.,* 2003). The time from infection until the death of the host depends on the host species, host physiological state, fungal species and virulence of the strain, dose of conidia suspension and abiotic factors (Ferron, 1978; Gillespie and Claydon, 1989; Blanford *et al.,* 2005). In order to regulate mosquito population size, researchers are focussing on increasing the understanding of the biology and ecology of pathogen-mosquito interaction (Roy *et al.,* 2006).

The *Hypomycetes* fungus *M. anisopliae* is recommended as one of the management approaches against insecticide resistant and susceptible mosquitoes (Scholte *et al.,* 2006; Farenhorst *et al.,* 2010). Based on the toxicity tests, Environmental Protection Agency declared no risk to humans when using mass products of microbial biopesticides containing *M. anisopliae* (EPA, 2003). The volatiles released from entomopathogenic fungi may alter behavioural response of the mosquitoes (Mburu *et al.,* 2011; George *et al.,* 2011). Some of the fungal volatiles are not repellent to *Anopheles* and *Culex* mosquitoes (Mnyone *et al.,* 2010), and some fungal dry spores may attract *An. stephensi* (George *et al.,* 2013). Several studies have investigated the effectiveness of fungal pathogens and how these are affected by salinity, temperature, relative humidity and breeding sites with variable water quality, in the context of low cost mass-production and formulation techniques, long shelf life, killing effect on target mosquitoes and non-target organisms (Zimmermann, 2007; Scholte *et al.,* 2004; Blanford *et al.,* 2011; Read *et al.,* 2009).

Dengue fever is one of the most rapidly spreading mosquito-borne diseases worldwide (WHO, 2009), and is transmitted by female *Ae. aegypti* mosquitoes. The behaviour of adult mosquitoes is highly anthropophilic, endophilic and endophagic. Due to the development of insecticide resistance there are several challenges to develop effective control methods (Darbro *et al.,* 2011). Dengue fever vector control strategies include chemical-based control measures, non-chemical-based control measures and biological control agents (Poopathi and Tyagi, 2006). Previous studies have reported that in *M. anisopliae* reduce the survival rate of *Ae. aegypti* mosquitoes, and also kill the insecticide-resistant mosquitoes (Blanford *et al.,* 2005; Farenhorst *et al.,* 2009; Howard *et al.,* 2010). With the background information given above, the present study was undertaken to evaluate impact of *M. anisopliae*-131 and *M. anisopliae*-30 strain on the attraction, survival and fecundity rate of non-blood and blood fed female *Ae. aegypti*.

MATERIALS AND METHODS

a) Mosquito culture establishment:

Aedes aegypti (Rockefeller strain) eggs were collected from the culture maintained at the insectary, at the University of Sweden Agricultural

Science, Alnarp, Sweden. The eggs were kept in plastic trays (25 cm x 25 cm x7 cm) filled to a depth of 5 cm with distilled water. After eclosion, the larvae were provided with finely powdered Tetramin® fish food as a source of food. After the larvae reached the pupal stage, the pupae were separated every day and introduced into a beaker half-filled with distilled water and kept inside the adult emergence cages. Filter paper was provided in distilled water-filled cups for oviposition. The adult mosquitoes were provided with 10% sucrose solution (w/v) as food. At 4 to 5 day old, adults were first starved at eight hours and then provided with a sheep blood meal for 30 min, using the Hemotek® membrane feeding system. The culture was maintained at 27 ± 1°C, 65-70% RH and on a 12:12 h photo-period. From the culture, blood and non-blood fed female adults were taken and used for the behavioural, survival and fecundity bioassays.

b) *Metarhizium anisopliae* culture establishment:

The *M. anisopliae*-131 strain was obtained from Addis Ababa University Ethiopia, whereas *M. anisopliae*-ICIPE-30 (*M. anisopliae*-30) was a gift from the International Centre of Insect Physiology and Ecology, Kenya. Both strains were cultured on Sabouraud dextrose agar media (dextrose 10 g; peptone 2.5 g; yeast extract 2.5 g; agar 20 g in 1 L H_2O) and kept at 27°C in an incubator for 15 days until sporulation (Plate 1). After sporulation, different sized dry spore plates (full, 1/4, 1/16) were prepared under aseptic condition and used for the behavioural bioassay (Plate 2). Survival and fecundity bioassays were carried out by using spore suspension initially prepared with 0.05% Triton X-100 and conidial concentration of 10^5, 10^7 and10^9 spores/mL (Figure 2A). The conidial concentration was determined by using a Neubauer haemocytometer. The fungal suspension was mixed vigorously by using a vortex mixer, and then 0.2 µl of the suspension was applied on the thoracic region of individual adult mosquitoes using a micropipette inside a safety cabinet.

c) Test of spore germination:

The viable conidia of the two strains were determined by sub-culturing the conidial suspension. The conidial suspension was serially diluted, so that 0.1 mL of 10^{-2} spore suspension inoculated three SDA plates. The spore suspension was uniformly spread on the surface area of the SDA plates by using L-shaped glass rod. After inoculation, plates were covered with sterilized cover slips, sealed with parafilm, and stored in a temperature-controlled incubator for 20-24 h. Percentage germination of spores was examined after the incubation period. From each plate, 300 spore counts were examined under a compound microscope (at 16X magnification). The conidial growth of more than 95% spores out of 300 conidia per plate produced visible germ-tube length at least three times the width (diameter) of the conidium in both strains of *M. anisopliae*.

d) Behavioral bioassay:

The behavioural response of female *Ae. aegypti* mosquitoes exposed to the volatiles emitted by *M. anisopliae* was studied in four treatment groups. In first treatment, non-blood fed mosquitoes were exposed to volatiles emanating from dry spores of *M. anisopliae*-131, and pure SDA media. In a second treatment, non-blood fed mosquitoes were exposed to volatiles emanating from the dry spores of *M. anisopliae*-30 and pure media. In a third treatment, non-blood fed mosquitoes were exposed to the volatiles emanating from the dry spores of *M. anisopliae*-131 and *M. anisopliae*-30. In a fourth treatment, blood fed mosquitoes were exposed to the volatiles emanating from dry spores of *M. anisopliae*-131 and *M. anisopliae*-30. Before each experiment, 10 mosquitoes were kept in each of eight individual release cages and exposed to 24 h of starvation with access to water, and allowed to acclimatize in the bioassay room. Two-choice bioassays were carried out from 6:00 pm to 7:00 pm to analyse the behavioural response of non-blood and blood fed mosquitoes. After a one-hour exposure to the volatiles emanating from the dry spores, the mosquitoes from the treatment and control arms were collected, killed and counted. The experiment was replicated eight times by using new dry spore plates, mosquitoes and viable conidia.

e) Dry spore plate preparation:

The *M. anisopliae*-131, *M. anisopliae*-30 and pure SDA media plates were prepared at full, 1/4 and 1/16 dry spore plates for paired comparisons to test the attraction of non-blood and blood fed *Ae. aegypti*. The differently sized plates of dry spores and pure SDA media plates were prepared in a sterilized safety cabinet by using sterilized knife. After preparation, the plates were immediately transferred to the bioassay room and used for the experiment.

f) Description of two choice behavioral bioassay method (Figure 1):

Computer fans were used to draw the volatiles from dry spore and SDA plates from the treatment and control arms, at the upwind end of the bioassay. The end of the two arms was closed with Plexiglas cylinder (8.9 cm diameter, 5 cm long) with one end sealed with nylon mesh. The most upwind section of two-choice bioassay consisted of two samples chambers of Plexiglas cylinders (9.9 cm diameter, 14.5cm long) into which the plates were placed. Dry spore plates of *M. anisopliae* versus pure SDA plates, or dry spore plates of *M. anisopliae*-131 versus *M. anisopliae*-30, of equal size, were placed into the two parallel arms at the same time for each experiment. In the Plexiglas cylinder, a nylon mesh screen was used to prevent the entry of mosquitoes to the treatment and control chambers. The rotatable nylon mesh screen valve at the other end of a mosquito collecting chamber (9.9 cm diameter, 6.5 cm long) was closed at the end of the experiments to count number of mosquitoes in each arm. A pair of Plexiglass cylinders were used as flight chambers (8.9 cm diameter and 25 cm length). At one end, both flight chambers were attached to a Plexiglass box, known as the decision chamber (30.5 cm × 22 cm × 13 cm). At the other side of the decision chamber, a single Plexiglass cylinder (9.9 cm diameter, 29.5 cm long) extends and is connected to the release cage (8.9 cm diameter, 10 cm long). One end was covered with rotatable nylon mesh and other end sealed with nylon mesh. The flow of *M. anisopliae* volatile was controlled by computer fan into the upwind end of the two arms in two choice bioassay systems. The air flow current was linear until it reached the downwind wall of the decision chamber with an airspeed determined at 10 cm s^{-1}.

g) Conidial suspension preparation for survival bioassay:

Fungal spore suspensions were prepared from 15 days old surface sporulation fungi in which 0.05% Triton X-100 was added. The homogenous spore suspensions were prepared using a vortex mixer. Unwanted material from the spore suspensions were removed by a series of three centrifugations at 300 rpm for 3 minutes. Conidial concentrations of 10^5, 10^7 and 10^9 spores/mL were prepared for the survival and fecundity experiments through serial dilutions using distilled water supplemented with 0.05% Triton X-100. The conidial concentration was determined by using a Neubauer haemocytometer (Figure 2A).

h) Effect of infection on the survival of mosquitoes:

Female adult *Ae. aegypti* mosquitoes were anaesthetized on ice for 7-10 min and inoculated with the spore suspensions, as described above. After inoculation, individual mosquitoes were transferred to plastic pots (12 cm diameter × 8 cm height), and provided with 10% sucrose *ad libitum*. Blood fed mosquitoes were provided with an oviposition substrate identical to that supplied for rearing (Figure 2B). Ten replicates for each spore concentration were run in total. The plastic pots were placed in a controlled climate cabinet at 27 ± 1°C and 65-70% RH until the completion of the experiment.

i) Detection of mycosis:

The fungal infection and its impact on mosquito survival and mortality was recorded. Cadavers (Figure 2C) were removed, dipped in 70% ethanol, and rinsed with distilled water to remove the remaining spores associated with the cuticle, and then each cleaned cadaver was placed on moist filter paper inside Petri dish and sealed with

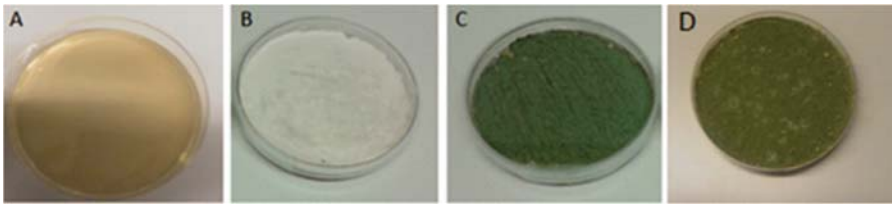

Plate 1. *Metarhizium anisopliae* growth on Sabouraud dextrose agar media at 25ºC.
A) Sabouraud dextrose agar media; B) Mycelium of *M. anisopliae* before sporulation, C) Sporulation of *M. anisopliae*-131; D) Sporulation of *M. anisopliae-ICIPE*-30

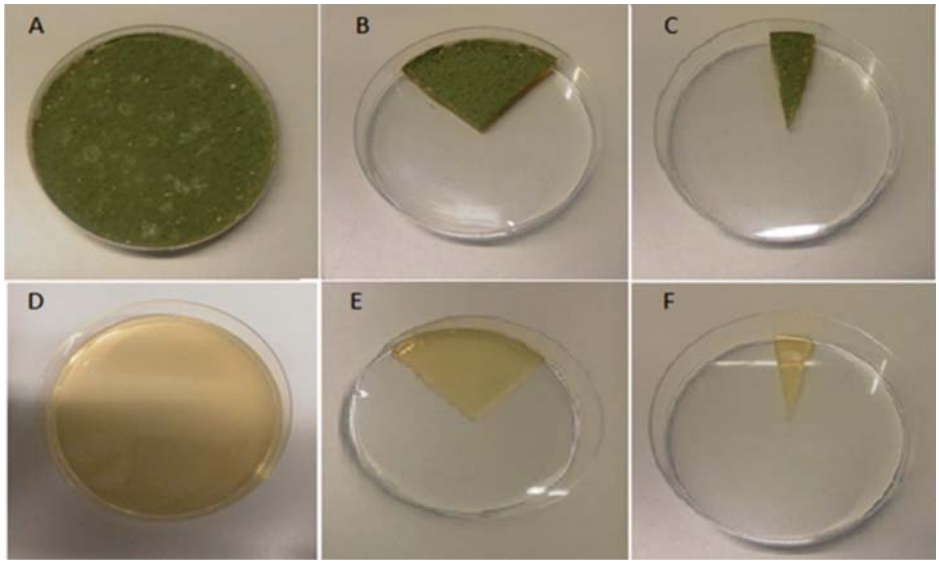

Plate 2. Dry spore plates of *M. anisopliae* and SDA media plates.
A, B and C showing full, 1/4 and 1/16 plates of *M. anisopliae*
D, E and F, showing full, 1/4 and 1/16 plates of SDA media

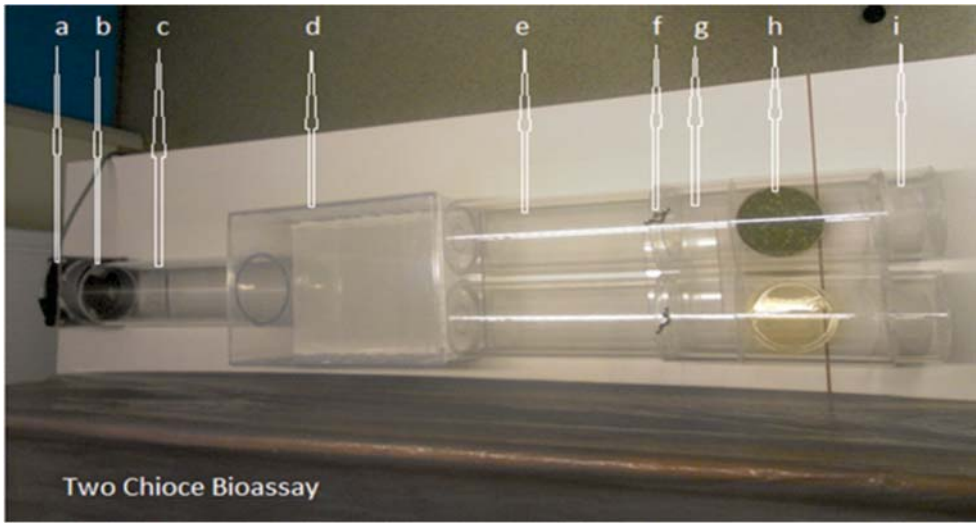

Figure 1. Two choice bioassay chambers
a) Computer fan regulating the air flow, b) Place to release adult mosquitoes, c) Release chamber, d) Decision chamber, e) Decision arm, f) Circular mesh, g) Mosquito collecting chamber, h) Dry spore and control Petri-plates keeping chambers, i) Air inlet covered with mesh

parafilm. To verify the infection, Petri plates were placed inside an incubator at 27 ± 1°C for 5 days. Pathogens were re-isolated for detection of mycosis and to assess the survival agents from emerging mycelium from the cadaver. The spore structure was examined under a compound microscope.

j) Fecundity bioassay:

The total number of batches and number of eggs per female from uninfected and infected mosquitoes were recorded for both fungal strains. The fecundity and survival bioassays were conducted simultaneously in the same plastic pot setup described above inside the cabinet.

k) Data analysis:

A preference index (PI) indicating the attractive response of female *Ae. aegypti* to the volatiles emanating from the two strains of *M. anisopliae* was calculated according to the formula: PI = (T-C)/(T+C), where T is the number of mosquitoes collected in the treatment arm, and C is the number of mosquitoes collected in the control arm. A linear regression analysis was used to determine the functional relationship between log spores concentration and attractive preference index (PI) using PROBAN (Van Ark, 1995). The homogeneity of the replicated experiments was determined by using a Log-rank Test (Elandt-Johnson, 2009) at a level of 95% significance. Subsequently, the results were pooled to calculate the average preference index and standard deviation. Mean percentage survival and survival curve analyses were calculated for the survival and fecundity experiments and the results were compared by using one-way analysis of variance followed by a Duncan's post-hoc test. Mean survival curve comparison was carried out using a Log-rank (Mantel-Cox) test.

RESULTS

a) Attraction of female *Ae. aegypti* to *M. anisopliae* volatiles:

The attraction, calculated as a preference index (PI ± SE), of non-blood fed female *Ae. aegypti* exposed to volatiles emanating from *M. anisopliae*-131versus that of SDA media demonstrates a dose-dependent response (F =11.23; df =1, 22; p = 0.0029; Figure 3). Similarly, the attraction of non-blood fed female *Ae. aegypti* to the volatiles emanating from *M. anisopliae*-30 versus that of SDA media was also dose-dependent (F = 31.56; df = 1, 22; p < 0.0001; Figure 3), and when the dose-responses of the two strains against SDA media were directly compared they did not significantly differ from one another (F = 2.69; df = 1, 44; p = 0.1084; Figure 3). The resulting shared curve also demonstrated a dose-dependent response (F = 6.60; df = 1, 45; p = 0.0136).

In a direct two-choice experiment, non-blood fed female *Ae. aegypti* were significantly more attracted to the volatiles emanating from *M. anisopliae*-30 compared to those of *M. anisopliae*-131 in a dose-dependent manner (F = 12.11; df = 1.000, 46.00; p = 0.0011; Figure 4). Similarly, the blood fed females also respond to the fungal volatiles in a dose-dependent manner. However, at the lower doses, blood fed females were more attracted to the volatiles emanating from *M. anisopliae*-131 than *M.anisopliae*-30. Conversely, at higher doses, blood fed females were more attracted to the volatiles emanating from *M. anisopliae*-30 than *M.anisopliae*-131, indicating a significant difference between the attraction of the two strains for blood fed females (F = 11.16; df = 1, 45; p = 0.0017). No significant difference in the overall attraction between the physiological states observed (F = 0.08 21; df = 1, 91, p = 0.7751).

b) Effect of *M. anisopliae* on the survival rate of non-blood fed *Ae. aegypti*:

The mean percentage survival of non-blood fed *Ae. aegypti* infected with three standard doses of *M. anisopliae*-131 and that of the controls is presented in figure 5. The mean percentage survival was 0% at 6 days post-infection (dpi) at the highest dose (10^9 spores/mL), 16 dpi at the intermediate dose (10^7 spores/mL) and 20 dpi at the lowest dose (10^5 spores/mL). In comparison, the mean percentage survival of controls (0.05% Triton X-100) was 83 ± 7% up to 28 days (Figure 5A). The survival of

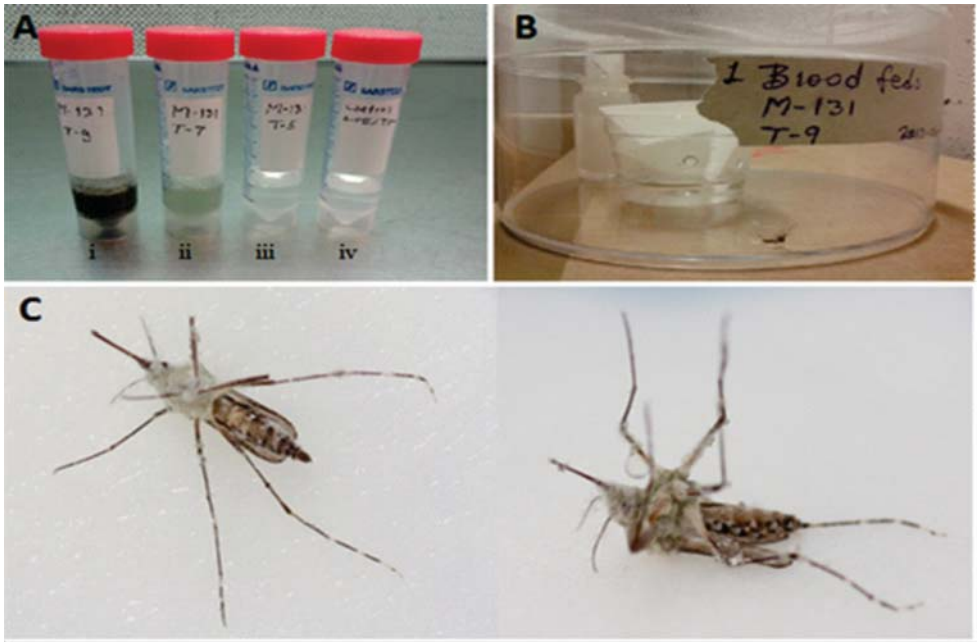

Figure 2.
A) Three different doses [(i). 10^9 spores/mL (ii). 10^7 spores/mL (iii). 10^5 spores/mL, and (iv). zero spores/mL)] of *M. anisolpiae* and control group
B) Bioassay chamber containing 10% sugar solution and moist filter paper in the small beaker for oviposition
C) *M. anisolpiae* mycelium growth from cadavers of female *Aedes aegypti*

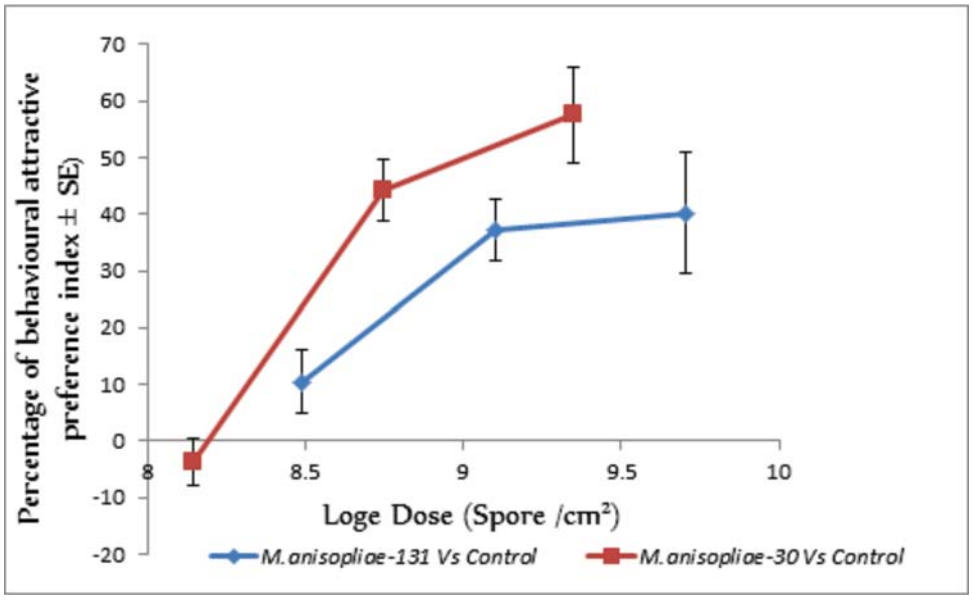

Figure 3. Behavioural responses of *Aedes aegypti* against dry spore volatiles of *Metarhizium anisopliae*

