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Studies on polymorphism of enzyme systems in populations of *Apis florea* F. (Hymenoptera: Apidae) from Chandigarh and foothills of Himachal Pradesh, North West India

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ABSTRACT: Biochemical characterization and enzyme polymorphism are useful tools in establishing intraspecific diversity in insect populations. The populations of *Apis florea* F. adult workers were selected from six varied topographic and climatic places of north western India including Chandigarh plain and foothills of Himachal Pradesh. These honey bees were analysed for proteome profile by SDS-Polyacrylamide gel electrophoresis (PAGE). Further four enzyme systems namely malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), esterase (EST) and hexokinase (HK) were analysed for enzyme polymorphism and isozymic variability in all the populations by running native PAGE and staining with specific substrate stains on the gels. Sixteen protein bands were reported in each population of *A. florea* using SDS PAGE and no difference was found in banding pattern of proteins of the populations studied. Enzymes stained on native PAGE from whole body homogenates revealed single isozymic form of ADH, two isozymic forms each of EST and MDH and three isozymic forms of HK in all the populations of *A. florea* collected from different study regions. There was significant difference in specific activity of enzymes from different regions but no polymorphism was found for the enzymes studied. Small changes in Rf values were found in the isozymes of the enzymes. The results suggest that none of these enzyme systems could be used as marker to differentiate the species at intraspecific level as the enzyme MDH, ADH, EST and HK did not exhibit polymorphism in *A. florea* from studied regions. In conclusion a single species of *A. florea* persisted throughout the area studied. However the slight changes in mobilities of individual bands and also changes in specific activity of enzymes reflect the adaptation ability of the bee according to climate variations.

KEY WORDS: *Apis florea,* biodiversity, protein profile, isozyme, polymorphism

INTRODUCTION

India is rich in honey bee biodiversity and several honey bee species exist here in nature. *Apis florea* F. is one among different honey bees present in India and is confined to tropical and sub tropical regions upto an altitude of 1600 meter above mean sea level. The flora and fauna varies from region to region, especially with change in altitude and thus some molecular changes do occur so as to make them adapt to a particular type of environment. The understanding of differences in protein types or levels may also be useful in studying variations in performance with respect to honey production,

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pollination or other services of honey bees. It exhibits migratory tendencies to far off places and may develop altitude or region specific adaptations. *Apis florea* preferably inhabits agricultural areas. It has been reported that honey bees are vulnerable to exposure of pesticides as well as human activities (Kumar and Kumar, 2014). Pesticides kill or cause changes at molecular level in honey bees in terms of honey production, pollination or other services. Tanksley and Orten (1983) described isozyme analysis as prominent tool in systematics and evolutionary biology of honey bees and regarded molecular markers superior to morphological markers in quantitative trait studies. According to Geigger (1988) enzyme electrophoresis could be used to compare populations and taxa with a set of genetic markers and zymograms. Enzyme electrophoresis has the advantage that the degree of genetic correspondence between populations or taxa of biological species could be quantified. Isozyme markers contained a huge amount of genetic information and different enzyme systems have been ranked as useful in studying taxonomic status of various other insects. Although isoenzyme electrophoresis is a well established technique that can reveal genetic variation with significant results for several populations of organisms, it has been applied for only a few honey bee species like *A. mellifera* (Sylvester, 1982; Ivanova *et al.,* 2011, 2012; Kumar and Kumar, 2013) but scarcely for species like *A. florea*. Till date electrophoretic studies in dwarf honey bees have been restricted by the number of enzymes, species and size of the sample. Indeed, multipronged studies and efforts to conserve the indigenous species and intraspecific diversity of dwarf honey bee are needed at local level because they are indispensable part of the ecosystem. Therefore the present investigation was conducted to confirm the electrophoretic profile peculiar to the populations and to determine and characterize isozymes of four enzyme systems *viz*. MDH, ADH, EST, and HK of *A. florea*.

MATERIALS AND METHODS

Study area

Workers of the dwarf honey bees, *Apis florea* F., were collected from north western India including foothills of Himachal Pradesh and Chandigarh plain regions (Table 1). Collection of samples was made with the sweep of butterfly net. From each study region approximately 50 live bees were collected and transferred to polythene bags. The bees were frozen at -20°C till further usage.