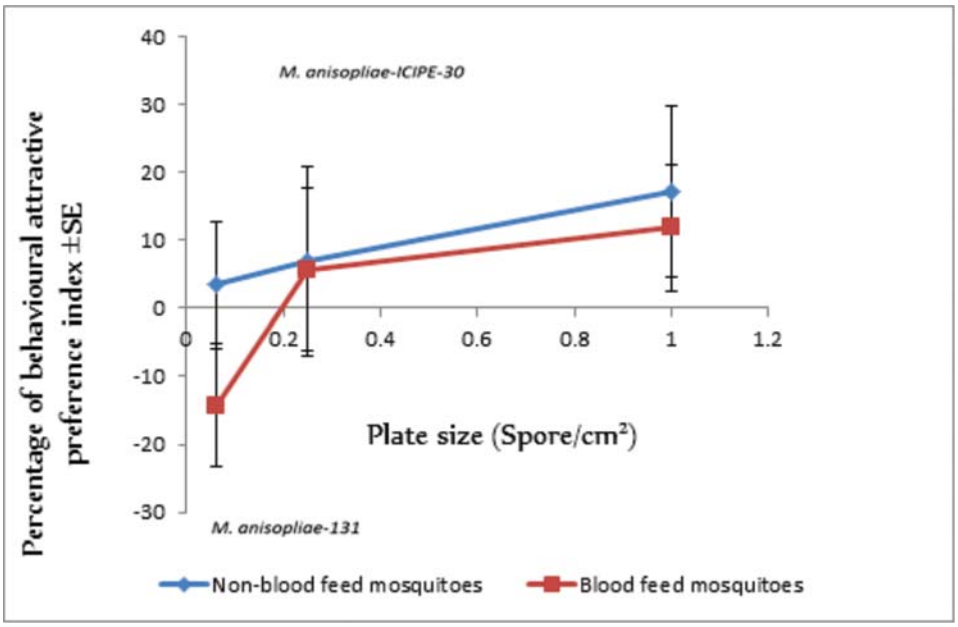

Figure 4. Comparison of *M. anisopliae*-131 and *M. anisopliae-ICIPE*-30 dry fungal spores volatiles attractive response against *Ae. aegypti* mosquitoes

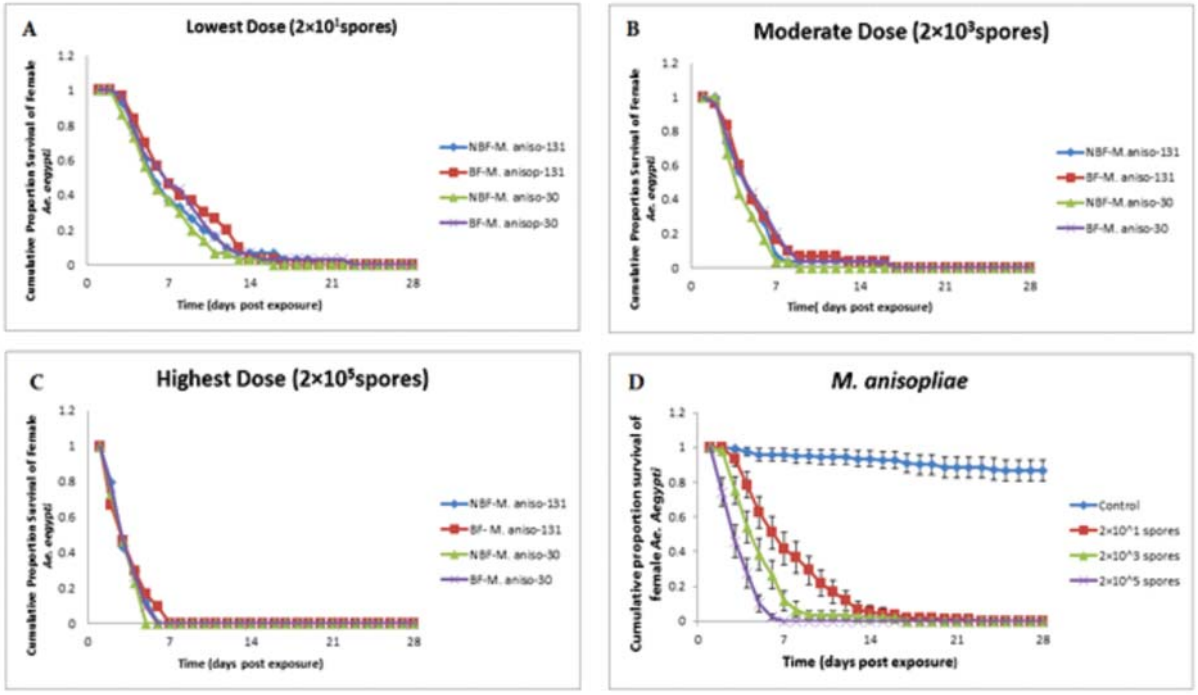

Figure 5. Overall pathogenicity of the two strains of *M. anisopliae* on blood fed and non-blood fed *Ae. aegypti*
A) Low dose ($2x10^1$ spores), B) Moderate dose ($2x10^3$ spores), C) High dose ($2x10^5$ spores), D) Combined survival curves of two strains and two physiological states at all three doses

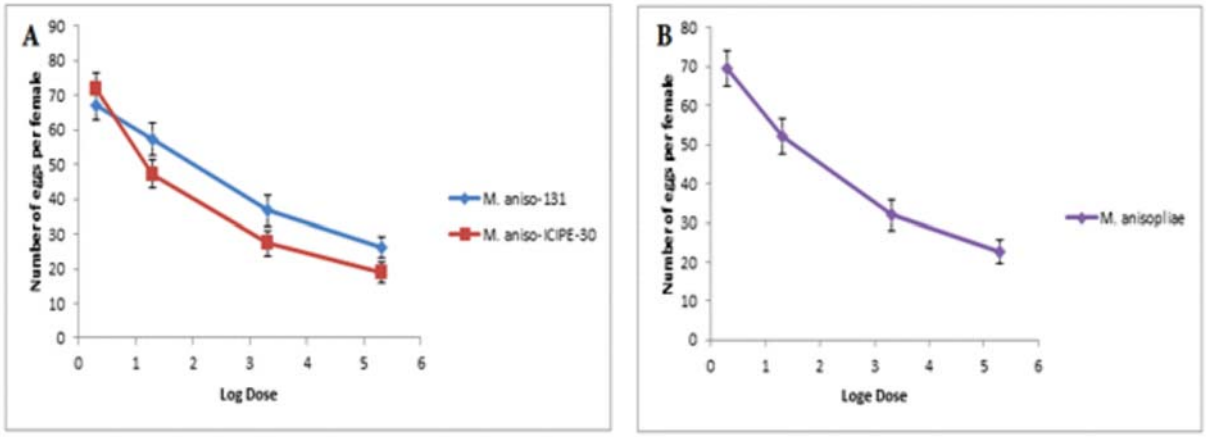

Figure 6. Effect of *M. anisopliae* on egg deposition by blood fed female *Ae. aegypti*
A) *M.* ansopliae-131 and *M. anisopliae-ICIPE*-30. B) Comparison between the two strains of *M. anisopliae* on the fecundity of infected *Ae. aegypti* showed no significant difference (P> 0.05) and the pooled one slope for all the data are significant different (P <0.05).

Ae. aegypti exposed to *M. anisopliae*-131 was significantly different from the controls ($\chi^2 = 66.5$, df = 3; $p < 0.0001$). Similarly, the survival of non-blood fed *Ae. aegypti* infected with *M. anisopliae*-30 was 0% for 5 dpi at the highest dose (10^9 spores/mL), 9 dpi at the intermediate dose (10^7 spores/mL) and 16 dpi at the lowest (10^5 spores/mL), compared to the controls, of which 87 ± 6% survived for for at least 28 dpi. This result also showed statistically significant difference ($\chi^2 = 68.44$, df = 3; $p < 0.0001$) (Figure 5B).

c) Effect of *M. anisopliae* on survival rate of blood fed *Ae. aegypti* :

The mean percentage survival of blood fed *Ae. aegypti* infected with three standard doses of *M. anisopliae*-131 and that of the controls is presented in figure 5. The mean percentage survival of blood fed female *Ae. aegypti* exposed to *M. anisopliae*-131 was 0% for 7 dpi at the highest dose (10^9 spores/mL), 17 dpi at the intermediate (10^7 spores/mL) and 17 dpi at the lowest dose (10^5 spore/mL), which is significantly different from the controls, with 87 ± 6% surviving 28 dpi ($\chi^2 = 74.91$, df = 3; $p < 0.0001$; Figure 5C). Similarly, the survival of blood fed *Ae. aegypti* exposed to *M. anisopliae*-30 was 0% for 6 dpi at the highest dose (10^9 spores/mL), 17 dpi at the intermediate dose (10^7 spores/mL) and 23 dpi at lowest dose (10^5 spores/mL), which was also significantly different from the controls with 90 ± 6% surviving 28 dpi ($\chi^2 = 77.06$; df = 3; $p < 0.0001$). Overall, the survival of the two strains and the two physiological states was significantly different and dependent on ($\chi^2 = 288.5$; df = 3; $p < 0.0001$).

d) Effect of *M. anisopliae* strains and conidia on the fecundity rate of female *Ae. aegypti:*

The number of eggs laid by blood fed female *Ae. aegypti* infected with the two strains of *M. anisopliae* is presented in figure 6. The mean number of eggs laid by blood fed *Ae. aegypti* infected with *M. anisopliae*-131 was 26 ± 3 at the higher dose (10^9 spores/mL), 37 ± 4 at the intermediate dose (10^7 spores/mL), and 57 ± 5 at the lower dose (10^5 spores/mL), which was significantly lower than the control group laying 67 ± 4 eggs (F = 54.19; df = 1.000, 118.00; $p < 0.0001$). In general, blood fed female *Ae. aegypti* exposed to *M. anisopliae*-30 laid fewer eggs than those exposed to *M. anisopliae*-131, and were significantly different (F = 77.4; df = 1.000, 118.000; $p < 0.0001$) from the control (72 ± 5), with 19 ± 3 eggs laid at the higher dose (10^9 spores/mL), 27 ± 3 at the intermediate dose (10^7 spores/mL), and 47 ± 4 at the lower dose (10^5 spores/mL). Blood fed females exposed to increasing doses of either of the strains demonstrated a dose-dependent

reduction in the number of eggs laid (F = 104.2; df = 1.000, 118.0; p < 0.0001). All eggs were laid within 5 days post-blood meal.

DISCUSSION

Developing eco-products to control vector mosquitoes and pests are gaining importance in recent times among the scientific communities. The current research was designed to obtain necessary information for the development of a myco-insecticide to control the dengue fever vector mosquito, *Ae. aegypti*. We show that *M. anisoplae* volatiles emanating from dry spore suspensions attract as well as reduce the survival and fecundity rate of female *Ae. aegypti* under laboratory conditions.

Several previous reports have shown the bio-potential of *M. anisopliae* and of other of the entomopathogenic fungi, such as *Lecanicillium, Longisporum* and *Beauveria bassiana*, against adult dengue mosquitoes (Milner *et al.*, 2003; Shah and Pell, 2003; Paula, 2008, Scholte *et al.*, 2005). The efficacy of these entomopathogenic fungi may be both increased or decreased depending upon whether the insects are attracted or repelled, respectively (Cory and Hoover, 2006). In this study, we show that both physiological states were attracted by the volatiles emanating from the two strains of *M. anisopliae*, with *M. anisopliae*-30 generally being the more attractive strain. Similar results have been observed by George *et al.* (2013), who showed that the malaria vector *Anopheles stephensi* is also attracted to the volatiles emanating from the dry spores of *M. anisoplae* on the filter-paper. This indicates that as long as *M. anisopliae* is not repellent, the conidia have a greater opportunity to infect mosquitoes using the attract and contaminate principle (Okumu *et al.*, 2010). To achieve this end, spore-treated cloth (Scholte *et al.*, 2005) and resting boxes (Lwetoijera *et al.*, 2010) can be used, as previously demonstrated for wild free-flying *Anopheles* mosquitoes.

Both fungal strains of *M. anisopliae* significantly reduced the survival of the two physiological states of female *Ae. aegypti*. Whereas the highest dose of the fungal treatment (10^9 spore/mL) did not prevent infected blood fed mosquito from laying eggs, it did reduce the average survival of adults to below the incubation period for the dengue virus, which ranges between 10-14 days (Watts, 1987; Paula *et al.*, 2011; Darbro *et al.*, 2011). Interestingly, this study suggested that the survival rate of blood-fed female *Ae. aegypti* is increased compared to that of non-blood fed following *M. anisopliae* infection. For blood fed mosquitoes, the digested blood meal may provide additional nutrients and a stronger immune response, which is a plausible explanation for the reduced mortality. This hypothesis is in agreement with that of Dana *et al.* (2005). Moreover, the increased mortality rate of non-blood fed mosquitoes may be associated with nutrient depletion but also related to immune strength, mechanical damage, and toxicosis (Ferron, 1978; Gillespie and Claydon, 1989).

Fungal infection significantly reduced the number of eggs laid as well as changed the behaviour of blood fed mosquitoes. Infected mosquitoes laid fewer eggs than the controls, however these eggs were scattered on the filter paper with other eggs placed in other regions of the plastic pot. This change was dependent on the conidia concentration but not strain, and is in line with that reported by Flores *et al*. (2004). In addition, Scholte *et al*. (2006) observed a reduction in fecundity of *An. gambiae* treated with *M. anisopliae*. This study concludes that both strains of *M. anisopliae* tested against *Ae. aegypti* are effective in reducing survival and egg laying. The development of a myco-insecticide using *M. anisopliae* has therefore a potential alternative eco-product for the management of dengue fever mosquitoes.

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Identification and molecular characterization of *Anopheles* mosquitoes in some rural areas of West Bengal, India

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ABSTRACT: During the year 2015-2016 a systemic survey has been undertaken to know the temporal and spatial distribution of *Anopheles* mosquitoes in Southern and Northern parts of West Bengal. *Anopheles vagus* and *An. subpictus* predominate in southern Bengal while *An. barbirostris* is more abundant in northern Bengal. *Anopheles* species were identified morphologically as well as by the sequencing of ITS 2 region of rDNA. © 2018 Association for Advancement of Entomology

KEY WORDS: *Anopheles*, distribution, molecular identification

INTRODUCTION

Thirty-six percent of the global population becomes the victim of the disease malaria and almost two thousand twenty million of people are at the risk of the same in ~90 countries. In the South-East Asian region India itself estimates for approximately two third of the confirmed malarial cases. In accordance with Singh and Sharma (2002) the central and eastern parts of India are the most vulnerable areas of the disease malaria. According to World malaria report, 2009 five states including West Bengal account for sixty percent of cases of malaria. The *Anopheles* is the only mosquito taxon that is root cause for the transmission of malaria. *Anopheles* also transmits dirofilarial nematodes and arboviruses of veterinary importance (Ramachandra, 1984). 444 formally named species and 40 unnamed species complexes are identified as distinct species of *Anopheles* (Harbach, 2004). India possesses about 58 morphologically identified species of *Anopheles*. It has been narrated that about 13 species are available in Kolkata and sub urban areas of West Bengal. Out of 58 species of *Anopheles* found in our country, six taxa are considered as major malaria vectors with different regional specificities. As for example, *Anopheles culicifacies* is a vector of rural areas in our country and generates about sixty five percent of malaria per year. *An. fluviatilis* is found in the plains as well as in foothills and is responsible for almost 15% of malarial cases, *An. minimus* is an important vector in northeast, *An. dirus* is found in the forest areas of northeastern states where as *An. sundaicus* is mainly present in Andaman and Nicobar islands, and *An. stephensi* is a dominant vector mosquito in urban areas like Kolkata of West Bengal. Population load of this genus is mainly generated by the *An. subpictus* and *An. vagus* in different areas of West Bengal (Paul *et al.*, 2015). Each of the 40 species of anophelines transmitting human malaria differ in their transmission potential (WHO, 2005). All these mosquito species except *An. stephensi* have been characterized as species

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complexes with number of sibling species and have different roles in the transmission of malaria. The density of these vector mosquitoes varies with the seasons as well as available habitats. Therefore studies of temporal and spatial abundance of different types of *Anopheles* mosquitoes are essential for formulating the controlling strategies. The Ribosomal DNA has been used extensively and very successfully for phylogenetic analysis of both closely and distantly related organisms. Due to overlapping morphological characteristics of malaria vectors and difficulties in their identification based on morphological features, it becomes essential to distinguish closely related groups of *Anopheles* using alternative methods other than morphological taxonomy. Among the available alternative methods, the cytological method of polytene chromosome based identification has been utilized to distinguish of the cryptic species. However there are few problems namely, it defines only female mosquitoes, further it cannot be utilized in females that are unfed or fully gravid; moreover the method requires high level of technical expertise. Biochemical assays are also developed for species identification in *Anopheles* in some cases. Therefore the Ribosomal DNA probes are used for species identification in *Anopheles* (Collins *et al.,* 1996). The Polymerase chain reaction (PCR) based diagnostic assay reflects its own advantage in taxonomy as it reliable and sensitive. The PCR methods have been effectively used to distinguish Anopheles species.The internal transcribed spacer 2 (ITS2) sequence, which like the internal transcribed spacer 1 (ITS1), evolves faster than coding sequence. So, for isolation and molecular characterization of closely related *Anopheles* mosquitoes the internal transcribed spacer 2(ITS-2) region of ribosomal DNA (r DNA) has widely been used (Walton *et al.,* 1999). ITS2 regions alone have been successfully utilized in distinguishing closely related mosquito species that belongs to various genera such as *Anopheles* (Marrelli *et al.,* 2006), *Culex* (Toma *et al.,* 2000) and *Aedes* (Beebe *et al.,* 2007). Recent developments in the field of DNA-based tools, such as allele-specific PCR, PCR restriction fragment length polymorphism and single-strand conformational polymorphism assay (Wilkerson, 2005) have proven to be potential techniques for the differentiation of numerous *Anopheles* species. The present investigations were concentrated for the proper identification of the *Anopheles* species and the comparative study of sequence variations in ITS2 of the different species of *Anopheles* found in the studied areas of both south and north Bengal.

MATERIALS AND METHODS

***Collection of mosquito*:** Adult *Anopheles* mosquitoes have been collected from different areas of West Bengal. The mosquitoes have been captured in early morning (6-8 am) from different biotopes like cattle sheds and human dwellings, near to cattle shed by using manual aspirator, when the mosquitoes take rest after feeding at night.

Location	Lattitude/ Longitude
1. Mogra (Hooghly District)	$22^0$59N/ $88^0$22E
2. Singur (Hooghly District)	22.82N/ 88.23E
3. Bhotpatti (Jalpaiguri District)	26.54N/ 88.72E
4. Berubari (Jalpaigur***i*** District)	26.42N/88.70E

It is established that Sibling species A or fresh water form of *An. subpictus* is a potential vector of malaria in some regions of Hooghly district of West Bengal (Chatterjee and Chandra, 2000). Again the sub Himalayan Dooars area of the Jalpaiguri district in West Bengal is an endemic area for malaria. Rudra *et al*. (2010) reported that *Anopheles minimus, An. varuna, An. vagus, An. maculatus, An. fluviatilis, An. hyrcanus, An. barbirostris, An. culicifacies* etc. have been recorded from the tea garden dwellings of the Jalpaiguri district. Therefore the abovementioned areas have been selected for our present survey.

Identification of species based on the external morphology:

Stereozoom of Dewinter Technologies were used for the identification and for the classification of the physiological stages. All larvae were reared up to adult stage in the water collected from the water bodies from where the larvae were collected. Dried

specimens were used in morphological and molecular identifications. The collected specimens were morphologically identified according to the identification key of Christophers (1933) and Nagpal *et al*. (2005).

DNA isolation and PCR amplification:

DNA was isolated from individual adult mosquito by phenol chloroform extraction method following the protocols of Ausubel *et al*. (1999), Neetu and Choudhury (2005), Choudhury and Sharma (2006) and standardized in our laboratory. The ITS2 region of r DNA was amplified using the specific forward and reverse primer (FP, RP) consisting of 20 - 21 base oligomers having the sequence 5' TGTGAACTGCAGGACACACAT-3' (CODE 46JB) and 5'- TGTGCTTAAATTCAGGGGGT-3' (code 47JB) respectively. A PCR master mix was prepared by mixing 10X PCR buffer, dNTP mix (100mM each), $MgCl_2$, Taq polymerase, double distilled water and the template DNA. The thermal cycling condition was: initial denaturation at 95^0C for 5 min followed by 40 cycles of denaturation at 95^0C for 30 sec/1 min, annealing at 50^0-60^0C for 1 min, extension at 72^0C for 2-5 min and again final extension at 72^0C for 10 min. The PCR product and standard DNA ladder was electrophoresed in 2% agarose gel and visualized by ethidium bromide.

Statistical analysis: Mean, Standard Deviation, Standard Error were calculated using Graphpad software and chi square test was performed to make it clear whether there were any significant variation of population density of different species of *Anopheles* in different seasons.

RESULTS AND DISCUSSION

Adult *Anopheles* mosquitoes were identified morphologically. In *An. subpictus* maxillary palp possesses three pale bands. Maxillary palp is with sub-apical pale band 0.33or less in length of pre-apical dark band which is 0.5 or more in length of apical pale band. In *An. vagus* the apical pale band is larger than sub-apical pale band that is equal to pre-apical dark band (Fig. 1). Maxillary palp of *An. barbirostris* is totally black and bushy (Fig. 4). In south Bengal the mean abundance of *An. subpictus* is maximum during the period of March-May (Fig. 2a) season while *An. vagus* predominates during June to August (Fig. 2b). In case of north Bengal *An. barbirostris* could be collected throughout the year but it is most abundant during September to November and *An. pseudowillmori* is the least abundant among the collected species and it has been mostly collected during the period of September to November (Fig. 2c and 2d).

Length of ITS 2 sequences and % of GC content of the collected specimens, *An. subpictus* and *An. vagus* collected from the South Bengal, respectively were 686 bp and 831 bp and both are rich in GC 55.9 and 56.8 % respectively. Length of ITS 2 sequences and % of GC content of the collected specimens from North Bengal, indicated that *An. barbirostris* collected from North Bengal richness in the ITS 2 sequence (872), but the *An. pseudowillmori* showed 452; whereas percentage of GC content was 54.7% in *An. barbirostris* and 52.1 % in *An. pseudowillmori*.

The Gene Accession Numbers of the collected species are given in Table 1.The sequences have also been subjected to Spectral Repeat Finder (SRF) and Tandem Repeat Occurrence Locator (TROLL) programs (Sharma *et al.*, 2004; Benson, 1999) for identifying the occurrence of interspersed and tandem repeats respectively. The SRF represents various repeats which are further categorized as dimers, trimers, tetramers, pentamers and polymers. In the present sequence AC repeat shows the highest copy in *An. subpictus* where as CA and TG repeats are mostly found in *An. vagus* (Table 2). Tetramers, pentamers as well as polymers are present in both of the species populations. In Northern Bengal *An. barbirostris* predominates but *An. pseudowilmori* has also been found. TG and CA dimers show highest copies in *An. barbirostris* where as in *An. pseudowilmori* AC dimers are highest in numbers and in this species no polymers are found (Table 3). It is known that *An. pseudowillmori* is one of the predominant malarial vectors in Tibet (Song, 2009) but record of its occurrence in West Bengal is very poor. But this study reveals the presence of this

Table 1. Collected species with the collection sites and their gene accession numbers

Site of collection	Name of species	Accession Number
Hooghly district, South Bengal	*An. subpictus*	KC191825
Hooghly district, South Bengal	*An. vagus*	KT 716079
Jalpaiguri district, North Bengal	*An. barbirostris*	KU378200.1
Jalpaiguri district, North Bengal	*An. pseudowillmori*	KU378201.1

Table 2. Spectral repeat finder (SRF) based ITS2 sequence characteristics of *An. subpictus* and *An.vagus*

Sequence	*An. vagus*	*An. subpictus*
Dimers		
AC	130	113
TG	138	116
CA	138	100
Trimers		
GTG	48	42
Tetramers		
CCTA	4	5
GCAT	13	8
CGTG	15	10
GTGC	23	13
TGCA	16	8
GCGT	16	10
Pentamers		
GGTGC	6	3
Polymers		
GACGTG	0	1
CTCGGCGTG	1	1

Table 3. Spectral repeat finder (SRF) based ITS2 sequence characteristics of *An. barbirostris* and *An. pseudowilmori*

Sequence	*An. barbirostris*	*An. pesudowillmori*
Dimers		
AC	109	51
TG	129	50
CA	129	50
Trimers		
GTG	28	13
Tetramers		
CCTA	2	2
GCAT	7	2
CGTG	6	3
GTGC	8	3
TGCA	8	0
GCGT	3	4
Petamers		
GGTGC	1	1
Polymers		
GACGTG	3	0
CTCGGCGTG	0	0

species in North Bengal and it shows 93% similarity with the existing sequence in gene bank. According to Zhang (1998) repetitive sequences are important in a number of regulatory functions and are principle causes of genomic instability. Tandem repeats were lacking in all the collected species which may reveal the genomic stability of the collected specimens. The tetramer TGCA has restriction site for a restriction enzyme HpyCH4IV. The present studies suggest that the distribution of sequence polymorphism throughout the populations of a species is a type of intra-genomic mechanism that can promote genotypic variations by constantly changing sequences. Chi square tests revealed that there exists significant difference in seasonal abundance of different *Anopheles* mosquitoes in South-Bengal within the groups *An. subpictus* and *An. vagus* ($\chi^2 = 45.797^{**}$ as against the table value 16.919). But there is no significant difference among seasonal abundance of different *Anopheles* mosquitoes in North-Bengal within the groups of *An. barbirostris* and *An. psudowillmori* ($\chi^2 = 3.928^{NS}$ as against the table value 16.919).

ITS2 rDNA is a non-coding DNA sequence that is reliable and dependable for differentiation of closely related species and restriction fragment length

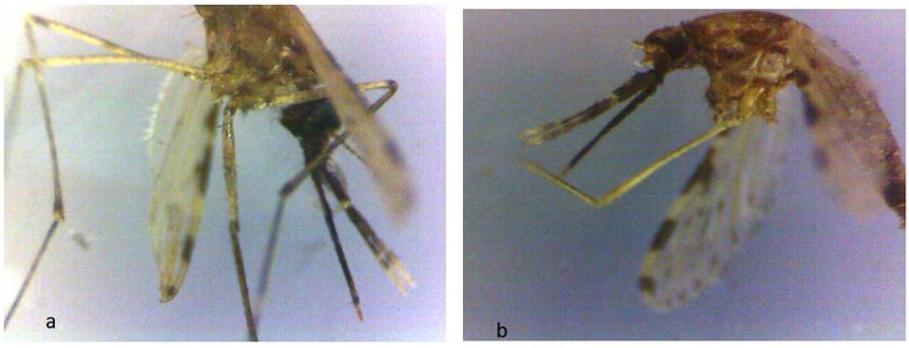
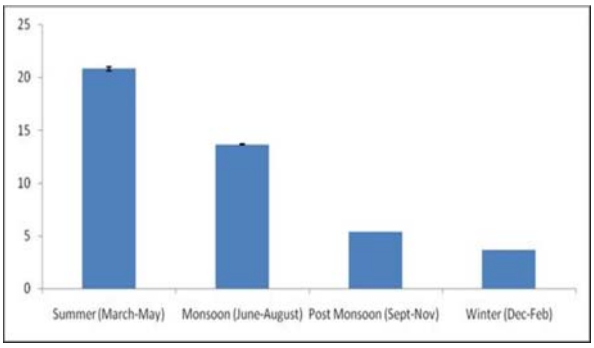
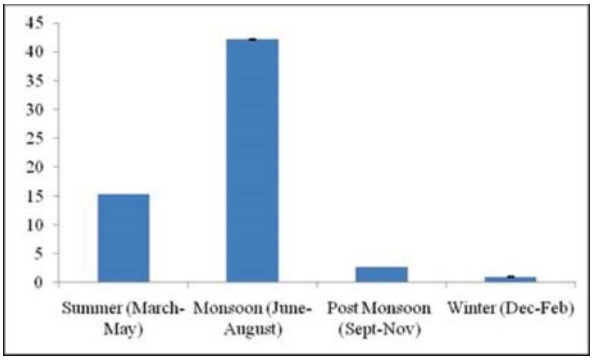


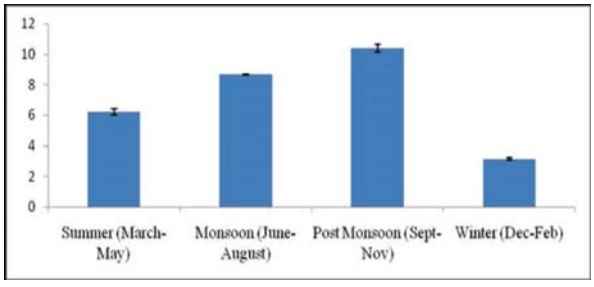
Fig. 1 Morphological identification of *Anopheles subpictus* and *An. vagus* collected from South Bengal.



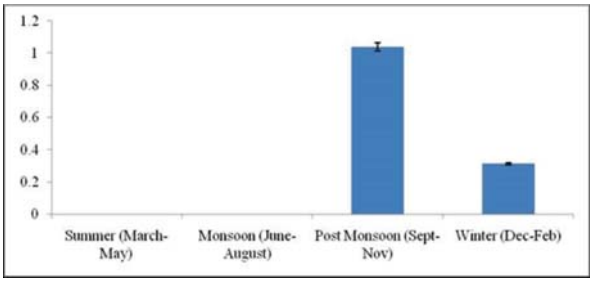
2a: An. subpictus



2b: An. vagus



2c: An. barbirostris



2d: An. pseudowillmori

Fig. 2 Graphical representation of the seasonal abundance of different types of *Anopheles* in *rural areas* during 2015-16 (Mean + Standard Error)

polymorphism of the ITS2 is a sensitive, specific and rapid method for molecular confirmation (Loaiza, 2010). ITS2 is a prudent choice to study phylogenetic relationship of closely related *Anopheles* species, as well as biodiversity and geographic races of a particular species of mosquitoes. The present investigation reveals that *An. subpictus* and *An.vagus* are predominant *Anopheles* mosquitoes in some rural and sub-urban areas of South Bengal. In rural areas *An.vagus* is most teeming in number during monsoon while *An.subpictus* predominates in sub-urban areas throughout the year. Analysis of ITS 2 indicates that both the species are GC rich and dimers are mostly found in SRF based ITS2 study. *An.subpictus* is one of the most abundant species in most parts of India. *An. subpictus* has been incriminated as a potent vector of malaria in Maldive Islands, Lakshadweep Islands etc. It is assumed (Panicker *et al.*, 1981) that this species might be responsible for the transmission of malaria in the coastal villages of Pondicherry and Tamil

(I) ITS 2 sequence of *An. subpictus*:

GTGGTATTGCTGACGCATATGGCGCATCGGACGTTTCAACCCGACCGATGCACACATCCT
TGAGTGCCTACTAGGTACTGAGAGATTCCTATAACTTGACTACAGACGGCGCCACAAACG
GGCTGACGGGCCATCCGTCGTCCGGCGTGCGACTGTGCAGCATGGCGTGCTCGGGTCTCG
GCGTGGACCCTTGGGCGCTGAAAGTGGACACTGTTTGGCGGCACCTGCGCGTGTGCTCTC
AGTGTTGACGTATGGTGAGGGTAGTGTCAAATCGCACGGTTCGACAACAAGCGTACCGTC
GAGTTTGGTGCAATCGGATGCCTACTACCATGGGCGGAGCCGGCGTGCATTCAACACTCG
ACGTCCTGTATCAACCGGATGCCAACTTGGTTGGTGGTGCCGGCGCAGACAGGACACTGA
ATCGATCTTGGTGGTACAACCCACATGTGGGTGATCAAGGAGGGGGTGTAAGTGTGGGAG
GGACACGAGGGTGGCGCCGACGCACACGCCGGCACTCACCCCCACGTCCCTCGTTGCGTG
TATTGCGGGTGATCCATAGAGTGATCTGTTTGAATAGTGGGTTGAACTGGGTATGAAAAA
AGTTACCAAAAAAGCTGACCACACTCCCAGTAGGCCTTCCATGATGTGTGACTAGATGTG
GGACTACACCCAGAATTTAAGCATAA

(II) ITS 2 sequence of *An. vagus*:

TAAGGAGTGCAAACGCTGAGAGACGCATGCGCAGTGCCGCAAGACTTGTCACATTCAGGCCCCAAGT
GTACGCGAGAGCACCGAGTCACGCCTGCTGCACAGTGCATCGCGGACAAAGATGCCCGTCAGCCCCTT
AGAGCGCCGTGTGCGTACAAGTCTATAGATAAATACTCAGTACACGTAGGCACTCACGATGTGTGCAT
CTGTCTGGTGAAACGTCCGTTGCCAAAGGCAATAAAAGGGAATTTTTTGAAATATTCCGCACACGAAC
ACCGATAAGTTGAACGCATATGGCGCATCGGACGTTTCAACCCGACCGATGCACACATCCTTGAGTGC
CTACTAGGTACTGAGATTTAACTATGACTTGACTACAGACGGCGCCACTAAAGGGCTGACGGGCCATC
CGTCGTCCGGCGTGCGACTGTGCAGCATGGCGTGCTCGGGTCTCGGCGTGGACCCTTGGGCGCTGAAA
GTGGACACTGTTTGGCGGCACCTGCGCGTGTGCTCTCAGTGTTGACGTATGGTGAGGGTAGTGTCAAG
TCGCACGGTTCGACAACAAGCGTACCGTCGAGTTTGGTGCAATCGGATGCCTACTACCATGGGCGGTG
CCGGCGTGCATTCAACACACTCGACGTCCCGTACCAACCGGATGCCTGTGAAGGCGGTGCCGGCGCAG
ACGGGACACTGAATTGATCTTGGTGATATTGGGGGATGATGGATGATGTGTGTCGCGAGTGACAACCG
GATGCCAGCGATGGCGGTGCCGGCGCACACGAGCGCTCACACACGCCTCTCCCCTCGGTCGCTTGTGG
CGTGTAACGCGTGTGA

Fig. 3 ITS2 sequences of the collected samples from South Bengal

Fig. 4 Mouth parts and tip of the hind limb of *An. barbirostris.*

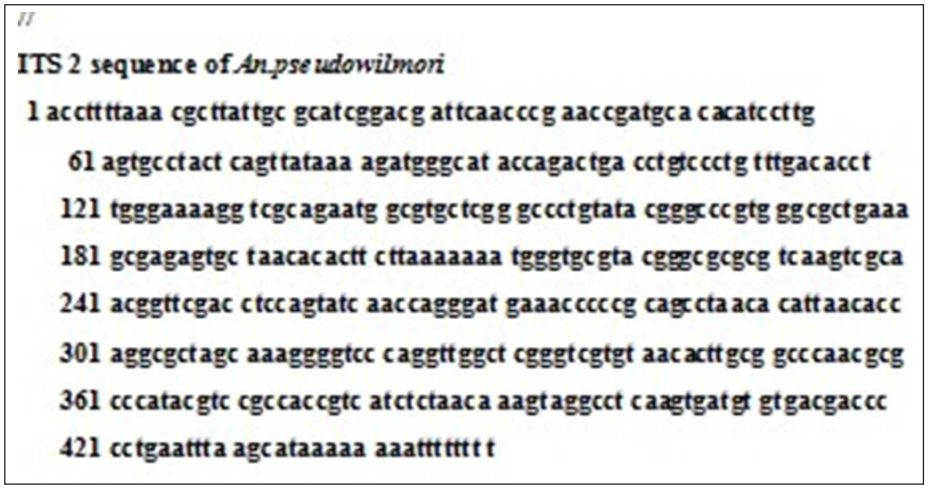


Fig. 5 ITS2 sequences of collected samples from North Bengal

Nadu. *An. subpictus* is also considered as a secondary vector in certain parts of India (Singh *et al.,* 2014) *Anopheles vagus*, is widely distributed in Asia. Evidence shows that it can function as a secondary vector. *Anopheles barbirostris* is a vector of malaria in Sri Lanka (*Amerasinghe et al., 1999),* India and Southeast Asia, and also a vector of Brugian filariasis in Southeast Asia (Lien *et al.*, 1977). In Tibet, *A. pseudowilmori* , both an indoor and outdoor species, is recognized as the principal malarial vector. The ability to efficiently and unequivocally identify the species is a priority for obtaining a clear understanding of malarial transmission in any region. These are therefore key areas for the application of this diagnostic AS-PCR assay that is relevant to vector control. Since vector incrimination is dependent upon accurate species identification, so, proper identification and study of biological characteristics are part and parcel.

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Red ant *Oecophylla smaragdina* (F.) (Hymenoptera: Formicidae) in the management of cowpea pests

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ABSTRACT: *Oecophylla smaragdina* treatment in the management of cowpea pests showed yields comparable with POP. More number of pods was harvested from T_2 (POP) followed by T_1 (red ant). Fresh weights of the pods were significantly low in control but T_1 and T_2 were on par. In the study on impact of selected pesticides on red ant showed low ant activity and number of live nests in the sprayed plants at one week after the treatment, compared to the pre-treatment count. Tobacco decoction @ 2.5 per cent did not seriously affect red ant activity.

KEY WORDS: Red ant, *Oecophylla smaragdina*, cowpea pests, indigenous knowledge

INTRODUCTION

Classical biological control has achieved some tremendous successes over the past century, yet scientists recognize that the opportunities are limited and greater attention is needed to increase the impact of native natural enemies (Greathead, 1991). The first written record of biological control dating from 304 AD is the use of red ant, *Oecophylla smaragdina* (F) (Hymenoptera: Formicidae) in Citrus (Huang and Yang, 1987). Though such time tested methods went into oblivion with the introduction of pesticides, they have been staging a comeback over the past two decades across the world (Mele, 2008). Red ants were extensively used for pest control in Africa and Asia on various crops like coconut, cocoa, coffee, citrus etc. (Mele and Cuc, 2000; Peng *et al.*, 1997; 1999; 2001). A paradigm shift in pest management have led to increased focus on *Oecophylla* in pest management of other crops like cashew, mango and timber crops also in addition to other tree crops (Mele, 2008; Sreekumar *et al.,* 2011).

Red ant, *O. smaragdina* is a self-perpetuating and effective biological control agent. The red ant is found in many different countries from Africa to Asia. Developing alternatives to pesticides is critical to maintaining agricultural production in view of the phasing out of low cost broad spectrum insecticides with newer but costly ones. This is all the more true for Kerala, since we are insisting in organic cultivation nowadays.Vegetable cowpea is an important crop of the state and harbours many pests such as aphids, pod borers, pod bugs etc. Vegetable cowpea is harvested on every alternate day without which the pods will become fibrous and non-marketable. For most of the pesticides, a minimum of five days is to be observed as waiting period which is not possible in the case of cowpea. At the same time, imparting faster methods of control is imperative in the case of cowpea to reduce crop damage and to protect aesthetic value of the produce. Sreekumar *et al*. (2006) reported that augmented control by red ant is being used by farmers in north Kerala in managing pests in kitchen gardens especially in cowpea but the effectiveness

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was not scientifically validated. Therefore studies were undertaken to know the effectiveness of *O. smaragdina* in managing the pests of cowpea and the effect of selected pesticides on *O. smaragdina* which will generate information on the use of pesticides to manage certain pest and diseases of cowpea without affecting red ant population.

MATERIALS AND METHODS

1. Effectiveness of *O. smaragdina* in managing the pests of cowpea:

An experiment was laid out in randomized block design with three treatments and seven replications. The cowpea variety Lola was raised and trailed on trellises separately. One replication contains one trellis and one trellis contains three plants. Red ants were harboured on the plant at young stage itself. Small branches of wild trees on which the nests built were cut carefully and collected directly in to plastic covers and tied properly. These nests were taken to the host plant and carefully tied on the host plant branches and trellises. Another method followed was to grow red ants on trees near the cow pea field by providing chicken offal and connecting the tree branches to cow pea trellis using nylon ropes. The plants were observed from the seedling stage to the end of the crop period. The observations noted were the number of damaged plant parts due to attack by major pests, number of adults and larval / nymphal stages of major pests and the yield. The yield parameters taken were the pod length, pod number and fresh weight of the pods. Aphid (*Aphis craccivora*) and leaf folder (*Nacoleia vulgaris*) were recorded as pests on cowpea plants during the crop period.

The treatments were, T_1: Crop harbouring red ant, T_2: Pest management as per Package of Practices Recommendations Crops (POP) of Kerala Agricultural University, that is need based application of chemical pesticide Malathion/ DDVP and T_3: Untreated control. The cultivation practices followed were as per the Package of Practices Recommendations Crops (POP) of Kerala Agricultural University viz. spacing of 2 X 2 metre trailed on pandal at the rate of three plants per pit, farm yard manure was applied at the rate of 20 tonnes per ha and lime at the rate of 250 g per ha which was applied at the time of first ploughing. NPK fertilizers were applied in the ratio of 20:30:10 kg per ha. Half the quantity of nitrogen, whole of phosphorus and potash was applied at the time of final ploughing. The remaining nitrogen was applied 15-20 days after sowing and irrigation was given properly in all the stages of growth.

2. Impact of selected pesticides on red ant:

The experiment was laid out in randomized block design with five treatments and four replications. One replication contains one trellis and one trellis contains three plants. The cowpea variety Lola was raised and trailed over trellises and red ant colonies were established on it. The following treatments - T1: DDVP 76 EC 0.076 %, T2: Bordeaux mixture 1 %, T3: Tobacco decoction 2.5 %, T4: Azadirachtin 0.03 EC 0.0003 %, T5: Control were applied on the crop. These pesticides were sprayed on the plants. The experiment was done in the flowering stage of the crop. The yield parameters were analysed using ANOVA.

Impact of the pesticides on the red ant was assessed by observing the ant activity and number of live nests made on the trellises. To measure the ant activity, the number of ant movements over 15 cm length of the chest height of the host plant in 120 seconds time period at 7 am and 11am was counted as described by Amida Saparya and Sreekumar (2017). Establishment of the ant was noted by observing the number of live nests and ant activity. The data were analysed using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

1. Effectiveness of *O. smaragdina* in managing the pests of cowpea:

a) Number of damaged plant parts due to attack by major pests

Maximum number of pods were attacked in T_3 (Control) followed by T_1 (Red ants) and minimum in T_2 (POP). But the values were not significantly different. Number of damaged leaves was significantly high in control. T_1 and T_2 were on par.

Less number of leaves were affected in T_2 in which POP recommendations were followed. In both the parameters, plants under T_3 (control) were affected more followed by T_1 and T_2 respectively. There is no significant difference found between the treatments in the case of damaged plant parts by leaf folder. Leaf folder infestation was found in the initial stage of cowpea (first month) against which no control measure was adopted (Table 1).

The major pests which infested the cowpea during the crop period were aphids and leaf folder. The attack of aphid was higher and that of leaf folder was negligible. Spraying of malathion was done in T_2 (POP) for the management of aphids. But no spraying was done for leaf folder even in POP recommended treatment. Peng and Christian (2006) reported that the treatment with red ant plus soft chemicals produced lower levels of rejected fruits than the treatment with chemical insecticides.

b) Pest population on plants

More number of aphids were found on T_3 (Control) and it was significantly higher than other two treatments. T_1 and T_2 were on par. Aphid population was found less in T_2 in which recommendations were followed based on Package of Practices compared to the other two treatments. The data on number of leaf folder larvae recorded on the cowpea plants was analysed and there was no significant difference between these three treatments (Table 1).

The number of aphids was higher compared to leaf folder. The population of leaf folder was too less to initiate any management measure. The population of aphid was more in control than in the other two treatments. The plants harboured with red ant also were infested by a higher population of aphids because of their association with red ant. Red ant used to feed on the honey dew produced by aphids and in turn they protect aphids from natural enemies which is the basis of their mutual relationship. But Mele and Cuc (2007) reported that this relationship never associated with the outbreak of aphids.

c) Yield parameters of cowpea

There was no significant difference between the treatments in the case of pod number and pod length. More number of pods were harvested from T_2 (POP) followed by T_1 (red ant) and T_3 (control). An average pod length of 42.61cm was recorded in T_2 and 41.12 cm in T_1 where as in T_3 it was only 37.61 cm. Fresh weights of the pods were significantly low in control but T_1 and T_2 were on par (Table 2). The mean pod number was not significant between treatments but the highest value was observed in T_2 (POP). The pod number is basically a varietal character. A higher pod number in T_2 though it is statistically insignificant is due to the better protection of the crop. The same is the trend with mean pod length also. The mean fresh weight of the pods was significantly high in T_2 (POP) which is on par with T_1 (red ant) which shows that red ant protect cowpea pods from attack by pests.

The cowpea harvested from red ant harboured plants had more lustre and more preferred by the consumers in the initial stage which lasted up to two months. Thereafter there was aphid infestation which reduced the aesthetic value of the produce entailing low consumer preference. The aphid

Table 1. Mean number of damaged cowpea plant parts and its population under different treatments

Treatments	Mean no. of damaged		No. of aphids
	Pod	Leaf	
T_1: Red ant	16.4	34.6	297.14
T_2: POP	15.4	16	120.42
T_3: Control	20	96.4	559.85
CD (0.05%)	14.802^{NS}	45.67^{**}	262.38^{**}

Table 2. Mean yield parameters of cowpea under different treatments

Treatments	Pod number	Pod length(cm)	Fresh weight (kg)
T_1: Red ant	157.8	41.12	2.23
T_2: POP	228.2	42.61	3.14
T_3: Control	157.8	37.61	1.49
CD (0.05%)	79.889^{NS}	5.740^{NS}	0.996^{**}

population lasted till the end of the harvesting season both in T_1 (red ant) and T_3 (control). In T_2 (POP) aphids were managed by spraying malathion two times.

2. Impact of selected pesticides on red ant:

a) Impact of selected pesticides on the number of live nests of red ant

The impact of pesticides was assessed by observing the number of live nests present and ant activity on cowpea. The numbers of live nests present were significantly lowest in DDVP treated plants consistently during the observation period of 7 days. The impact on nest building was found more in T_1 (DDVP 0.076 %) from the next day after spraying till the seventh day which was followed by T_4 (Azadirachtin 0.0003 %). The live nests present on all the plants before spraying was on par. One week after the treatment, number of live nests present on the sprayed plants were low compared to the pre-treatment count. The impact was low in T_3 (Tobacco decoction 2.5 %) (Table 3).

b) Impact of selected pesticides on the activity of red ant

The red ant activities in different trellis were on par on the day before the spray. The impact on the activity of red ants was found more in T_1 (DDVP 0.076 %) followed by T_4 (Azadirachtin 0.03 %), T_2 (Bordeaux mixture 1 %), T3 (Tobacco decoction 2.5 %) and T_5 (Control) respectively immediately after spraying. T_5 was found significantly high compared to all other treatments on the first readings taken after spraying. The impact was found lesser in T_5 followed by T_3 and T_2 one week after spraying. The treatment T_1 has much impact on the activity of red ants from the first day followed by T_4.

The impact of DDVP was more on the ant activity and nest building of red ants. The mean numbers of nests were 2.75 before spraying and from the next day of spraying itself it become 0.5 till the seventh day. The mean ant activity was 56.75 before spraying and it is reduced to 7.50 on the seventh day. The results are in agreement with the findings of Mele and Cuc (2007) who reported that only less toxic and highly selective pesticides should be used in the fields where red ants are present and organophosphates and pyrethroids should be avoided. Mele and Cuc (2000) reported that nearly all chemicals are harmful to *Oecophylla*. When compared to DDVP, Azadirachtin which is a derivative of neem has less influence on red ant activity and nest building. Azadirachtin is recommended in organic farming

Table 3. Impact of selected pesticides on the nest building of red ant

Treatments	Mean number of live nests of red ant on cowpea trellises								Mean
	Before spray	Day one	Day two	Day three	Day four	Day five	Day six	Day seven	
T_1: DDVP 0.076 %	2.75	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
T_2: Bordeaux mixture 1%	2.75	1.50	1.00	1.00	1.00	0.50	0.50	0.50	0.85
T_3: Tobacco decoction 2.5%	2.50	2.00	1.75	1.75	1.75	1.75	1.75	1.75	1.78
T_4: Azadirachtin 0.03 %	2.75	1.00	1.00	0.50	0.50	0.50	0.50	0.25	0.60
T_5: Control	2.75	1.75	1.25	1.25	1.25	1.25	1.25	1.25	1.32
SE(+/-)	0.228	0.344	0.247	0.230	0.230	0.211	0.211	0.196	
CD(0.05)	0.49 ^{NS}	0.75*	0.54*	0.502*	0.502*	0.461*	0.461*	0.427*	

Table 4. Impact of selected pesticides on the activity of red ant

Treatments	Mean activity of red ant on cowpea trellisesbefore and days after spray									Mean
	Before	same day	One	Two	Three	Four	Five	Six	Seven	
T_1: DDVP 0.076% *	56.75	14.25	12.50	8.50	10.75	8.75	8.50	10.25	7.50	10.13
T_2: Bordeaux mixture 1%	44.50	23.00	17.50	18.75	20.75	15.00	15.75	15.50	16.00	17.78
T_3: Tobacco decoction 2.5%	50.25	25.00	27.75	33.75	33.00	32.00	23.25	31.50	32.25	29.81
T_4:Azadirachtin 0.03%	62.00	20.50	17.25	13.75	9.25	11.25	10.75	9.00	7.50	12.41
T_5: Control	54.75	57.25	40.50	42.25	43.25	42.25	46.25	37.00	43.25	44
SE(+/-)	2.407	2.480	3.685	3.779	3.912	4.369	4.449	4.246	3.702	
CD(0.05)	5.244	5.403	8.031	8.234	8.524	9.521	9.695	9.251	8.067	

*Immediate mortality was noticed after the spray of DDVP

practices. Spraying of Azadirachtin reduced the number of live nests from 2.75 to 0.25 on the seventh day. The ant activity was reduced from 62 on the day before spray to 7.5 on the seventh day. Bordeaux mixture is an essential plant protection chemical which is used for managing many diseases of cowpea such as anthracnose, web blight, *Cercospora* and *Alternaria* leaf spot etc. From the data, it can be discerned that spraying of BM (1%) reduced the number of live nests from 2.75 to 0.5 on the seventh day. The ant activity was reduced from 44.5 on the day before spray to 16 on the seventh day. Tobacco decoction is usually prepared and applied by farmers for pest management in cowpea. The impact of Tobacco decoction on the nest building and ant activity was less. The ant activity was 50.25 before spraying which reduced to 32.25 on the seventh day (Table 4).

The study showed that the yields in plants as per POP recommendation and in plants in which red ants introduced for pest management are comparable. There are limitations in the use of pesticides in cowpea since waiting period is less. Harbouring red ant is an organic way of pest management which is desirable. Management of cowpea pests by tobacco decoction 2.5 % did not seriously affect ant activity.

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A comparison of sweep net, yellow pan trap and malaise trap for sampling parasitic Hymenoptera in a backyard habitat in Kerala

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ABSTRACT: The trapping efficiency of three main parasitic hymenopteran sampling gadgets, the sweep net (SN), yellow pan traps (YPT) and malaise trap (MT) was assessed in two periods-December 2013 to May 2014 and from December 2014 to May 2015. The collections were made once a month and the traps were standardized as follows-SN-100 sweeps were taken from each site, YPT- 25 traps were set in each site for a period of 24 hours and one MT was employed at each site for a period of 1 week. SN and YPT were found to be suitable for quantitative estimation of parasitoids whereas MT was more suitable for qualitative estimates. Even though each trap seemed to indicate significant collection rate for certain genera, for a comprehensive collection, a combination of the three traps are recommended. Further comparison of traps in a combination of several types of habitats is advisable for an all-encompassing assessment.

KEYWORDS: Sweep net, yellow pan trap, malaise trap, parasitic Hymenoptera

INTRODUCTION

The parasitic Hymenoptera are one of the most species rich and abundant components of terrestrial ecosystems and are estimated to comprise up to 20% of all insect species (LaSalle and Gauld, 1991). Despite this, they are a poorly studied group owing to their small size and available taxonomic expertise being limited. Increased efforts towards their study should be an integral component of future research programmes with the aim of assessing and conserving the world's biodiversity (LaSalle and Gauld, 1991). A major portion of the studies on parasitic Hymenoptera focuses on its taxonomy. Many new species are emerging in many of the families of parasitic Hymenoptera from around the world which clearly points to the fact that a lot of its diversity is still awaiting discovery. The common methods to collect parasitic Hymenoptera include sweep net, malaise trap, yellow pan trap (Narendran, 2001) and occasionally pit fall traps, flight intercept traps, beating tray and vacuum samplers (Shweta and Rajmohana, 2016). This study assessed the trapping efficiency of parasitic Hymenoptera identified upto genera from a backyard with mixed vegetation consisting of coconut trees, teak trees, a couple of mango trees and shrubs such as *Tridax procumbens*, *Mimosa pudica*, *Wedelia trilobata etc* in Kozhikode district, Kerala.

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When selecting an appropriate sampling method, one should closely consider the design of the respective sampling tools and their costs, as well as the ecological traits and habitat conditions of the target taxa (Gullan and Cranston, 2010). Sweep net (SN) is considered to be a simple and cost-effective method to collect parasitic Hymenoptera from vegetation (Narendran, 2001; Yi *et al.*, 2012). It is useful when comparing the species abundance and richness of small, vegetation-dwelling arthropods between different areas with similar vegetation types (Evans *et al.*, 1983; Siemann *et al.*, 1997). Yi *et al.* (2012) reported that it is a time-consuming method which is most suitable for open habitat types and is carried out at day time as it requires a good vision, thus causing some limitation to its wider applicability, e.g. for catching nocturnal taxa (Bartholomew and Prowell, 2005; Roulston *et al.*, 2007).

Yellow pan traps (YPT) work on the principle of yellow color being attractive to insects (Kennedy *et al.*, 1961; Hollingsworth *et al.*, 1970). The traps are filled three-fourth with a mild detergent solution to break the surface tension. Many insects get attracted to the yellow color and get collected in the soap solution. The contents are then filtered the same day and the parasitic hymenopterans are preserved in 70% ethyl alcohol.

Malaise traps (MT) make use of the negatively geotactic and positively phototactic behaviour of flying insects (Narendran, 2001). The insects in flight get intercepted by chance and move towards a collecting jar often filled with a killing agent (Campos *et al.*, 2000; Yi *et al.*, 2012). It is a passive method, as the trap is kept at fixed place and is expensive compared to SN and YPT.

The choice of appropriate approaches to collect different groups of insects has been in contention for much time. Noyes (1989) observed that even though sweep net was the most effective single method for sampling Hymenoptera, malaise trap was very effective in forest edges and yellow pan trap was effective in habitats with increased visibility of the traps. Idris *et al.* (2001) studied the effectiveness of malaise traps, yellow pan traps, flight-interception traps and sweep nets in sampling Ichneumonoidea in Malaysia and suggested to use all suitable sampling methods in order to get better collections. Wells and Decker (2006) compared yellow pan traps, malaise traps and flight interception traps to capture Hymenoptera on the island of Dominica and found yellow pan traps to be most effective followed by malaise traps and least by flight interception traps. Mazon and Bordera (2008) estimated the effectiveness of yellow pan traps and malaise traps to collect Ichneumonidae in a national park in Spain, and reported that since the relative abundance of the most common species differed in both the traps, a combination of both traps was ideal. Yi *et al.* (2012) provided a detailed review of sampling methods commonly used to collect insects along with their advantages and disadvantages. In a recent assessment of malaise traps over yellow pan traps to collect Velvet ants, Vieira *et al.* (2017) found the former to be more effective than the latter, even though yellow pan trap succeeded in collecting a few species that were rare in MT.

In India, very few works has been conducted in this regard. Pannure and Chandrashekara (2013) compared efficiency of sweep nets and pan traps to sample bee fauna in Karnataka and found sweep nets to capture more bee fauna compared to pan traps. Shweta and Rajmohana (2016) compared sweep net, yellow pan trap and malaise trap to capture Platygastridae in two urban habitats in Kerala concluding that to get a diverse collection of platygastrids, the use of MT was better over SN and YPT. Manoj *et al.* (2017) compared the pitfall trap and malaise trap to capture Platygastridae in forests of Western Ghats and found that malaise traps were ineffective to collect platygastrids in forests compared to pitfall traps. This is the first time in India that the trapping efficiency is being explored, taking into account the entire gamut of parasitic Hymenoptera. It is hoped that this work will pave the way for studies on trapping efficiencies in a wide range of habitats which will address the question whether habitat characteristics influence trapping efficiencies.

MATERIALS AND METHODS

The collections were made from a backyard habitat at Mayanad (11º16'57.42'' N 75º51'02.64''E; elevation 96 MASL; approximate distance from the Arabian sea 12 km; approximate area 1500m^2), Kozhikode district in the south Indian state of Kerala. Sampling was done for 12 months (once every month), from December 2013 to May 2014 andfrom December 2014 to May 2015 (non-monsoon months). The traps were standardized as follows-SN-100 sweeps were taken from each site. One to and fro motion of the SN was considered as one sweep. The SN measured 60 cm with a rounded bottom. The frame was made of aluminium and the sides measured 48 cm X 46 cm X 48 cm. The handle, also made of aluminium, measured up to 4 feet. The sweeps were made in the fore-noon when insect activity was prominent (9.30 am to 11.30 pm). YPT- 25 traps were set in each site, half-filled with water (approximately 20 ml) to which added 2 ml of commercially available detergent. Each rectangular trap measured 2.5cm deep with sides 14 X 8 cm. The spacing between each YPT was standardized to 1m distance. The traps were set for a period of 24 hours (Example: traps set at 10 am on one day were serviced at 10 am the following day). MT- measure approximately 6 feet wide, 3 feet and 6 inches high at one end and 6 feet and 6 inches high at the other end. One MT was employed at each site for a period of 1 week. The specimens were collected in the preservative-70% ethyl alcohol.

The alcohol containing the preserved sweep net, yellow pan trap and malaise trap collections, were transferred in small quantities into a watch glass under the microscope to ensure that even minute parasitoids (especially those belonging to Chalcidoidea) were included. They were then, preliminarily sorted into families, using keys in Narendran (2001), Goulet and Huber (1993), Gullan and Cranson (2010). The classification of Hymenoptera is as per Aguiar *et al.* (2013). The sorted collections were stored in labelled Tarson plastic vials. The taxa involved in the study were identified to their highest taxonomic resolutions possible, with the help of experts present in the home institute as well as from other institutes. The dried specimens were mounted on pointed triangular cards and studied under Olympus SZ 61 and Leica M 205-A stereomicroscopes; at a magnification of 60 to 160X.The specimens studied are deposited at the National Zoological Collection at Zoological Survey of India, Kozhikode.

The Shapiro-Wilk Normality test was applied to the data using R studio (R Core Team, 2016). As the data turned out to be not normal with P-value < 0.05, non-parametric tests were applied. The trap-wise capture rate for the three traps (SN, YPT, MT) was calculated. The mean for the genera was computed. The over-all capture of parasitic Hymenoptera for the traps was tested using Kruskal-Wallis H-test. When significant differences were found, a Mann-Whitney U-test was applied to determine which pairs of methods were different significantly (Weiss, 2007). The Kruskal-Wallis and Mann-Whitney U-test were done using MegaStat Version 10.0 (Orris, 2005).

RESULTS

A total of 1260 individuals belonging to 7 superfamilies, 19 families and 160 genera were studied. Out of the 441 parasitoids collected by SN, the most dominant superfamily was Chalcidoidea (55%) followed by Platygastroidea (23%). The superfamilies Ceraphronoidea, Ichneumonoidea, Cynipoidea and Diaprioidea were less dominant occupying 10%, 9%, 2% and 1% respectively. More than half of the parasitoids collected by SN, belong to superfamily Chalcidoidea, which included several families.

Out of the 446 parasitoids collected by YPT, the most dominant superfamily was Chalcidoidea (39%) followed by Diaprioidea (27%). The superfamilies Platygastroidea, Ceraphronoidea and Ichneumonoidea occupied 20%, 9% and 5% of the collections respectively. It was observed that even though YPT was not effective in capturing diverse superfamilies, there was more evenness between the collected superfamilies from the YPT collections.

With respect to MT, a total of 373 parasitoids were collected, with the dominant superfamilies being Chalcidoidea (36%) followed by Platygastroidea (28%). The superfamilies Ichneumonoidea, Ceraphronoidea, Diaprioidea, Evanioidea and Cynipoidea occupied 21, 7, 4, 3 and 1% respectively. MT was found most effective in capturing a wide range of parasitoid superfamilies, especially the superfamily Evanioidea, in comparison to SN or YPT. The parasitoids collected belonged to the families Agaonidae, Aphelinidae, Chalcididae, Encyrtidae, Eucharitidae, Eulophidae, Eupelmidae, Eurytomidae, Mymaridae, Pteromalidae, Torymidae, Trichogrammatidae, Braconidae, Ichneumonidae, Ceraphronidae, Diapriidae, Evaniidae, Figitidae and Platygastridae (Fig. 1).

Many of the genera were represented by single individuals, which were not suitable for meaningful statistical comparison. A complete list of the genera collected is appended (Appendix 1).

The average capture rate of the traps was calculated by dividing the number of parasitic wasps collected by one trap with the total number of parasitic wasps collected by all the traps. The average capture rate for YPT and SN was 0.353 and 0.350 respectively (very similar values) and least for MT (0.296). Kruskal-Wallis H-test revealed significant differences among the different methods of collection, based on the overall average capture rate for each genus (H = 18.532, df = 2, P-value = 0.001).

Applied Kruskal-Wallis H-test for testing for a significant difference between the capture rate of the traps for the collected genera. The post-hoc Mann Whitney U-test was applied further, for the genera that showed significant Kruskal-Wallis H-test values (Mean number of parasitoids captured per month presented in Table 1 and Appendix 1).

MT appeared to be significantly good at collecting the braconid genus *Neoclarkinella* Rema and Narendran and the platygastrid genus *Ceratobaeus* Ashmead.

YPT had significantly high collection rate for the parasitoids belonging to the diapriid genera *Basalys* Westwood and *Trichopria* Ashmead. MT seemed to be better over SN to collect the genus *Basalys* Westwood. YPT infact appeared to be highly suitable to collect *Basalys* Westwood with a very low P-value (1.02×10^{-5} and mean = 9.25). The mymarid genus *Acmopolynema* Ogloblin and the pteromalid genus *Dipara* Walker were significantly highly collected by YPT. The genera *Anagyrus* Howard (Encyrtidae) and *Aprostocetus* Westwood (Eulophidae) were significantly better collected by SN (Table 1).

DISCUSSION

Different sampling methods are used for collecting different insect taxa, with appropriate sampling techniques being the key for effective monitoring and biodiversity research (Yi *et al.*, 2012). Often, the sampling method to be adopted is greatly influenced by the nature of the habitat in which the study is conducted. A home-garden or backyard is defined as the traditional land use system around a homestead, where several species of plants are grown and maintained by the household members and their products are primarily intended for the family consumption (Thirumarpan and Weeraheva, 2014). The backyard in this study at Mayanad had a heterogeneous plant composition. In a previous study, Shweta and Rajmohana (2016) made a comparison of traps to capture parasitic Hymenoptera belonging to family Platygastrirdae from a backyard and found that MT was suitable to capture a wide range of genera, followed by SN and YPT. But, since the study focussed only on parasitoids belonging to family Platygastridae, the results could not be extended to represent the entire range of parasitic Hymenoptera commonly collected from backyards.

Among the three traps compared, SN and YPT were more efficient for exhaustive quantitative data whereas MT was more efficient for qualitative data in the backyard habitat.

It was interesting to note that in the study by Shweta and Rajmohana (2016) from urban habitats, the genus *Ceratobaeus* Ashmead was best collected using malaise traps, an observation that is supported

Table 1. Kruskal-Wallis and Mann-Whitney tests on the variations in the collection for parasitic Hymenoptera

Sl no	Genus	Mean no. of parasitoids			Trapping method	Mann-Whitney	Kruskal-Wallis	
		SN	YPT	MT		P-value	H	P-value
1	*Neoclarkinella* Rema and Narendran	0	0	0.75	SN/YPT YPT/MT SN/MT	+ **0.0366** **0.0366**	4.557	0.0328
2	*Basalys* Westwood	0	9.25	0.83	SN/YPT YPT/MT SN/MT	**1.02×10^{-5}** **0.0002** **0.0363**	26.760	1.55×10^{-6}
3	*Trichopria* Ashmead	0.5	1.33	0.25	SN/YPT YPT/MT SN/MT	**0.0227** **0.0070** 0.9390	9.340	0.0094
4	*Anagyrus* Howard	2.42	0.33	0.25	SN/YPT YPT/MT SN/MT	**0.0268** 0.6837 **0.0128**	8.617	0.0135
5	*Aprostocetus* Westwood	2.92	0.33	0.5	SN/YPT YPT/MT SN/MT	**0.0055** 0.4236 **0.02**	10.137	0.0063
6	*Acmopolynema* Ogloblin	0	0.67	0.08	SN/YPT YPT/MT SN/MT	**0.0068** **0.0273** 0.3593	10.876	0.0043
7	*Ceratobaeus* Ashmead	0	0	0.42	SN/YPT YPT/MT SN/MT	+ **0.0156** **0.0156**	6.053	0.0139
8	*Dipara* Walker	0.25	1.92	0	SN/YPT YPT/MT SN/MT	**0.0628** **0.0164** 0.3593	8.345	0.0154

Note: + Statistical interpretation impossible; Significant Mann-Whitney P-value indicated in bold

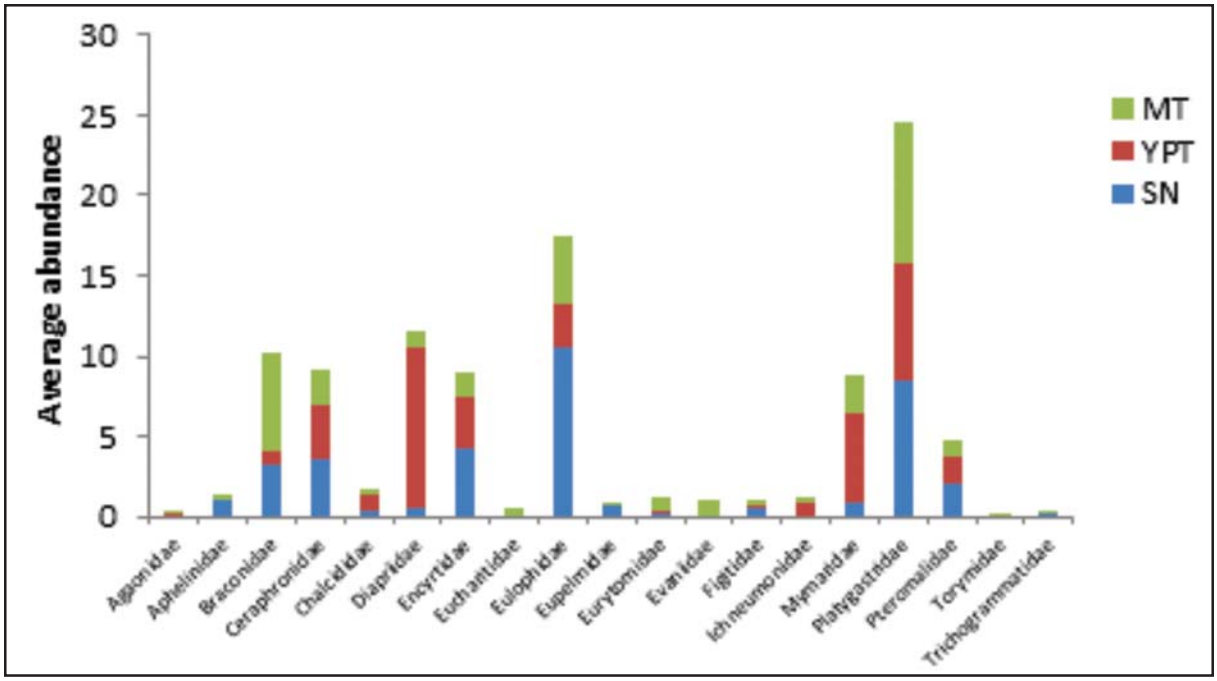

Figure 1. Relative abundance of parasitic hymenopteran families from SN, YPT and MT

by the present study. This shows that despite change in habitat characteristics, some traps tend to be highly efficient to capture certain genera. *Ceratobaeus* Ashmead is an egg parasitoid of spiders, but a more detailed investigation into the bioecology of *Ceratobaeus* Ashmead will be required to successfully explain the reason for the significant ability of MT to capture *Ceratobaeus* Ashmead over SN and YPT.

Compared to MT or SN, YPT was found to be more suitable to collect the diapriid genera- *Basalys* Westwood and *Trichopria* Ashmead. Masner (1976), Jervis and Kidd (1986) and Noyes (1989) mention that yellow pan traps are particularly efficient in sampling Diapriidae. This can be because the dipteran larvae/pupae, on which the diapriids attack are seen in soil. Rajmohana *et al.*(2013) also mentions that *Basalys* Westwood is particularly common in yellow pan trap collections. Even though it was not possible to observe a significant bias in YPT for all the diapriid genera, two of the most abundantly collected genera (*Basalys* and *Trichopria*) were clearly better collected by YPTs.

Noyes (1982) and Rameshkumar *et al.* (2015) states that due to their minute size, only yellow pan traps and malaise traps yield sufficiently good field collections of mymarids. In this study, YPT appeared to capture a sizable number of parasitoids belonging to genus *Acmopolynema* Ogloblin more significantly than MT and SN respectively. Cooper and Whitmore (1990) added that SN is more biased towards heavier and larger sized insects. Since mymarids are very small, they were not very abundant in SN collections. Callahan *et al.* (1966) found that invertebrates may be damaged by sweep-nets during collection and suggested the use of vacuum sampling to sweep-netting. It is possible that the active to-and-fro sweeping nature adopted for employing the sweep net may accidently damage the minute and fragile bodies of mymarids.

The genus *Dipara* Walker was also best collected by YPT over MT and SN. In a previous taxonomic study on species of *Dipara* Walker (Sureshan and Narendran, 2005), the collections were made exclusively from yellow pan traps possibly pointing to the propensity for YPT to collect *Dipara* Walker more than SN or MT. The species of *Dipara gastra* collected as part of this study was a brachypterous form (female) (Sureshan, 2013). We presume a better possibility for brachypterous parasitoids (like *D. gastra*) to easily get attracted to yellow pan traps placed directly on the ground and to gain access into it by crawling. It is possible that the host of this species could also be seen in close association with soil. According to Boucek (1988) and Noyes (2017) larvae of beetles could be its possible host.

The genera *Anagyrus* Howard (Encyrtidae) and *Aprostocetus* Westwood (Eulophidae), were better collected by SN in the present study. The genus *Anagyrus* Howard is almost entirely parasitic on Pseudococcidae (Hemiptera) whereas *Aprostocetus* Westwood has wide host range, and are known to parasitize Diptera (Cecidomyiidae), Hymenoptera, Cynipoidea, Coleoptera, Coccoidea and eriophyid mites. Several species are gall formers (Noyes, 2017). Evans *et al.* (1983) and Siemann *et al.* (1997) mention the effectiveness of SN to compare the species abundance and richness of vegetation dwelling small arthropods. Buffington and Redak (1998) observed that SN is biased towards foliar insects near the tips of vegetation. Overall, the SN seemed to represent an assemblage of hymenopterans that are parasitoids on hosts that are foliage dwelling and tend to be not exclusively soil-inhabiting.

The relative abundance of the parasitoids was different in the trapping methods. Where SN and YPT seem to be very suitable for quantitative estimation, MT was ideal for a wider qualitative estimation. A combination of different methods is highly recommended for a comprehensive sampling of groups like parasitic Hymenoptera where different genera vary in behaviour, ecological niche and their choice of hosts.

ACKNOWLEDGEMENTS

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Appendix 1. List of the identified genera with their mean (*-12 taxa identified only up to subfamily)

Superfamily	Family	Genus	Mean		
			SN	YPT	MT
Ceraphronoidea	Ceraphronidae	*Aphanogmus* Thomson	2.17	0.92	1.33
Ceraphronoidea	Ceraphronidae	*Ceraphron* Jurine	1.42	2.08	0.92
Ceraphronoidea	Ceraphronidae	*Cyoceraphron* Dessart	0.00	0.33	0.00
Chalcidoidea	Aphelinidae	*Aphelinus* Dalman	0.08	0.00	0.00
Chalcidoidea	Aphelinidae	*Coccophagus* Westwood	0.25	0.00	0.08
Chalcidoidea	Aphelinidae	*Encarsia* Forster	0.58	0.00	0.08
Chalcidoidea	Aphelinidae	*Coccobius* Ratzeburg	0.00	0.08	0.00
Chalcidoidea	Aphelinidae	*Pteroptrix* Westwood	0.08	0.00	0.08
Chalcidoidea	Chalcididae	*Antrocephalus* Kirby	0.08	0.25	0.00
Chalcidoidea	Chalcididae	*Hockeria* Walker	0.17	0.67	0.17
Chalcidoidea	Chalcididae	*Dirhinus* Dalman	0.08	0.00	0.00
Chalcidoidea	Chalcididae	*Neochalcis* Kirby	0.00	0.00	0.17
Chalcidoidea	Encyrtidae	*Anagyrus* Howard	2.42	0.33	0.25
Chalcidoidea	Encyrtidae	*Leptomastix* Forster	0.25	0.08	0.00
Chalcidoidea	Encyrtidae	*Copidosoma* Ratzeburg	0.67	0.83	0.42
Chalcidoidea	Encyrtidae	*Metaphaenodiscus* Mercet	0.08	0.08	0.00
Chalcidoidea	Encyrtidae	*Cheiloneurus* Westwood	0.00	0.00	0.17
Chalcidoidea	Encyrtidae	*Ooencyrtus* Ashmead	0.25	0.25	0.08
Chalcidoidea	Encyrtidae	*Adelencyrtus* Ashmead	0.08	0.00	0.08
Chalcidoidea	Encyrtidae	*Rhopus* Foerster	0.00	0.00	0.17
Chalcidoidea	Encyrtidae	*Anomalicornia* Mercet	0.00	0.08	0.00
Chalcidoidea	Encyrtidae	*Callipteroma* Motschusky	0.17	0.00	0.00
Chalcidoidea	Encyrtidae	*Leptomastidea* Mercet	0.00	0.00	0.08
Chalcidoidea	Encyrtidae	*Adektitopus* Noyes & Hayat	0.00	0.33	0.00
Chalcidoidea	Encyrtidae	*Neodusmetia* Kerrich	0.00	0.75	0.08
Chalcidoidea	Encyrtidae	*Metaphycus* Mercet	0.00	0.89	0.00
Chalcidoidea	Encyrtidae	*Paraclausenia* Hayat	0.08	0.00	0.00
Chalcidoidea	Encyrtidae	*Acerophagus* Smith	0.00	0.00	0.08
Chalcidoidea	Encyrtidae	*Aenasius* Walker	0.08	0.08	0.00
Chalcidoidea	Encyrtidae	*Trechnites* Thomson	0.00	0.08	0.00
Chalcidoidea	Encyrtidae	*Blepyrus* Howard	0.08	0.00	0.00
Chalcidoidea	Encyrtidae	*Proleurocerus* Silvestri	0.08	0.00	0.08
Chalcidoidea	Eucharitidae	*Schizaspidia* Westwood	0.08	0.00	0.08
Chalcidoidea	Eucharitidae	*Neolosbanus* Heraty	0.00	0.00	0.33
Chalcidoidea	Eulophidae	*Aceratoneuromyia* Girault	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Anaprostocetus* Graham	0.75	0.00	0.00
Chalcidoidea	Eulophidae	*Aprostocetus* Wstwood	2.92	0.33	0.50
Chalcidoidea	Eulophidae	*Dutereulophus* Schulz	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Elachertus* Spinola	0.33	0.58	0.25
Chalcidoidea	Eulophidae	*Elasmus* Westwood	0.50	0.00	0.58
Chalcidoidea	Eulophidae	*Euplectrus* Westwood	0.67	0.33	0.17
Chalcidoidea	Eulophidae	*Hyssopus* Girault	0.00	0.08	0.08

Superfamily	Family	Genus	Mean		
			SN	YPT	MT
Chalcidoidea	Eulophidae	*Leptocybe* Fisher & LaSalle	0.08	0.00	0.25
Chalcidoidea	Eulophidae	*Nesolynx* Ashmead	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Parahorismenus* Girault	0.00	0.00	0.17
Chalcidoidea	Eulophidae	*Pediobius* Walker	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Quadrastichus* Girault	0.42	0.00	0.17
Chalcidoidea	Eulophidae	*Sigmophora* Rondani	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Stenomesius* Westwood	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Sympiesis* Forster	0.29	0.00	0.29
Chalcidoidea	Eulophidae	*Tetrastichus* Haliday	3.75	1.00	0.42
Chalcidoidea	Eulophidae	*Metaplectrus* Ferriere	0.00	0.08	0.17
Chalcidoidea	Eulophidae	*Euplectrophelinus* Girault	0.00	0.00	0.17
Chalcidoidea	Eulophidae	*Trichospilus* Ferriere	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Kostjurixia* Narendran	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Tamarixia* Mercet	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Mestocharella* Girault	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Perthiola* Boucek	0.08	0.00	0.00
Chalcidoidea	Eulophidae	*Pleurotroppopsis* Girault	0.08	0.00	0.08
Chalcidoidea	Eulophidae	*Pseudosecodes* Girault & Dodd	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Rhynchentedon* Girault	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Oomyzus* Rondani	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Platyplecrus* Ferriere	0.33	0.00	0.08
Chalcidoidea	Eulophidae	*Aulogymnus* Forster	0.00	0.00	0.08
Chalcidoidea	Eulophidae	*Euplecromorpha* Girault	0.00	0.08	0.08
Chalcidoidea	Eulophidae	*Piekna* Boucek	0.08	0.00	0.00
Chalcidoidea	Eupelmidae	*Anastatus* Motschulsky	0.00	0.00	0.08
Chalcidoidea	Eupelmidae	*Calosota* Curtis	0.08	0.00	0.08
Chalcidoidea	Eupelmidae	*Neanastatus* Girault	0.58	0.00	0.17
Chalcidoidea	Eupelmidae	*Zaischnopsis* Ashmead	0.00	0.00	0.08
Chalcidoidea	Eurytomidae	*Eurytoma* Illiger	0.17	0.17	0.83
Chalcidoidea	Mymaridae	*Anagrus* Haliday	0.00	1.00	0.00
Chalcidoidea	Mymaridae	*Gonatocerus* Nees	0.75	2.17	1.00
Chalcidoidea	Mymaridae	*Camtoptera* Forster	0.00	0.00	0.08
Chalcidoidea	Mymaridae	*Mymar* Curtis	0.08	0.2	0.58
Chalcidoidea	Mymaridae	*Polynema* Haliday	0.00	0.33	0.33
Chalcidoidea	Mymaridae	*Achmopolynema* Oglobin	0.00	0.67	0.08
Chalcidoidea	Mymaridae	*Ooctonus* Haliday	0.00	0.00	0.08
Chalcidoidea	Mymaridae	*Anaphes* Haliday	0.08	0.17	0.17
Chalcidoidea	Mymaridae	*Ptilomymar* Annecke and Doutt	0.00	0.00	0.00
Chalcidoidea	Platygastridae	*Alaptus* Westwood	0.00	0.00	0.08
Chalcidoidea	Pteromalidae	*Dipara* Walker	0.25	1.92	0.00
Chalcidoidea	Pteromalidae	*Dinarmus* Thompson	0.08	0.00	0.00
Chalcidoidea	Pteromalidae	*Systasis* Walker	0.08	0.00	0.00
Chalcidoidea	Pteromalidae	*Spalangia* Latreille	0.08	0.00	0.00

Superfamily	Family	Genus	Mean		
			SN	YPT	MT
Chalcidoidea	Pteromalidae	*Propicroscytus* Szelenyi	0.75	0.08	0.67
Chalcidoidea	Pteromalidae	Eunotinae Ashmead*	0.08	0.00	0.08
Chalcidoidea	Pteromalidae	*Pteromalus* Swederus	0.58	0.00	0.25
Chalcidoidea	Pteromalidae	*Netomocera* Boucek	0.00	0.08	0.00
Chalcidoidea	Trichogrammatidae	*Trichogramma* Westwood	0.17	0.00	0.08
Chalcidoidea	Torymidae	*Podagrion* Spinola	0.00	0.00	0.08
Chalcidoidea	Agaonidae	*Ceratosolen* Mayr	0.00	0.17	0.08
Cynipoidea	Figitidae	*Kleidotoma* Westwood	0.08	0.00	0.08
Cynipoidea	Figitidae	*Leptopilina* Forster	0.42	0.08	0.00
Cynipoidea	Figitidae	*Endecameris* Yoshimoto	0.00	0.00	0.17
Cynipoidea	Figitidae	*Hexacola* Forster	0.08	0.00	0.00
Cynipoidea	Figitidae	*Rhoptomeris* Forster	0.00	0.00	0.08
Cynipoidea	Figitidae	*Micruroides* Yoshimoto	0.00	0.00	0.08
Diaprioidea	Diapriidae	*Basalys* Westwood	0.00	9.25	0.83
Diaprioidea	Diapriidae	*Trichopria* Ashmead	0.50	1.33	0.25
Diaprioidea	Diapriidae	*Entomacis* Forster	0.00	0.08	0.00
Evanioidea	Evanidae	*Parevania* Kieffer	0.08	0.00	0.83
Evanioidea	Evanidae	*Evania* Fabricius	0.00	0.00	0.08
Ichneumonoidea	Braconidae	Alysiinae Leach*	0.00	0.00	0.17
Ichneumonoidea	Braconidae	*Apanteles* Forster	0.33	0.33	0.92
Ichneumonoidea	Braconidae	Opiinae Blanchard*	0.25	0.08	0.33
Ichneumonoidea	Braconidae	*Bracon* Fabricius	0.17	0.08	0.17
Ichneumonoidea	Braconidae	*Orgilonia* van Achterberg	0.00	0.00	0.08
Ichneumonoidea	Braconidae	*Phaenodus* Forster	0.08	0.00	0.50
Ichneumonoidea	Braconidae	*Choeras* Mason	0.08	0.08	0.25
Ichneumonoidea	Braconidae	*Gnamptodon* Haliday	0.42	0.00	0.33
Ichneumonoidea	Braconidae	*Cardiochiles* Nees	0.00	0.08	0.17
Ichneumonoidea	Braconidae	*Parahormius* Nixon	0.00	0.00	0.25
Ichneumonoidea	Braconidae	*Glyptapanteles*Ashmead	0.08	0.00	0.25
Ichneumonoidea	Braconidae	Lysiterminae Tobias*	0.00	0.00	0.08
Ichneumonoidea	Braconidae	*Spathius* Nees	0.33	0.00	0.08
Ichneumonoidea	Braconidae	*Rhaconotus* Ruthe	0.33	0.00	0.00
Ichneumonoidea	Braconidae	Doryctinae Forster*	0.08	0.08	0.25
Ichneumonoidea	Braconidae	*Aspidobracon* van Achterberg	0.33	0.00	0.08
Ichneumonoidea	Braconidae	*Phanerotoma* Wesmael	0.17	0.00	0.08
Ichneumonoidea	Braconidae	*Neoclarkinella* Rema & Narendran	0.00	0.00	0.75
Ichneumonoidea	Braconidae	*Testudobracon* Quicke	0.25	0.00	0.00
Ichneumonoidea	Braconidae	*Diolocogaster* Ashmead	0.00	0.00	0.08
Ichneumonoidea	Braconidae	*Acanthormius* Ashmead	0.17	0.00	0.42
Ichneumonoidea	Braconidae	Rhyssalinae Forster*	0.08	0.08	0.17
Ichneumonoidea	Braconidae	*Canalirogas* van Achterberg & Chen	0.00	0.00	0.17
Ichneumonoidea	Braconidae	Pambolinae Marshall*	0.08	0.00	0.00
Ichneumonoidea	Braconidae	Ophioninae Shuckard*	0.00	0.00	0.08

Superfamily	Family	Genus	Mean		
			SN	YPT	MT
Ichneumonoidea	Braconidae	*Heterospilus* Haliday	0.08	0.00	0.08
Ichneumonoidea	Braconidae	*Adesha* Cameroon	0.00	0.00	0.08
Ichneumonoidea	Braconidae	Euphorinae Forster*	0.00	0.00	0.17
Ichneumonoidea	Braconidae	*Euagathis* Szepligeti	0.00	0.29	0.00
Ichneumonoidea	Ichneumonidae	*Acromia* Townes	0.00	0.08	0.00
Ichneumonoidea	Ichneumonidae	*Lepotobatopsis* Ashmead	0.00	0.08	0.00
Ichneumonoidea	Ichneumonidae	Cryptinae Kirby*	0.08	0.08	0.08
Ichneumonoidea	Ichneumonidae	Orthocentrinae Forster*	0.00	0.08	0.00
Ichneumonoidea	Ichneumonidae	*Isotima* Foerster	0.00	0.33	0.17
Ichneumonoidea	Ichneumonidae	*Menaforia* Seyrig	0.00	0.08	0.00
Ichneumonoidea	Ichneumonidae	*Scenocharops* Uchida	0.00	0.08	0.00
Ichneumonoidea	Ichneumonidae	*Metopius* Panzer	0.00	0.00	0.08
Ichneumonoidea	Ichneumonidae	Ichneumoninae Latreille*	0.00	0.08	0.00
Platygastroidea	Platygastridae	*Baryconus* Forster	0.08	0.00	0.25
Platygastroidea	Platygastridae	*Calliscelio* Ashmead	0.17	0.17	0.25
Platygastroidea	Platygastridae	*Chakra* Rajmohana & Veenakumari	0.17	0.25	0.83
Platygastroidea	Platygastridae	*Ceratobaeus* Ashmead	0.00	0.00	0.42
Platygastroidea	Platygastridae	*Cremastobaeus* Ashmead	0.00	0.00	0.08
Platygastroidea	Platygastridae	*Dicroscelio* Kieffer	0.25	0.08	0.42
Platygastroidea	Platygastridae	*Duta* Nixon	0.25	0.17	0.17
Platygastroidea	Platygastridae	*Encyrtoscelio* Dodd	0.08	0.08	0.00
Platygastroidea	Platygastridae	*Gryon* Haliday	1.25	1.42	0.92
Platygastroidea	Platygastridae	*Idris* Forster	0.42	0.67	0.92
Platygastroidea	Platygastridae	*Leptacis* Forster	0.67	0.25	0.33
Platygastroidea	Platygastridae	*Macroteleia* Westwood	0.17	0.00	0.00
Platygastroidea	Platygastridae	*Narendraniola* Rajmohana	0.00	0.00	0.08
Platygastroidea	Platygastridae	*Microthoron* Masner	0.00	0.00	0.08
Platygastroidea	Platygastridae	*Odontocolus* Kieffer	0.00	0.17	0.50
Platygastroidea	Platygastridae	*Palpoteleia* Forster	0.00	0.00	0.08
Platygastroidea	Platygastridae	*Paratelenomus* Dodd	0.08	0.42	0.17
Platygastroidea	Platygastridae	*Platygaster* Latrielle	0.67	0.08	0.25
Platygastroidea	Platygastridae	*Platyscelio* Kieffer	0.00	0.08	0.00
Platygastroidea	Platygastridae	*Scelio* Latreille	0.67	0.58	0.33
Platygastroidea	Platygastridae	*Synopeas* Forster	1.17	0.58	0.25
Platygastroidea	Platygastridae	*Telenomus* Haliday	2.25	2.00	1.58
Platygastroidea	Platygastridae	*Titta* Mineo O' Connor & Ashe	0.00	0.58	0.00
Platygastroidea	Platygastridae	*Trimorus* Ashmead	0.00	0.08	0.00
Platygastroidea	Platygastridae	*Trissolcus* Ashmead	0.08	0.08	0.00
Platygastroidea	Platygastridae	*Paridris* Kieffer	0.00	0.00	0.17
Platygastroidea	Platygastridae	*Leptoteleia* Forster	0.00	0.17	0.00
Platygastroidea	Platygastridae	*Isolia* Forster	0.08	0.00	0.08
Platygastroidea	Platygastridae	*Allotropa* Forster	0.00	0.00	0.17
Platygastroidea	Platygastridae	*Iphetrachelis* Haliday	0.00	0.00	0.17
Platygastroidea	Platygastridae	*Heptascelio* Forster	0.00	0.00	0.08

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First record of the genus *Leofa* Distant (Hemiptera: Cicadellidae: Deltocephalinae) from Pakistan

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ABSTRACT: The leafhopper genus *Leofa* Distant is newly reported from Pakistan. Three species of the subgenus *Leofa* Distant, *Leofa* (*Leofa*) *mysorensis* Distant, *Leofa* (*Leofa*) *naga* Viraktamath and Viraktamath and *Leofa* (*Leofa*) *truncata* Viraktamath and Viraktamath are recorded. It's another subgenus *Leofa* (*Prasutagus*) is also reported for the first time with *Leofa (Prasutagus) pulchellus* (Distant) from Pakistan. A key is given to distinguish the two subgenera.

KEY WORDS: *Leofa* Distant, Cicadellidae, Deltocephalinae, new report

INTRODUCTION

The grass feeding leafhopper genus *Leofa* was established by Distant (1918) with *Leofa mysorensis* Distant as its type species along with another four brachypterous species. Pruthi (1930) added one new macropterous species. Viraktamath and Viraktamath (1992) revised and redescribed the genus, designated lectotypes for Distant's and Pruthi's species and considered them as junior synonyms of *L. mysorensis*, in addition to describing four new species from peninsular India. Chalam and Rao (2005) subsequently added one more species to this genus. The grassland leafhopper genus *Prasutagus* was established by Distant (1918) for *Prasutagus pulchellus* Distant, 1918 and Vilbaste (1975) described the genus *Oneratulus* for *Jassus? curtulus* Motschulsky from Sri Lanka.

The genus *Leofa* was placed earlier in the tribe Stenometopiini but subsequently transferred to Chiasmini by Zahniser (2008). He also synonymised *Oneratulus* Vilbaste, 1975, *Prasutagus* Distant, 1918, and *Tortotettix* Theron, 1982 with *Leofa* Distant 1918 and considered *Prasutagus* as one of the four genera he recognised. Duan *et al.* (2009) reviewed the subgenus *Leofa* (*Prasutagus*) Distant and described two new species from China and recently (Duan *et al.*, 2012) described another new species from Thailand and provided a checklist to the genus *Leofa* Distant.

The genus *Leofa* is characterized by having the forewing brachypterous to macropterous, ocelli close to eye, pygofer devoid of modified or macrosetae and connective V-shaped, with a very short stem or stem absent.

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In a recent collection of leafhopper fauna of Pakistan, three species in the subgenus *Leofa* (*Leofa*) Distant and one species in *Leofa (Prasutagus)* Distant represent new records to Pakistan. The material examined is deposited in the Entomological Museum of Northwest A&F University (NWAFU), Yangling, Shaanxi, China.

Leofa Distant, 1918

Leofa Distant, 1918: 86; Zahniser, 2008: 8, 10 Type species: *Leofa mysorensis* Distant, 1918.

Oneratulus Vilbaste, 1975: 233. Type species: *Jassus*? (sic!) *curtulus* Motschulsky, 1863: 98. Synonymy by Zhaniser, 2008: 8.

Prasutagus Distant, 1918: 53; Zahniser, 2008: 8. Type species: *Prasutagus pulchellus* Distant, 1918. Synonymy by Zhaniser, 2008:8.

Tortotettix Theron, 1982: 27. Type species: *Tortotettix dispar* Theron, 1982. Synonymy by Zahniser, 2008: 8.

Remarks: Zahniser (2008) gave description of the genus including a key for the four included subgenera.

Key to subgenera of *Leofa* from Pakistan

1. Submacropterous; pygofer with a well developed dorsal appendage ……*Leofa (Prasutagus)*

- Brachypterous; pygofer without dorsal appendage……*Leofa (Leofa)*

Leofa (*Prasutagus*) *pulchellus* Distant (Figs. 1-4)

Prasutagus pulchellus Distant, 1918: 53-54, fig. 57. India.

Leofa(*Prasutagus*) *pulchellus*, Zahniser, 2008: 18; Duan *et al.*, 2012: 39.

Material examined: Pakistan: Punjab: 2♂, 2♀, Kallar Kahar, 11 .vii. 2017, coll. Hassan Naveed.

Remarks: This species closely resembles *L. (P) curtulus* (Motchulsky) (= *Deltocephalus oneratus* Melichar, 1903) according to figures of Vilbaste (1975). The syntypes of *D. oneratus* were examined by M. Webb and C. Viraktamath and Zahniser (2008) confirmed that these two are different. Furthermore, Duan *et al.* (2012) provided detailed information on the syntypes. This is the first record of the species from Pakistan.

Leofa mysorensis Distant, New record to Pakistan (Figs. 5-8)

Leofa mysorensis Distant, 1918: 86; Viraktamath and Viraktamath, 1992: 5, figs. 10-19. India

Leofa affinis Distant, 1918: 87. Synonymy by Viraktamath and Viraktamath, 1992: 5.India.

Leofa sanguinalis Distant, 1918: 87. Synonymy by Viraktamath and Viraktamath, 1992: 5. India.

Leofa unicolor Distant, 1918: 88. Synonymy by Viraktamath and Viraktamath, 1992: 5. India.

Leofa pedestris Distant, 1918: 88. Synonymy by Viraktamath and Viraktamath, 1992: 5. India.

Leofa parwala Pruthi, 1930: 26. Synonymy by Viraktamath and Viraktamath, 1992: 5. India.

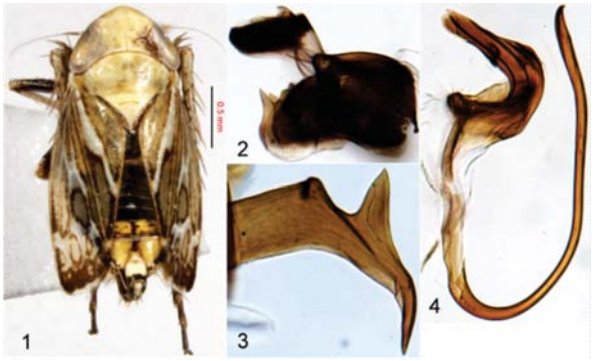
Material examined: Pakistan: Punjab: 2♂, 1♀, Piplan, 26 .vi. 2016, coll. Hassan Naveed; 1♂, 1♀, Kallar Kahar, 11 .vii. 2017, coll. Hassan Naveed.

Remarks: This species was described by Distant (1918) from India as the type species for the genus *Leofa*. Although the species is variable in colour pattern, there is little variation in the male genitalia and the female seventh sternite is usually bisinuate at the posterior margin. The aedeagal shaft of this species is rather slender and sinuate with an asymmetrically placed gonopore. This is the first record of the species from Pakistan.

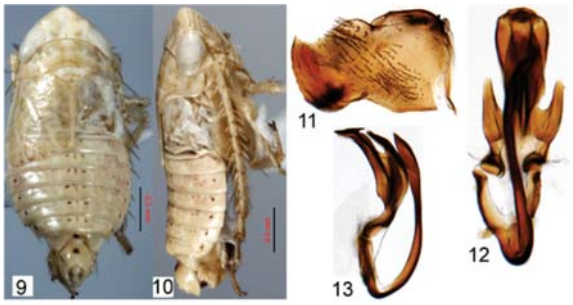
Leofa naga Viraktamath and Viraktamath (Figs. 9-13)

Leofa naga Viraktamath and Viraktamath, 1992: 9–10, figs 31–40.

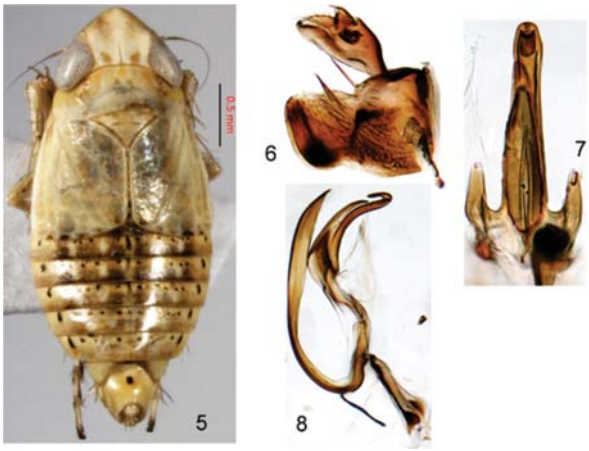
Material examined: 2♂, 2♀, Pakistan: Bhakkar City, 20 .vi. 2016, coll. Hassan Naveed; 1♀, Piplan, 22 .vi.2016, coll. Hassan Naveed; 2♂, 4♀, Chiniot,



Figs. 1-4. *Leofa (Prasutaga) pulchella* Distant
1. Habitus, dorsal view; 2. Pygofer lobe, lateral view;
3. Pygofer process, lateral view; 4. Aedeagus, lateral view



Figs. 9-13. *Leofa naga* Viraktamath and Viraktamath
9, 10. Habitus, dorsal & lateral view; 11. Pygofer lobe, lateral view; 12. Aedeagus, ventrocaudal view; 13. Aedeagus, lateral view



Figs. 5-8. *Leofa mysorensis* Distant
5. Habitus, dorsal view; 6. Pygofer lobe, lateral view; 7. Aedeagus, caudal view;
8. Aedeagus and connective, lateral view

27 .v. 2016, coll. Hassan Naveed; 2♂, 2♀, Kallar Kahar, 10 .vii.2017, coll. Hassan Naveed.

Remarks: This species closely resembles *L. mysorensnis* but can be differentiated by the aedeagus with well-developed caudal hood and laminate lateral expansion of the aedeagal shaft. This is the first record of the species from Pakistan.

***Leofa truncata* Viraktamath and Viraktamath (Figs. 14-19)**

Leofa truncata Viraktamath and Viraktamath, 1992: 4, figs. 1-9. India.

Material examined: Pakistan: Punjab: 2♂, Piplan, 24 .vi. 2016, coll. Hassan Naveed; 1♂, 1♀, Kallar Kahar, 11 .vii.2017, coll. Hassan Naveed.

Remarks: This species is considerably different from other known species of the genus *Leofa* Distant in the shape of the pygofer and subgenital plates. The species epithet 'truncata' indicates the truncate apex of subgenital plates. It also differs in the pygofer having a deep groove and a distinctive aedeagus shape. This is the first record of the species from Pakistan.

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Figs. 14 - 19. *Leofa truncate* Viraktamath and Viraktamath;
14. Habitus, dorsal view; 15. Pygofer lobe, lateral view; 6. Aedeagus, caudal view;
17. Aedeagus and connective, lateral view; 18. Style; 19. Subgenital plate, ventral view

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Key to the Indian species of the whitefly genus *Martiniella* Jesudasan and David (Hemiptera: Aleyrodidae) with description of a new species

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ABSTRACT: A new whitefly species, *Martiniella multituberculata* breeding on *Knema attenuata* from Varathahalli (Karnataka, India) is described and illustrated. Further, key to the Indian species of *Martiniella* is presented. © 2018 Association for Advancement of Entomology

KEYWORDS: Taxonomy, Aleyrodidae, *Martiniella*, new species, India

INTRODUCTION

Jesudasan and David (1990) erected the whitefly genus *Martiniella* for two species of *Aleurotuberculatus* viz., *A. canangae* and *A. macarange* described by Corbett (1935), with the former being the type species. Martin (1999) synonymised *Martiniella* with *Aleuroclava* while Sundararaj and Pushpa (2011) reinstated its generic status indicating that the presence of very much enlarged, jointed, cephalic and first abdominal setae form a distinct diagnostic character in separating *Martiniella* from all known species of *Aleuroclava.* Following this Vimala and Sundararaj (2015) revealed that the base of the seta is nothing but an elongated extension of the cuticle in the form of elongate tubercle bearing the seta at its apex and redefined the diagnostic features of the genus *Martiniella*. This genus is represented by 12 hitherto described species and is so far known from Hong Kong, India, Malaysia, Sri Lanka and Taiwan (Vimala and Sundararaj, 2015). In India, it is so far represented by seven species, *viz.*, *M. ayyari* Sundararaj and David, *M. fletcheri* (Sundararaj and David), *M. indica* (Singh), *M. lefroyi* Sundararaj and David, *M. papillata* Sundararaj and Dubey, *M. sepangensis* (Martin and Mound) and *M. tripori* (Dubey and Sundararaj) (Vimala and Sundararaj, 2015). In this paper a new species *M. multituberculata* breeding on *Knema attenuata* from Varathahalli (Karnataka: India) in south India is described and illustrated, raising the total number of Indian species of *Martiniella* to eight. A key to the Indian species of the genus is given.

Genus *Martiniella* Jesudasan and David, 1990

Type species: *Aleurotuberculatus canangae* Corbett, 1935. *J. Fed. Malay. St. Mus.* 17: 827–828; by original designation.

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Martiniella canangae (Corbett) Jesudasan and David, 1990. *FIPPAT Entomology Series*, 2: 1-13.

Aleuroclava canangae (Corbett) Martin, 1999. *CSIRO Entomology Technical Paper,* 38: 197 pp.

Martiniella canangae (Corbett) Sundararaj and Dubey, 2004. *Entomon*, 29 (4): 357-360.

Aleuroclava canangae (Corbett) Martin and Mound, 2007. *Zootaxa*, 1492: 10.

Martiniella canangae (Corbett) Sundararaj and Pushpa, 2011: 509. In: *Advancements in Invertebrate Taxonomy and Biodiversity.* Gupta, Rajiv K. (Ed.), Agrobios (International), 552 Pp; Vimala and Sundararaj, 2015. *Entomon*, 40 (4): 221-234.

Key to puparia of the Indian species of *Martiniella*

1. Thoracic tracheal pores/clefts/folds indicated 2

-. Thoracic tracheal pores/clefts/folds not indicated. 5

2. Dorsal area not smooth, with papillae or tubercles 3

-. Dorsal area smooth, without papillae or tubercles ..... ***ayyari*** Sundararaj and David

3. Submedian area smooth, without papillae or granules, only subdorsum with papillae and granules; thoracic tracheal pore region not with pouch-like structure 4

-. Entire dorsum not smooth, with papillae, granules and tubercles; thoracic tracheal pore region invaginated with pouch-like structure... . . . . ***multituberculata*** sp. nov.

4. Submargin with a row of papillae; abdominal segments I to V with median tubercles; vasiform orifice subcordate . . . .***papillata*** Sundararaj and Dubey

-. Submargin without a row of papillae; abdominal segments I to V without median tubercles; vasiform orifice subrectangular. . .. . ***lefroyi*** Sundararaj and David

5. Submargin without large subcircular lobes . . . 6

-. Submargin with three pairs of large subcircular lobes.. . . . . ***tripori*** (Dubey and Sundararaj)

6. Abdominal segments with median tubercles. . 7

-. Abdominal segments without median tubercles . . . . ***indica*** (Singh)

7. Median tubercles on abdominal segments not extending along the segment sutures; subdorsum without microtubercles; caudal furrow closed at its anterior end . . .. ***sepangensis*** (Martin and Mound)

-. Median tubercles on abdominal segments extending along the segment sutures; subdorsum with microtubercles; caudal furrow not closed at its anterior end. . . . . ***fletcheri*** (Sundararaj and David)

Description of new species:

***Martiniella multituberculata* sp. nov.**
(Figs. 1-6)

urn:lsid:zoobank.org:act:67EFB127-F17C-4CAC-9809-250C8C28BC08

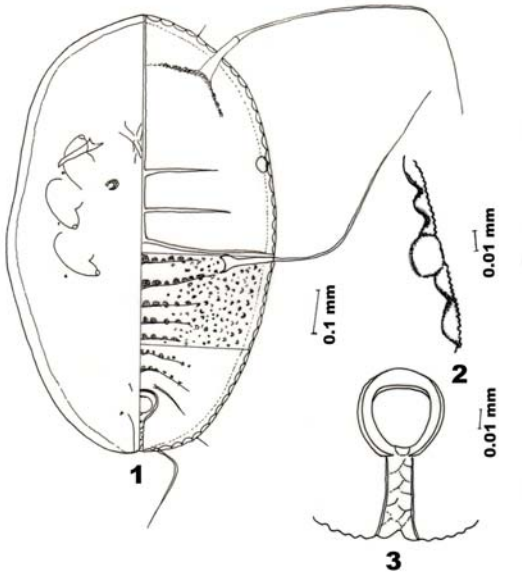
Puparium: Elliptical, slightly narrowing at posterior region, broadest at metathoracic segment region; white, transparent without any wax secretion; 0.49-0.58 mm long, 0.31-0.37 mm wide; found singly one or two per leaf, on the under surface of leaves.

Margin: Finely crenulate, 39-40 crenulations in 0.1 mm. Anterior and posterior marginal setae respectively, 10 μm and 14 μm long. Thoracic tracheal pore regions slightly emarginated and invaginated into a prominent pouch-like structure, caudal tracheal pore distinct.

Dorsum: Submargin separated from the dorsal disc by a thin submarginal ventral fold, with semicircular markings; dorsum with numerous granules and

microtubercles, abdominal segments I-VII with prominent median tubercles, and microtubercles along the thoracic and abdominal sutures distinct. Longitudinal moulting suture reaching margin, transverse moulting suture reaching submargin. Thoracic tracheal furrows indistinct, caudal tracheal furrow distinct, cylindrical shape, tassellated, 38-48 µm long, 15-18 µm wide. Pores and porettes not evident.

Chaetotaxy: Two pairs of long tuberculate setae-cephalic setae 415-420 µm long (basal long elevated tubercle 43-44 µm long and the seta at apex 372- to 376 µm long) and first abdominal setae 390-394 µm long (basal long elevated tubercle 42-44 µm and the seta at apex 348 to 350 µm long); a pair of eighth abdominal setae cephalolaterad of vasiform orifice 75 µm long and a pair of submarginal caudal setae 110-118 µm long.

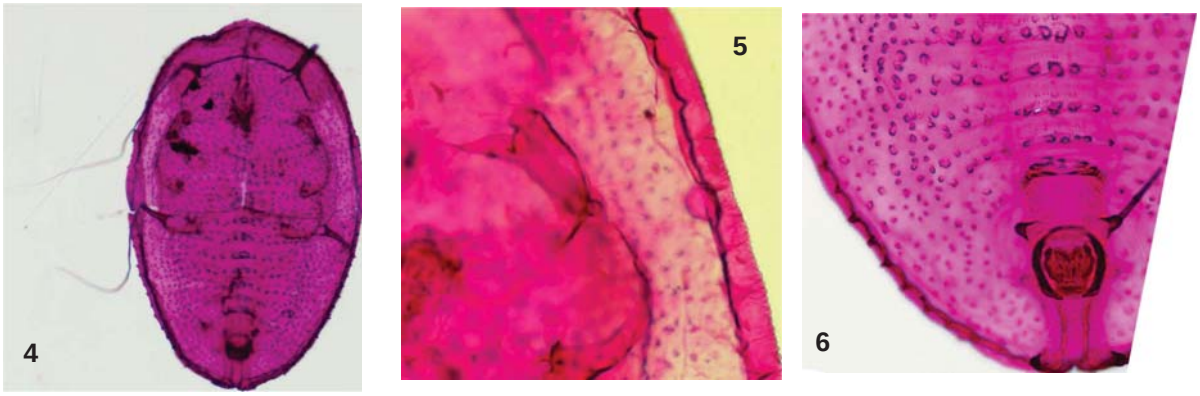


Figs. 1-3: Line diagram, *Martiniella multituberculata* sp. nov. 1. Puparium, 2. Margin at thoracic tracheal pore region, 3. Vasiform orifice

Vasiform orifice: Cordate, distinctly notched at caudal end with its lateral walls ridged, 36-39 µm long, 35-38 µm wide; operculum cordate, 20-24 µm long, 22-26 µm wide, filling the orifice, lingula tip slightly exposed, included.

Venter: A pair of ventral abdominal setae 5 µm long, 26 µm apart. Antennae reaching base of prothoracic legs. Thoracic tracheal folds not indicated while caudal tracheal fold distinct. All the four pairs of spiracles and adhesive sacs visible.

Material examined: Holotype- One puparium, mounted on slide, *Knema attenuata*, 24.x.2013, R. Sundararaj, will be deposited in the collection of National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India. **Paratypes:** Nine mounted puparia, data same as holotype, deposited one each in the collections of National Forest Insect Collection (NFIC# 22050), Forest Entomology Division, Forest Research Institute, Dehradun; Zoological Survey of India, Kolkata (5631/H15) and the remaining in the collection of Institute of Wood Science and Technology, Bangalore.



Figs. 4-6: Mounted images, *Martiniella multituberculata* sp. nov.,
4. Puparium, 5. Margin at thoracic tracheal pore region, 6. Vasiform orifice

Comments: This species closely resembles *M. papillata* Sundararaj and Dubey in shape, indication of thoracic tracheal pore regions, and presence of median tubercles on abdominal segments but differs from it in having dorsum fully covered with microtubercles and granules with microtubercles extending along the abdominal and thoracic segment sutures, thoracic tracheal pore regions with invaginated pouch-like structures and by the presence of very long cephalic, first abdominal, eighth abdominal and caudal setae. It is also close to *M. fletcheri* (Sundararaj and David) in having entire dorsum tuberculated with microtubercles extending along the abdominal and thoracic segment sutures but differs by the presence of distinct thoracic tracheal pore regions with invaginated pouch-like structures and tessellated caudal furrow.

Etymology: Named to reflect the nature of its dorsum.

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Distribution and abundance of benthic macroinvertebrates in Angereb reservoir ecosystem in Gondar, Ethiopia

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ABSTRACT: Benthic macroinvertebrates are organisms that are found at the bottom of water body living on or in the substrate. They have many applications like bioindicators of aquatic biodiversity. Investigations on distribution and abundance of benthic macroinvertebrates in Angereb reservoir in Gondar, Ethiopia, revealed that Angereb reservoir was poor in macroinvertebrate diversity in that only 16 taxa of macroinvertebrate belonging to 13 orders and 5 classes were recorded. Class Insecta was the most diverse group (9 taxa) followed by Gastropoda (3 taxa), Clitellata (2 taxa) and the least diverse classes were Malacostraca and Rhabditiphora having one taxa each. The genus Physa covers the maximum percentage abundance (12.8%) followed by family Chironomidae (10%). The pH and conductivity of Angereb reservoir water were varied among stations significantly and nitrate concentration seasonally. In addition, phosphate concentration of was higher than the standard value of lakes and reservoirs. The diversity indices showed that the macroinvertebrate diversity varied among the sampling stations. The Angereb reservoir water was polluted which was evidenced by its dominance by pollution tolerant macroinvertebrate, genus Physa. The physicochemical characteristics of water determined the abundance and composition of macroinvertebrates in Angereb reservoir.

KEY WORDS: macroinvertebrates, Angereb reservoir, diversity index, physico-chemical parameters

INTRODUCTION

Reservoirs are artificial water bodies that have dynamics and structural features of lakes and rivers (Callisto *et al.*, 2005). They have economic importance, source of water supply, power, and used to increase agriculture and aquaculture productivity. The conservation of their ecosystem and biodiversity can be implemented by their biological and ecological status (Paz *et al.*, 2008). Benthic macroinvertebrate are one of the most important biomonitoring tools (Gabriel *et al.*, 2017). Duran and Suicmez (2007) conducted a study in Cekerek stream in Tokat of Turkey and they found biological and chemical results in good agreement with regard to water quality.

Benthic macroinvertebrates are organisms that are found at the bottom of water body living on or in the substrate (Idowu and Ugwumba, 2005). Benthic macroinvertebrates can be used as indicator of changes to all the biodiversity in aquatic ecosystems (Chatzinikolaou *et al.*, 2006). They are linking agents of primary producers, detrital deposits and

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higher trophic levels of aquatic food webs (Stoffels *et al.,* 2005). They also serve as food material for other invertebrates and vertebrates (Moulton *et al.,* 2010). Macroinvertebrates are bioindicators of aquatic environmental changes (Duran and Suicmez, 2007; Oliveira and Callisto, 2010; Wibowo and Setijanto, 2017) and used to measure hydrological and anthropogenic influences on reservoirs (Martinez-Sanz *et al.,* 2012).

Diversity of benthic fauna is determined by the potential of habitat to be colonized, the presence of different ecological niches and quantity and quality of food (Slavevska-Stamenkoviæ *et al.,* 2010). In particular, the abundance of benthic macroinvertebrate in aquatic ecosystems depends on environmental variables like conductivity, alkalinity, temperature, and pH (Graça *et al.,* 2004; Weatherhead and James, 2001). The accumulation of organic matter and type of substratum (Graça *et al.,* 2004; Okorafor *et al.,* 2012) and the physical and chemical properties of the substratum are the other determinant factors of macroinvertebrate diversity (Chatzinikolaou *et al.,* 2006). In the current study area (Angereb reservoir) there is no published work on the distribution and abundance of benthic macroinvertebrates. Therefore, this study was conducted to assess the distribution and abundance of benthic macroinvertebrate in Angereb reservoir.

MATERIALS AND METHODS

Angereb reservoir is found in the eastern direction of Gondar town (Figure 1) having an area of 7653.73 ha. It was found 748 km away from Addis Ababa, Ethiopia. Angereb watershed belongs to Blue Nile basin and it has an average altitude of 2125 m.a.s.l. Angereb reservoir was constructed during early 1980 to solve drinking water shortage of Gondar town. Currently, it is the major source of drinking water supply for Gondar town (Amare, 2005).

Physico-chemical parameters of the reservoir water including pH, conductivity, temperature, turbidity, and dissolved oxygen were measured in an *in situ* monthly following Wetzel and Likens (2000). At the same time a liter of water sample was collected from each of the stations and nutrient analysis of nitrate, phosphate and ammonia were conducted following the method recommended by APHA and AWWA (1999).

The sediment samples with benthic macroinvertebrates were collected by using Ekman Grab bottom sampler of area 0.023m^2. Sampling was done by monthly basis from five different stations of Angereb reservoir for seven consecutive months within two seasons dry (February-May, 2015) and rainy (June-August, 2015). In each station, triplicate sediment samples were collected. The sediment sample with benthic macroinvertebrate was washed using the reservoir water through 250mm mesh size metal sieve. The retained benthic macroinvertebrates were transferred into well cleaned plastic pot and fixed with 8% formalin. Then macroinvertebrates were transferred to clean tray, sorted by forceps, identified with naked eyes and using dissecting microscope and counted. They were identified using standard taxonomic keys (Elliott *et al.,* 1988*;* Hynes, 1997; Kerovec, 1986) to the possible taxa level. Important biodiversity indices including Shannon – Weaver (1949) and Simpson (1949) were calculated as follow:

Species diversity (Shannon - Weaver, 1949):
$H' = -\Sigma\ Pi\ lnPi$

Where, $Pi = S / N$

S = number of individuals of one species; N = total number of all individuals in the sample

In = logarithm to base

Simpson's index (D): $\Sigma(pi)^2$; Where, pi is the proportion of the i^{th} taxon (pi = ni/N), ni is the value of i^{th} taxon

RESULTS

Among the physico-chemical characteristics of Angereb reservoir from each of the sampling stations, the pH of water varied significantly, it was lower (4.98) at station 5 during dry season and higher (8.00) at station 2 during rainy season with

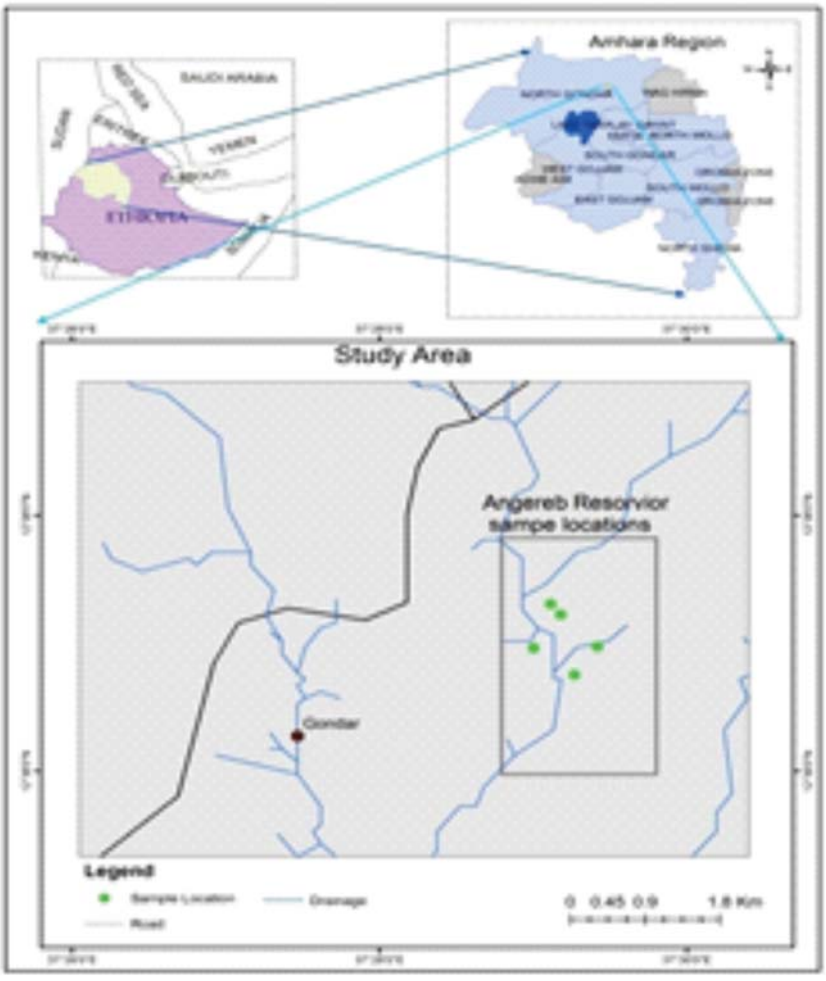

Fig. 1. Map of the study area (Angereb reservoir, Gondar)

the mean value 6.68 The surface water temperature of Angereb reservoir water is within the range of 19°C during May, 2016 at stations 4 to 28.8°C at station 1 during June, 2016 and the mean vale were 24.51°C. Conductivity showed remarkable spatial variation in that the minimum (97 ms/cm) was at station 1 and the maximum (450 ms/cm) was at station 5 and the mean value was 245.11 ms/cm. Turbidity was also varied from 42 NTU at station1 to 360 NTU station 4. Moreover, the lower dissolved oxygen (4.89 mg/l) was at station 3 and 8.42 mg/l at station 4. Chemical analysis of Angereb reservoir water indicated that there was significant monthly variation of nitrate concentration, ranging from 0.06 mg/l at station 3 and 2.30 mg/l at station 2. Phosphate varied from 0.01 mg/l at station 5 to 7.13 mg/l at station 1 with the mean value 1.08 mg/l and ammonia was in the range of 0.06 to 4.04 mg/l (Table 1).

In this study a total of 7487 individuals of benthic macroinvertebrates belonging to 5 classes and 13 orders were recorded. Class Insecta was the most diverse group (9 taxa) followed by Gastropoda (3 taxa), Clitellata (2 taxa) and the least diverse classes were Malacostraca and Rhabditiphora having a taxa for each. Again, Physa covers the maximum percentage abundance (12.8%) followed by Chironomidae (10%). All the other taxa have relative abundance less than 10%. Macroinvertebrates percentage abundance among sampling stations revealed that Chironomidae was dominant (15.5%) in station 1 and Macromia (13.2%) in station 2 whereas Physa was abundant

Taxa	Codes used in RDA figure
Macromia	Macromia
Neoperla	Neoperla
Chironomidae	Chironom
Syrphidae	Syrphida
Corduliidae	Cordulii
Dytiscidae	Dytiscid
Elimidae	Elimidae
Notonectidae	Notonect
Barbronia	Barbron
Corixidae	Corixida
Lumbriculidae	Lumbricu
Biomphalaria	Biomphal
Melanoides	Melanoid
Physa	Physa
Rhynchodemidae	Rhynchod

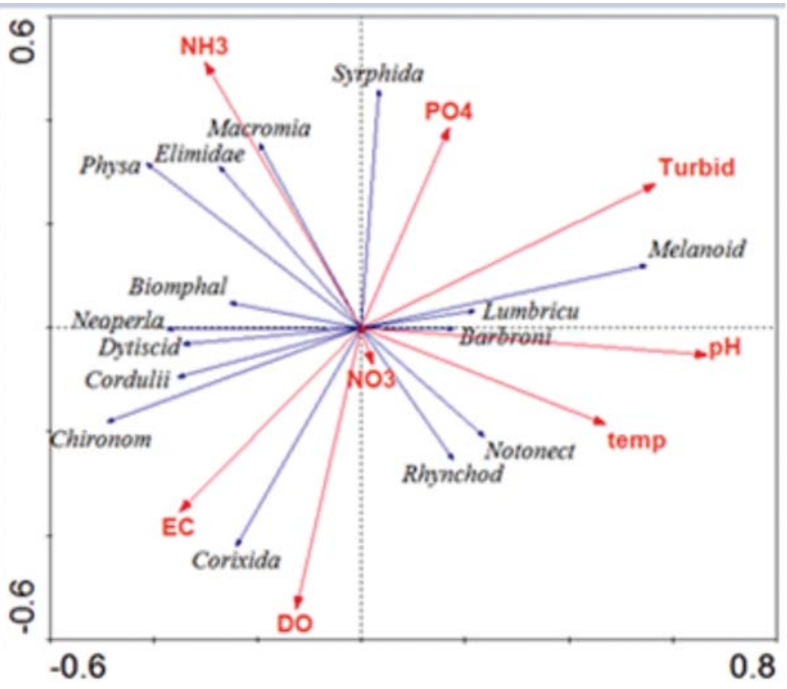

Fig.2. Biplot of redundancy analysis (RDA) analysis performed on benthic macroinvertebrates and environmental variables (DO= dissolved oxygen, EC= electrical conductivity, NH_3= ammonia, PO_4= phosphate, Turb= turbidity, pH= pH value, temp= temperature and NO_3= nitrate).

Relative abundance of macroinvertebrate classes indicated that class Insecta cover the maximum percentage (41%) followed by Gastropoda (33%), the least abundant classes were Rhabditophora and Malacostraca accounting 7% each.

In this study, the overall diversity (Shannon–Weaver) and richness (Simpson's) indices were found to be 2.60 and 0.918, respectively. In addition, indices of each sampling stations calculated showed that diversity index ranged from 1.98 to 2.51, among the five stations, station 4 (where Angereb river joins the reservoir and macrophytes are abundant) had higher diversity index (H' = 2.51). Whereas, station 2 (close to the water pumping generator installed) had lower diversity (H' = 1.98). In addition, the highest richness was recorded at station (D = 0.913) and the lowest at station 2 (D = 0.087).

Table 1. Water quality parameters of Angereb reservoir water (February-August, 2015)

Variable	Minimum	Maximum	Mean	Std. Deviation
pH	4.98	8.00	6.68	0.90
Temperature (!)	19.00	28.80	24.51	2.36
Conductivity (mS/cm)	97	450	245.11	84.54
Turbidity (NTU)	42.00	360.00	186.39	62.97
Dissolved Oxygen (mg/l)	4.89	8.42	6.44	0.90
Nitrate (mg/l)	0.06	2.30	0.69	0.54
Phosphate (mg/l)	0.01	7.13	1.08	1.50
Ammonia (mg/l)	0.06	4.04	1.64	1.17

Table 2. Abundance of benthic macroinvertebrates in Angereb reservoir water (February-August, 2015).

Taxa	Station 1		Station 2		Station 3		Station 4		Station 5		Total No.	Overall %
	No.	%	No.	%	No.	%	No.	%	No.	%		
Amphipoda	122	9.6	114	11.2	133	10.8	100	5.5	21	1	490	6.5
Macromia	56	4.4	141	13.8	111	9	93	5.1	142	6.6	543	7.3
Neoperla	37	2.9	51	5	30	2.4	167	9.1	173	8.1	458	6.1
Chironomidae	196	15.5	58	5.7	42	3.4	162	8.9	291	14	749	10.0
Syrphidae	71	5.6	64	6.3	51	4.1	44	2.4	11	0.5	241	3.2
Corduliidae	23	1.8	41	4	31	2.5	56	3.1	82	3.8	233	3.1
Dytiscidae	28	2.2	5	0.5	38	3.1	25	1.4	38	1.8	134	1.8
Elimidae	28	2.2	37	3.6	36	2.9	29	1.6	15	0.7	145	1.9
Notonectidae	46	3.6	28	2.7	12	1	27	1.5	12	0.6	125	1.7
Barbronia	39	3.1	39	3.8	12	1	17	0.9	24	1.1	131	1.8
Corixidae	91	7.2	51	5	52	4.2	155	8.5	319	15	668	8.9
Lumbriculidae	142	11.2	124	12.2	115	9.4	133	7.3	177	8.3	691	9.2
Biomphalaria	90	7.1	17	1.7	111	9	203	11.1	289	14	710	9.5
Melanoides	145	11.5	89	8.7	176	14.3	145	7.9	140	6.5	695	9.3
Physa114	9	43	4.2	181	14.7	354	19.3	265	12	957	12.8	
Rhynchodemidae	38	3	117	11.5	98	8	120	6.6	144	6.7	517	6.9

Redundancy analysis (RDA) was performed to test the relationship of the explanatory variables (macroinvertebrates) and response variables (physico-chemical variables). Accordingly, in the biplot of RDA, the first two axes explain 46.0% and 68.3% cumulative percentage variance of species-environment relation. The classes Syrphidae strongly correlated with phosphate, Melanoides with turbidiy, Lumbriculidae and Barbronia with pH and Corixidae with conductivity (Fig. 2).

DISCUSSION

Relatively lower pH was recorded in station 5 which might be due to high concentration of acid-forming chemicals like phosphate and nitrate as observed from the analysis of this study. The pH has great role in water quality determination as it influences other chemical reactions like solubility and metal toxicity. Freshwaters with a pH range of 6.0 - 9.0 have been noted to be productive and suit for fish culture (Fakayode, 2005). Therefore, based on the current study, Angereb reservoir water is comfortable for fish culture at least with regard to its pH value. Temperatures of water bodies affect the degree of proliferation and survival of aquatic microorganisms and solubility of gases and salts (Pelczar and Noel, 2005). The maximum conductivity recorded at station 5 might be due to temporal accumulation of too much suspended and dissolved solids from different parts of the reservoir for out let. In this study the phosphate concentration (1.08mg/l) of Angereb reservoir water was higher than the standard value of lakes and reservoirs 0.025mg/l and onset of higher concentration of phosphate is one of the preconditions for algal bloom (Devi *et al.*, 2008; USEPA, 2010).

Angereb reservoir has lower macroinvertebrate diversity (16 taxa) that was less than 20 taxa recorded in Gilgel Gibe I reservoir of Ethiopia (Ambelu and Goethals, 2013). This might be as a result of absence or inaccessibility of substrates and shortage of food materials for macroinvertebrates (Esenowo and Ugwumba,

2010). Domination of the reservoir by the pollution tolerant genus Physa (Clarke, 1981) during the overall study period is indication of the pollution of the angereb reservoir water. This finding was in line with my personal observation that both solid and liquid wastes were released from the villagers' kebele 13 of Gondar town in the northwest direction of angereb reservoir. In addition, Mengesha with other authors realized the absence or poor soil and water conservation practice in angereb watershed (Mengesha *et al.,* 2013). Chironomidae was the second abundant taxa next to Physa which might be related with the old age of the reservoir that accounts 30 years since its construction. Similar observation was reported in mantovo reservoir of the republic of Macedonia (Smiljkov *et al.*, 2008).

The highest diversity (H' = 2.51) was recorded at station 4and the lower diversity (H' = 1.98) was recorded at station 2. High diversity of macroinvertebrates at station 4 might be due to presence of macrophytes that are the determinant factor of macroinvertebrate diversity in a habitat (HorsÁk and HÁjek, 2003). In addition, station 4 is a place where the river joins the dam (reservoir water) so that it is arrival place for invertebrate diversities from the river (Smiljkov *et al.,* 2008). The lowest macroinvertebrate diversity (H' = 1.98) and richness (D = 0.087) at station 2 might be due to the disturbance from the water pumping generator and anthropogenic effects while operating the pumping generator. The current results in figure 3 revealed that the water physicochemical characteristics of water determined the abundance and composition of macroinvertebrates in a given aquatic ecosystem. This finding was in line with Oðuzkurt and Özhan (2008) report.

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Short Communication No. ent. 43109

Sex determination of home invading nuisance beetles, *Mesomorphus villiger* Blanchard and *Luprops tristis* Fabricius (Coleoptera: Tenebrionidae) based on pupal morphology

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ABSTRACT: A methodology based on pupal morphology for sexing two darkling beetles, *Mesomorphus villiger* and *Luprops tristis* is described. With this methodology, sexing of teneral adults is possible and is safer than the crude sternal notch methodology that involves forceful extrusion of genitalia of adults which often lead to the death of beetles.

KEY WORDS: Darkling beetles, pupa, sexing, *Mesomorphus villiger, Luprops tristis*, Tenebrionidae

Home invasion followed by formation of huge aggregation in residential buildings with the onset of monsoon season, nocturnal movements, prolonged inactivity in dormant state to tide over the rainy season, release of an irritating, odoriferous quinonic secretion that causes mild skin burns make the two darkling beetle species *Mesomorphus villiger* Blanchard 1853 and *Luprops tristis* Fabricius 1801, (Coleoptera: Tenebrionidae) serious nuisance pests in many regions of south India (Seena and Sabu, 2013 and Abitha *et al.,* 2010).

Sexing of live adults of *M. villiger* and *L. tristis* for experimental studies are done following the sternal notch methodology (Vinod *et al.*, 2008 and Arunraj and Sabu, 2012) which involves forceful extrusion of genitalia of adult. However, this crude method often affects the growth and survival of the beetles (personal observations) and not useful for sexing teneral adults. Search for alternate methods revealed that pupal morphological features used for sexing *Tenebrio molitor* (Bhattacharya *et al.*, 1970), *Alphitobius diaperinus* (Esquivel *et al.*,2012) and wax blooming beetles *Colposcelis microderoides microderoides* and *Anatolica politaborealis* (Wang *et al.,* 2013) could be used for sexing in *M. villiger* and *L. tristis*.

Pupae were obtained from field collections and from colonies maintained under laboratory conditions. Pupae were examined under a stereo zoom microscope (Labomed CZ 70; Labomed India Ltd, http://www.labomed.in) with the ventral surface facing up, to identify the differences. Sexed pupae were divided into two groups according to the morphological differences of the 8th abdominal sternite and were allowed to develop to the teneral adult stage. Confirmation of sexing was carried out by inspection of the adult genitalia with the sternal notch methodology.

Male and female pupae of *M. villiger* and *L. tristis* could be conclusively distinguished based on the morphological differences of the ventral region of the 8th abdominal sternite. A pair of prominent papillae is present on the 8th abdominal sternites in female pupae of both *M.villiger* and *L.tristis*, whereas male pupae are without similar structures

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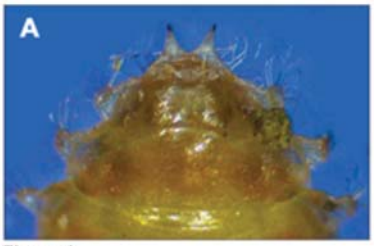


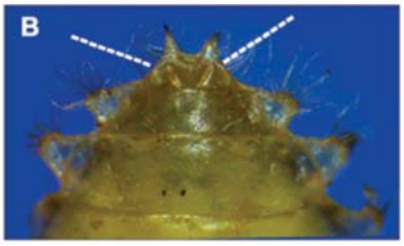
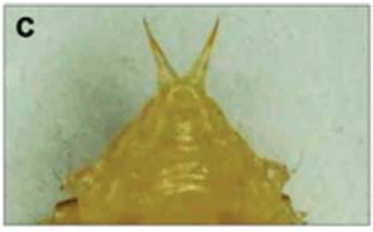


Figure 1



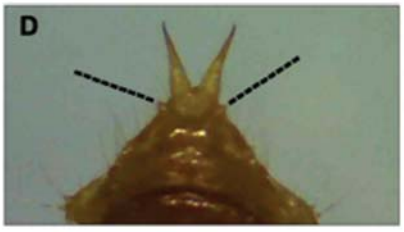


Figure 2

Fig. 1. Abdominal sternite VIII of *Mesomorphus villiger* : (A) male pupa without and (B) female pupa with paired pygopods; Fig. 2. Abdominal sternites VIII of *Luprops tristis*: (C) male pupa without and (D) female pupa with paired pygopods

(Figure 1&2). These prominent papillae are the paired pygopods. This is the simplest and least destructive method for sex differentiation of both *M.villiger* and *L.tristis.* However, univolitnism and the seasonality of pupal stage of both pest species with their occurrence confined to the pre summer periods (Sabu *et al*., 2008) make sexing based on pupal morphology difficult during other seasons and is a limitation of this methodology. During other periods, sexing of adults based on sternal notch methodology is the only option.

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A new record of the longhorn beetle *Astathes (Tetraophthalmus) bimaculata* (Coleoptera: Cerambycidae) from Maharashtra, India

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ABSTRACT: The long horned beetle *Astathes bimaculata* included in flat faced long horned beetles belongs to the subfamily Lamiinae of the family Cerambycidae. *A. bimaculata* is a very rare species in India, but it is common in Southern India and is a new record from Maharashtra state.

KEYWORDS: Cerambycidae, Coleoptera, *Astathes bimaculata*, Maharashtra

The members of Cerambycidae are commonly known as long-horned or longicorns or long-horn beetles. They are widely distributed, but mostly found in tropical region. The members of the Cerambycidae family are xylophagous and phytophagous (Ozdikmen and Caglar, 2004). Both the larva and adult of Cerambycidae are serious pests in forest area. The larvae of this family are voracious feeders and they often bore into trees (Mango, Citrus, Bamboo and Ain etc.). Cerambycidae includes numerous species almost more than 35,000 species under 4,000 genera distributed in 11 subfamilies (Lawrence, 1982) out of which more than 20,248 of lamiid species under 3,052 genera are known (Roguet, 2012). A total of 1500 species of long horn beetles are described from India (Beeson, 1941; Gahan, 1906; Breuning, 1960-62, 1963a, 1963b, 1964, 1965, 1966; Mukhopadhyay and Biswas, 2002), but in the recent past and many new records of Cerambycidae described and reported from India (Ghate and Sen, 2006; Ghate *et al*., 2011, 2012; Ghate and Mitra, 2013; Ghate and Agarwala, 2015; Vives and Ghate, 2015). The long- horn beetle *Astathes bimaculata* was first described by Fabricius in 1792, and after this species was identified by (Rondom and Breuning, 1970). The species *Astathes bimaculata* recently reported from Kerala by (Sen and Ghate, 2005). This species has been, in recent years, called as *Tetraophthalmus* and some directly place it in the genus *Tetraophthalmus* (http://cerambycidae.org/taxa/bimaculata- (Fabricius-1793).

The long-horned beetle *Astathes bimaculata* collected using insect net. The identification was done with the help of the available literature (Gahan, 1901; and Sen and Ghate, 2005).

Single specimen *Astathes bimaculata* female was collected on 10/vi/ 2016, in the mining area, near Chandgad city. Coll. Sadashiv More (Department of Zoology, R. B. Madkholkar Mahavidyalaya, Chandgad, Maharashtra, India).

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Fig.1 & 2. *Astathes bimaculata* (dorsal view)

Fig. 3. *Astathes bimaculata* (ventral view)

Fig. 4 & 5. *Astathes bimaculata* (elytra)

***Astathes bimaculata* (Fabricius, 1793)**

Cerambyx maculatus Fabricius, 1793 ([Syntypes] India Orientali ZMUC)

Astathes externa Pascoe, 1859 ([Holotype] India BMNH)

Astathes bimaculata Gahan, 1901 (Sen and Ghate, 2005)

Astathes (Tetraophthalmus) bimaculata Breuning, 1956

Diagnostic Character:

It is small sized insect with yellowish and orange colored body. Antennae somewhat fuscous and covered with black and golden pubescence with a little shorter than the body and they are not reaching

Fig. 6 & 7. *Astathes bimaculata* (lateral view)

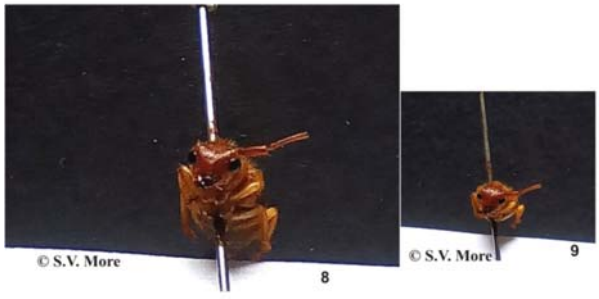

Fig. 8 & 9. *Astathes bimaculata* (front view)

the apex of elytra, first three segments of antennae are nitid, others are punctured and setose, apical segment sharply pointed (Fig. 1 & 2). Head reddish to orange, punctate, and covered with black and golden pubescence; eyes faceted (Fig. 8 & 9), ocelli black in color (Fig. 1 & 2). Thorax orange in colour, with short, blunt tubercle on each side of the prothorax (Fig. 1 & 2). Elytra yellowish, transverse violaceous patch on each side near the apex region on the elytra (Fig. 4 - 7), and covered with long, stiff, black and golden pubescence, the apex of each elytron rounded (Fig. 4). Scutellum slightly U shaped, with yellow in colour. Legs are yellowish, shorter and slender and covered with golden pubescence (Fig. 2), claws widely separated, with slightly black color. All the body covered with long, stiff, black and golden pubescence (Fig. 4). Ventral side the body covered with pale colors (Fig. 3).

Length: 12mm; Breadth: 5mm

Distribution**:** Southern India (Tranquebar, Madras, Trivandrum and Bangalore), Maharashtra.

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We are indebted to Dr. H. V. Ghate, Department of Zoology, Modern College, Pune, Maharashtra, for identification and support of this work. We are also thankful to authorities of R. B. Madkholkar Mahavidyalaya, Chandgad, Maharashtra, for providing laboratory facilities.

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Short Communication No. ent. 43111

Relative toxicity of commonly used pesticides to different stages of predator *Cheilomenes sexmaculata* (Fabricius) in cotton

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ABSTRCT: Experiment was conducted to evaluate toxicity of thirteen insecticides *viz*., thiamethoxam 25 WG, buprofezin 25 SC, clothianidin 50 WDG, diafenthiuron 50 WP, fipronil 5 SC, imidacloprid 70 WG, flonicamid 50 WDG, lamda-cyhalothrin 5 EC, methyl parathion 50 EC, thiacloprid 21.7 SC, cypermethrin 10 EC, indoxacarb 15.8 EC and chlorantranilliprole 18.5 SC against different stages of predatory coccinellid, *Cheilomenes sexmaculata*. Indoxacarb 15.8 E@ 0.008% was highly toxic at 72 hours after treatment against eggs (55.19% mortality). While, indoxacarb 15.8 E@ 0.008% and methyl parathion 50 EC@ 0.05% ware highly toxic at 72 hours after treatment against grubs and adults, with 92.96 and 83.33 per cent mortality of grubs and adults, respectively. Buprofezin 25 SC@ 0.05% caused the lowest mortality at 24, 48 and 72 hour after treatment, of all stages tested, hence can be considered as safer insecticide.

KEY WORDS: *Cheilomenes sexmaculata*, pesticides toxicity, cotton

To develop a sound pest management programme, the knowledge on the safety and adverse effect of pesticides to important natural and promising bio-control agent is essential. Therefore, attempt was made to study the effect of commonly used/new pesticides to eggs, grubs and adults of *Cheilomenes sexmaculata* a predator in cotton ecosystem.

Laboratory experiments were conducted under ambient and protected conditions at Bio-control Laboratory, Department of Agricultural Entomology, N. M. College of Agriculture, Navsari Agricultural University, Navsari during 2016-2017. Eggs, grubs and adults of predatory Coccinellids *Cheilomenes sexmaculata* were collected from research farm of Main Cotton Research Station, N.A.U., Surat. Collected insects were pre conditioned in the laboratory for about 3-4 hours before treatment.

Efficacy of thirteen commercial formulations of insecticides at recommended field dose using distilled water was evaluated under laboratory condition. Insecticides tested were insecticides viz., thiamethoxam 25 WG, buprofezin 25 SC, clothianidin 50 WDG, diafenthiuron 50 WP, fipronil 5 SC, imidacloprid 70 WG, flonicamid 50 WDG, lamda-cyhalothrin 5 EC, methyl parathion 50 EC, thiacloprid 21.7 SC, cypermethrin 10 EC, indoxacarb 15.8 EC and chlorantranilliprole 18.5 SC.

For study bioassay of eggs, the spray fluid of each insecticide prepared at desired strength was sprayed

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on the respective glass slide (7.5 x 2.5 cm) with the help of Potter's Tower spraying at a constant air pressure of 2.5 kg/m^2. A set of control treatment without any insecticidal application was maintained. The glass slides thus treated were thoroughly dried under ceiling fan and placed in a glass petridish (9.0 x 2.0 cm). Each treatment was replicated three times. Observations on the mortality of eggs were recorded at 72 h after spraying. For study of bioassay on grub and adult, third instar grub and adult of *C. sexmaculata* were used. Each of the insecticidal spray fluid was uniformly sprayed with hand sprayer on cotton leaves. The leaves were kept in plastic bowl (15.0 x 8.0 cm) and kept under ceiling fan for drying. After drying, 10 grub (3rd instar) and newly emerged adults of *C. sexmaculata* were released on treated leaves and the plastic bowl covered with lid having aeration facility and allowed to remain in contact with sprayed leaves for 45 minutes. The grub and adult were transferred to petri dish containing *A. gossypii* that served as food for the predator and the food changed daily. A control treatment without any insecticidal spray also maintained to compare the mortality. Mortality was recorded at 24, 48 and 72 hours after treatment. The mortality data was corrected with the help of Abbott's (1925) formula.

The mortality data was corrected with the help of Abbott's (1925) formula.

$$\text{Corrected mortality (\%)} = \frac{\text{\% Mortality (T)} - \text{\% Mortality(C)} \times 100}{100 - \text{\% mortality (control)}}$$

The data obtained were analyzed with appropriate methods of transformation using Completely Randomized Block Design with three repetitions.

Data on the toxicity of insecticides against to eggs exhibited 44.81 % and 55.19 % mortality in methyl parathion 50

EC @ 0.05% and indoxacarb 15.8 EC @ 0.008% and lowest mortality of eggs observed in buprofezin 25 SC @ 0.05% (17.04%) Neetan and Aggarwal (2012) reported that buprofezin 25 SC @ 0.05% and imidacloprid 70 WG @ 0.014% recorded lower per cent mortality of eggs of *C. sexmaculata* at 72 hours after exposure and categorized them harmless. They also reported that buprofezin 25 SC recorded significantly higher (76.66%) hatching of eggs of *C. sexmaculata* followed by imidacloprid (70.00%). As per the report of Awasthi *et al.* (2013) found that indoxacarb 15.8 EC was toxic to different predatory stage of Coccinellids. At 72 hours after exposure, the insecticide lamda-cyhalothrin 5 EC @ 0.0025%, cypermethrin 10 EC @ 0.01%, methyl parathion 50 EC@ 0.05% and indoxacarb 15.8 EC @ 0.008% recorded 67.78 to 92.96 per cent mortality in grubs. The lowest mortality was recorded in buprofezin 25 SC @ 0.05% (21.11%) which was on par with clothianidin 50 WDG @ 0.025% (28.15%) and flonicamid 50 WDG @ 0.015% (28.15%) (Table 1). Pandi *et al.* (2013) reported buprofezin at recommended dose @ 0.013% considered as safer for grub of *C. sexmaculata*, because it produced 22% mortality at 72 hrs after treatment and it categorized under harmless category. Whereas, in present study cypermethrin 10 EC @ 0.01% (71.11% mortality) and slightly harmful. Tank *et al.* (2007) reported cypermethrin 0.015 per cent recorded 74.33 per cent larval mortality of *C. sexmaculata.*

At 72 h after treatment, methyl parathion 50 EC @ 0.05% was recorded 83.33 per cent mortality and found more toxic as compared to rest of insecticides. The lower mortality were recorded in chlorantranilliprole 18.5 SC @ 0.006% (20.00%) which was on par with buprofezin 25 SC @ 0.05% (23.33%), flonicamid 50 WDG @ 0.015% (26.67%) and fipronil 5 SC @ 0.01% (30.00%) (Table 2). Megha *et al*. (2014) reported methyl parathion was highly toxic and recorded 100 percent mortality to adult of *C. sexmaculata* after 72 hr. Gour and Pareek (2005) revealed that cypermethrin 10 EC was found to be most toxic to the adults of the *C. septempunctata* and rated as highly toxic insecticides. Galvan *et al*. (2005) and Galvan *et al*. (2006) reported adverse effects of indoxacarb on *Harmonia axyridis*. Similar results were reported by Jalali *et al*. (2009). Emamectin benzoate was moderately toxic to predatory Coccinellids. Sharma and Kaushik (2010) found emamectin benzoate toxic to natural enemies including lady bird beetle on brinjal.

Table 1. Relative susceptibility of *C. sexmaculata* egg and grub to pesticides used in cotton

Tr. No.	Treatments	Conc. (%)	Formulation (ai/10 lit. water)	Per cent cumulative mortality of *C. sexmaculata* hour after exposure							
				Egg		Grub					
				72hours		24 hours		48 hours		72 hours	
T_1	Thiamethoxam 25 WG	0.01%	4 g	24.07	(29.29)	16.67	(23.84)	23.33	(28.77)	42.59	(40.67)
T_2	Buprofezin 25 SC	0.05%	20 ml	20.37	(26.40)	6.67	(12.58)	13.33	(21.13)	21.11	(26.92)
T_3	Clothianidin 50 WDG	0.025%	5 g	31.11	(33.88)	13.33	(21.14)	13.33	(21.14)	28.15	(31.81)
T_4	Diafenthiuron 50 WP	0.05%	10 g	24.07	(29.29)	20.00	(26.55)	26.67	(30.98)	49.63	(44.77)
T_5	Fipronil 5 SC	0.01%	20 ml	24.07	(29.29)	13.33	(21.14)	13.33	(21.14)	38.89	(38.30)
T_6	Imidacloprid 70 WG	0.014%	2 g	41.48	(38.06)	36.67	(37.21)	56.67	(48.83)	57.04	(49.03)
T_7	Flonicamid 50 WDG	0.015%	3 g	24.07	(29.29)	13.33	(21.14)	16.67	(23.85)	28.15	(31.81)
T_8	Lamda-cyhalothrin 5 EC	0.0025%	5 ml	38.15	(41.99)	43.33	(41.13)	66.67	(54.76)	67.78	(55.40)
T_9	Methyl Parathion 50 EC	0.05%	10 ml	51.85	(46.04)	56.67	(48.82)	60.00	(50.75)	82.22	(65.25)
T_{10}	Thiacloprid 21.7 SC	0.005%	2 ml	34.44	(35.89)	13.33	(21.13)	23.33	(28.76)	53.33	(46.90)
T_{11}	Cypermethrin 10 EC	0.01%	10 ml	37.78	(37.89)	46.67	(43.06)	53.33	(46.90)	71.11	(57.81)
T_{12}	Indoxacarb 15.8 EC	0.008%	5 ml	55.19	(46.90)	53.33	(46.90)	56.67	(48.83)	92.96	(77.34)
T_{13}	Chlorantranilliprole 18.5 SC	0.006%	3 ml	24.07	(29.29)	10.00	(18.43)	20.00	(26.55)	38.89	(38.49)
S Em+				2.12		2.63		2.13		3.65	
C.D. at 0.5%				6.18		7.63		6.20		10.60	
C.V. %				10.55		15.43		10.62		13.59	

Figures in the parentheses are the arc-sine transformed values

Table 2. Relative susceptibility of *C. sexmaculata* adult to pesticides used in cotton

Tr. No.	Insecticidal treatments	Conc. (%)	Formulation (ai/10 lit. water)	Per cent cumulative mortality of *C. sexmaculatus* adult hour after exposure					
				24 hour		48 hour		72 hour	
T_1	Thiamethoxam 25 WG	0.01%	4 g	13.33	(21.13)	20.00	(26.06	33.33	(35.20)
T_2	Buprofezin 25 SC	0.05%	20 ml	6.67	(12.28)	13.33	(21.13)	23.33	(28.76)
T_3	Clothianidin 50 WDG	0.025%	5 g	26.67	(30.98)	30.00	(32.98)	36.67	(37.21)
T_4	Diafenthiuron 50 WP	0.05%	10 g	13.33	(21.13)	23.33	(28.76)	33.33	(35.20)
T_5	Fipronil 5 SC	0.01%	20 ml	10.00	(18.42)	23.33	(28.76)	30.00	(32.98)
T_6	Imidacloprid 70 WG	0.014%	2 g	43.33	(41.13)	53.33	(46.90)	56.67	(48.82)
T_7	Flonicamid 50 WDG	0.015%	3 g	13.33	(21.13)	20.00	(26.55)	26.67	(30.98)
T_8	Lamda-cyhalothrin 5 EC	0.0025%	5 ml	33.33	(35.20)	53.33	(46.90)	56.67	(48.82)
T_9	Methyl Parathion 50 EC	0.05%	10 ml	33.33	(35.20)	76.67	(61.19)	83.33	(66.12)
T_{10}	Thiacloprid 21.7 SC	0.005%	2 ml	20.00	(26.55)	33.33	(35.20)	36.67	(37.21)
T_{11}	Cypermethrin 10 EC	0.01%	10 ml	43.33	(41.13)	46.67	(43.06)	63.33	(52.75)
T_{12}	Indoxacarb 15.8 EC	0.008%	5 ml	43.33	(41.13)	53.33	(46.90)	53.33	(46.90)
T_{13}	Chlorantranilliprole 18.5 SC	0.006%	3 ml	10.00	(18.42)	16.67	(23.84)	20.00	(26.06)
S Em+				2.48		2.47		2.69	
C.D. at 0.5%				7.22		7.20		7.80	
C.V. %				15.35		11.91		11.48	

Figures in the parentheses are the arc-sine transformed values

It may be concluded that **t**he insecticides methyl Parathion 50 EC, indoxacarb 15.8 EC, Cypermethrin 10 EC and lamda-cyhalothrin 5 EC showed higher mortality of coccinellid predators. Buprofezin 25 SC was the least toxic insecticide and hence, considered as safe.

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Evaluation on the effect of mineral nutrition in the management of spotted pod borer *Maruca vitrata* (Fabricius) and Blue butterfly *Lampides boeticus* (L) of cowpea

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ABSTARCT: Experiments on the effect of mineral nutrients such as K, Mg, Ca, S, Cu, Zn, B and Si in the management of spotted pod borer and blue butterfly pest of cowpea, conducted during two seasons *viz*., rainy and summer season revealed that the treatment effects in which T_{12} (NPK+ Ca+ Mg+ 3 spray of foliar nutrients) significantly influenced pod borer infestation with minimum percent of infested pods in both seasons (9.9%, 0.0%) over absolute control (41.26%) and control(56.74%) in rainy and summer season respectively. In summer, pod borer incidence was comparatively low and treatments which received nutrient spray were found free of borer infestation. Pod borers per plant exhibited the same trend as that of percentage of pods infested.

KEY WORDS: Spotted pod borer, blue butterfly, mineral nutrition, cowpea pests

Cowpea, *Vigna unguiculata* belongs to family Leguminosae is one of the major vegetable crops grown widely throughout Kerala. It is rich in nutrients, containing a fairly high percentage of proteins. Due to rich protein content, its leaves and shoots support development of pest, with improved fecundity and longevity (Breukel and Post, 1959). This threatens both quality and quantity of the seed and pod yield. Among various insect pests, *Maruca vitrata* is highly damaging to the growing cowpea because of their vast host range and cosmopolitan distribution. It causes damage to the pods, flower buds and flowers of the plant resulting in 20 – 88 per cent yield loss (Jayasinghe *et al*., 2015). Evidence of suppression of pest attack by use of mineral nutrients has been reported by different researchers (Gogi *et al.*, 2012; Habashy *et al*., 2010; Ebaid *et al*., 2006; Ghallab *et al*., 2014; Sarwar, 2011; Basagli *et al.*, 2003; Horng *et al*., 1990; Najafabadi *et al*., 2011). The timing, amount and type of mineral used can suppress or stimulate pest population, depending upon the pest species and the crop concerned. Knowledge about a plant's nutrition combined with dynamics of a pest can often provide an excellent basis for successful pest management (El-zik and Frisbie, 1985). Elements, when applied in adequate quantities, can impart resistance to pest and diseases (Span and Schumann, 2010).

Developing alternatives to pesticide is critical to maintain agriculture production. This is all the more true for Kasaragod district, which has been declared as an organic district and where synthetic

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insecticides are no more an option. With this background the present research work is proposed with objective to evaluate the effect of mineral nutrients particularly K, Ca, Mg, S, Cu, Zn, B and Si in the management of borer pests of cowpea viz., *Maruca vitrata* (Fab.) and *Lampides boeticus* (L.).

Experiment was conducted in CRD with 12 treatments including control and absolute control with 3 replications each in pot culture. The experiment was done in two seasons, *viz.* in the rainy season of 2016 (July - October) and summer season of 2017(January – April). The plants were raised in pots in three nutrient regimes namely, potting mixture, potting mixture + NPK and potting mixture + NPK + Ca + Mg. Later, the mineral nutrients were applied as foliar spray with 0.2 per cent concentrated solution prepared at Department of Soil Science, College of Agriculture, Padnekkad, Kasaragod containing K, Zn, Cu, B and Si at different time intervals *viz.* the branching stage, the peak branching stage and the flower bud initiation stage. The treatments were : T_1 - potting mixture (absolute control), T_2 - P.M + one nutrient spray, T_3 - P.M + two nutrient spray, T_4 - P.M + three nutrient spray, T_5 - P.M + NPK (control), T_6 - P.M + NPK + one nutrient spray, T_7 - P.M + NPK + two nutrient spray, T_8 - P.M + NPK + three nutrient spray, T_9 - P.M + NPK + Ca + Mg, T_{10} - P.M + NPK + Ca + Mg + one nutrient spray, T_{11} - P.M + NPK + Ca + Mg + two nutrient spray, T_{12} - P.M + NPK + Ca+ Mg + three nutrient spray. Monitoring of pests was done at biweekly intervals. Preliminary soil analysis was conducted to know the status of K, Ca, Mg, S, Cu, B, Zn and Si. The analysis of plant samples were carried out after the crop. About 1.5 times

Table 1. The percentage of infested pods in rainy season 2016 (n=6)

Treatments	15 DASP	30 DASP	45 DASP	60 DASP	Silicon in %
T_1	59.99 (.885)	28.00 (.557)	25.98 (.534)	41.73 (.702)	0.18
T_2	8.03 (.287)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.26
T_3	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.33
T_4	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.35
T_5	74.99 (1.04)	68.14 (.971)	32.25 (.603)	56.74 (.853)	0.26
T_6	14.99 (.397)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.27
T_7	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.33
T_8	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.41
T_9	0.00 (.008)	10.75 (.334)	12.63 (.363)	45.75 (.742)	0.38
T_{10}	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.44
T_{11}	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.48
T_{12}	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.00 (.008)	0.56
C.D.	23.05	10.83	8.57	23.78	0.03

Table 2. The percentage of infested pods in summer season 2017

Treatments	15 DASP	30 DASP	45 DASP	60 DASP	Silicon in %
T_1	33.83 (.621)	33.56 (.618)	28.55 (.563)	41.26 (.697)	0.21
T_2	30.81 (.588)	19.53 (.458)	26.48 (.540)	32.69 (.608)	0.27
T_3	26.88 (.545)	30.59 (.586)	26.82 (.544)	25.73 (.531)	0.32
T_4	17.70 (.434)	14.99 (.397)	21.72 (.484)	16.25 (.414)	0.41
T_5	46.27 (.748)	50.69 (.792)	32.26 (.604)	28.22 (.560)	0.32
T_6	25.75 (.532)	17.06 (.425)	23.85 (.510)	20.69 (.472)	0.29
T_7	21.75 (.485)	21.14 (.477)	20.31 (.467)	15.59 (.405)	0.37
T_8	18.85 (.449)	11.75 (.349)	21.44 (.481)	11.31 (.342)	0.44
T_9	27.37 (.550)	29.97 (.579)	45.34 (.738)	30.33 (.583)	0.36
T_{10}	23.54 (.506)	16.48 (.418)	20.38 (.468)	18.98 (.450)	0.47
T_{11}	33.84 (.620)	19.18 (.453)	19.99 (.463)	36.74 (.651)	0.46
T_{12}	11.75 (.350)	9.99 (.321)	16.06 (.412)	9.99 (.321)	0.54
C.D.	27.56	22.29	24.73	18.50	0.05

n=6;*DASP- days after spraying; *DAS- days after sowing

Table 3. Plant nutrient analysis in rainy season 2016

Treatment	Potassium %	Calcium %	Magnesium %	Sulphur %	Copper ppm	Zinc ppm	Boron ppm	Silicon %
T_1	0.59	0.10	0.14	0.20	3.21	19.07	49.15	0.21
T_2	0.63	0.11	0.15	0.21	7.25	20.25	54.65	0.27
T_3	1.41	0.12	0.19	0.24	6.25	22.26	55.32	0.32
T_4	1.62	0.16	0.20	0.24	5.26	22.96	58.38	0.41
T_5	0.99	0.12	0.15	0.23	5.12	21.28	53.36	0.32
T_6	1.68	0.17	0.18	0.24	5.19	22.28	54.35	0.29
T_7	1.29	0.16	0.18	0.23	6.29	23.36	54.85	0.37
T_8	1.49	0.19	0.18	0.26	7.91	23.98	57.56	0.44
T_9	1.29	0.15	0.17	0.27	7.25	22.32	56.21	0.36
T_{10}	1.72	0. 17	0.20	0.30	5.26	24.64	58.24	0.47
T_{11}	1.58	0.21	0.18	0.29	6.58	25.01	57.36	0.46
T_{12}	1.26	0.22	0.16	0.26	7.95	23.32	54.10	0.54
C.D.	0.02	0.01	0.01	0.03	2.20	NS	1.59	0.05

Table 4. Plant nutrient analysis in summer season 2017

Treatment	Potassium %	Calcium %	Magnesium %	Sulphur %	Copper ppm	Zinc ppm	Boron ppm	Silicon %
T1	0.65	0.14	0.14	0.24	4.70	22.07	46.89	0.18
T2	0.75	0.19	0.15	0.24	7.06	24.95	62.05	0.26
T3	1.73	0.20	0.17	0.26	5.41	27.74	63.89	0.33
T4	1.40	0.15	0.16	0.25	6.95	25.04	64.34	0.35
T5	1.05	0.15	0.14	0.22	5.01	24.45	47.42	0.26
T6	1.77	0.21	0.17	0.25	5.19	25.07	57.74	0.27
T7	1.39	0.17	0.15	0.30	6.89	26.07	53.21	0.33
T8	1.55	0.18	0.15	0.27	8.91	24.53	57.44	0.41
T9	1.38	0.18	0.16	0.26	7.71	23.85	56.99	0.38
T10	1.87	0. 19	0. 17	0. 31	4.48	26.38	58.10	0.44
T11	1.65	0. 24	0. 18	0. 26	6.32	28.45	60.75	0.48
T12	1.49	0.20	0.17	0.25	8.49	23.49	54.10	0.56
C.D. 0.05%	0.24	0.02	0.01	0.05	0.913	1.744	7.65	0.03

recommended quantity of N, P, K, Ca and Mg were added (as per Package of Practices recommendations, KAU (2011) were incorporated to the pot. Details of the quantity of chemical fertilizers per pot are as follows: Urea - 1.74g, Rajphos - 5.90 g, Muriate of potash - 1.80 g, Calcium carbonate - 10.13 g, Magnesium sulphate - 3.19 g. Observations on the damage caused by *Maruca vitrata* and *Lampides boeticus* were recorded as the number of infested pods and total number of pods.The plant samples were collected at the end of crop season and subjected to nutrient analysis for estimating the content of K, Ca, Mg, S, Cu, Zn, B and Si by using standard procedures (Jackson ,1958; Issac and Kerber, 1971; Bhargava and Raghupathi, 1995; Emmel *et al.*, 1977; Bingham, 1982; Korndorfer *et al.*, 2011). Data collected from the experiments were analysed using ANOVA after arcsine transformation.

Maximum infested pods was recorded in the control (T_5) with 46.27 % and 50.69 % on 15th and 30th day after spraying. Minimum infested pods was observed in T_{12} which were fertilized with 3 sprays + NPK + Ca + Mg with 11.75 %, 9.99 %, 16.06 % and 9.99 % on 15th, 30th, 45thand 60th day after spraying. In the summer season, maximum infested pods were found in control (T_5) followed by absolute control (T_1) and NPK + Ca +Mg (T_9). Treatments which has received nutrient spray was found to be free of infestation on 30th, 45thand 60th day after spraying in summer. Foliar nutrition (0.2 per cent) as one spray without and with NPK (T_2 and T_6) has shown some infestation on 15th day after spraying in summer season and it recovered after another 15 days (Table 1 and 2), which is in confirmation with the result of Sarwar (2011) who reported that use of K fertilizer @ 50 kg/ha is useful in the recovery of damage caused by larvae of rice stem borer and contributes to larger volume of yield. This indicates that foliar application of mineral nutrients has a significant effect on management of borer pest in cowpea and 2 to 3 times foliar fertilization with mineral nutrients containing Si, B, Zn, K and Cu is beneficial. The results are in line with the report of Mochiah *et al*. (2011) who observed that plants treated with NPK show highest leaf damage (31%) compared to absolute control (11 %) with pests such as *Plutella xylostella, Brevicoryne brassicae, Hellula undalis* and *Pieris rapae* in cabbage.

The highest K and S contents were recorded in T_{10}. Highest Si, B and Zn were recorded in $T_{12,}$ T_4 and T_{11} respectively, whereas lowest K, Ca Mg, B, Cu, Zn and Si were recorded by absolute control (Table 3 and 4). Significant differences in Si content in plants were noticed with foliar application of mineral nutrient mixture. 3 sprays of the mineral nutrient mixture along with soil application of NPK+ Ca + Mg recorded maximum concentration of silicon in the plant(0.54 %, 0.56 %), whereas, absolute control recorded minimum silicon content. Similar finding was reported by Lalithya *et al.* (2014) in sapota. So the high content of silicon might have increased the cell wall thickness. Silicon interferes with larval boring and feeding of rice striped borer (Ukwungwu and Odebiyi, 1985). Painter (1951), Takahashi (1996) and Epstein (1999) reported that Si deposited in the epidermal tissue may provide support and protection against pest as a mechanical barrier. Mandibles of larvae of the rice stem borer are damaged when the Si content of rice plants is high (Jones and Handreck, 1967). Zinc fertilization might have induced resistance through antibiosis or feeding inhibition to borer pest. Shu *et al*. (2009) reported that the application of Zinc has shown remarkable negative effect on the reproduction of phytophagous insect *Spodoptera litura* when they were reared on a diet containing 300-700 mg/kg of Zn. Application of zinc at 30 kg/ha markedly decreased the infestation of stem borer of rice, while application at 20-25kg/ha showed slightly more white head and dead heart incidence, but differed significantly from unfertilized plot. From the earlier reports and present study, it is clear that mineral nutrient such as Zn, Si and K are effective in the management of borer pest of cowpea.

Although more research is needed, this study suggests that mineral nutrients can influence the relative resistance of cowpea var. kanakamony to insect pests. Understanding how mineral nutrition improves plant health may lead to new and better integrated pest management and integrated soil fertility management designs.

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