Preparation of sample

The mean body weight of *Apis florea* from Chandigarh, Gagret, Daulatpur, Parwanoo, Hamirpur and Chintpurni were 27.2±0.676 mg, 27.13±0.516 mg 27.06±0.593 mg, 26.66±0.487 mg, 26.8±0.560 mg and 29.06 ± 0.961 mg respectively. Fifteen honey bees were weighed and homogenized and centrifuged at 4°C. Supernatant obtained was preserved at -20ºC for further enzyme analysis.

Estimation and separation of proteins

Protein content of different samples was estimated by the method of Lowry *et al.* (1951). BSA (20mg/100ml) was used as standard. SDS PAGE analysis

Table 1. Study region, altitude and geographic coordinates from where samples of *A. florea* bees were collected

S. No.	Collection Area	Altitude	Latitude	Longitude
1	Chandigarh	365 m	30°43’59.93"N	76°46’45.90"E
2	Gagret	439 m	31°39’37.88"N	76°03’35.09"E
3	Daulatpur	521 m	31°46’58.52"N	75°59’23.51"E
4	Parwanoo	672 m	30°50’17.02"N	76°57’30.60"E
5	Hamirpur	785 m	31°41’10.23"N	76°31’16.71"E
6	Chintpurni	975 m	31°48’34.53"N	76°07’27.90"E

of proteins from six different populations was carried out using 10% separating gel as per Laemmli *et al.* (1970) method. The resolved proteins were visualized by coomassie brilliant blue R-250.

Spectrophotometric determination and substrate specific staining on native PAGE for enzymes

Enzyme activity was determined in 10% homogenate for the following enzymes: Malate dehydrogease (MDH), Alcohol dehydrogenase (ADH), Esterase (EST) and Hexokinase (HK). Enzyme activity was estimated spectrophotometrically using suitable methods described below. All assays were done in triplicate at optimum temperature. One unit of enzyme activity was expressed as mM NADPH consumed min^{-1} mg^{-1} protein by using an extinction coefficient of 6.22 $mM^{-1}cm^{-1}$.

Malate dehydrogenase

MDH activity was determined by the method of Bisswanger (2004). The assay mixture was prepared by mixing 0.1 M potassium phosphate, pH 7.5 (9.1 ml); 0.01M NADH (0.2 ml) and 0.1 M oxaloacetic acid (0.5 ml) in a test tube. 0.02 ml of homogenate was added to 0.98 ml assay mixture. The change in absorption was recorded at 340 nm for 3 min at 30 sec interval at 25ºC.

MDH was stained for its isoenzymes according to the procedure described by Bergmeyer and Burnt (1974). Ingredients such as 50 mM Tris –HCl, pH 8.5 (50 ml); NAD (10 mg/ml); NBT (10 mg/ml); PMS (2 mg/ml) and L-malic acid (150 mg/ml, neutralized with NaOH) were combined in a container and poured onto the gel. The gel was incubated at 37°C until blue coloured bands appeared.

Alcohol dehydrogenase

Activity of ADH was determined by the method of Bergmeyer *et al.* (1983). The assay mixture was prepared by taking 0.1 M potassium phosphate, pH 7.5 (9.5 ml); 0.01 M NADH (0.1 ml); 0.5 M acetaldehyde (0.2 ml), in a test tube. Added 0.02 ml of enzyme source to 0.98 ml of assay mixture and noted the change in absorption at 340 nm for 3 min at 30 sec interval at 25ºC.

Isozymes of alcohol dehydrogenase were stained according to the procedure described by Kagi and Vallee (1960), with few modifications. Mixed 50 mM Tris-HCl, pH 8.5 (50 ml); NAD (40 mg); ethanol (2 ml); NBT (10 mg) and PMS (4 mg) in a container and placed the gel in it. Incubated the gel in the staining solution at 37°C till blue coloured bands appeared on the gel.

Esterase

EST activity was determined using the method of Kanwar *et al.* (2005). Mixed 5mM *p*-nitrophenyl palmitate (50 µl), 0.05 M Tris buffer, pH 8.5 (2.7 ml) and enzyme solution (50 µl) in a test tube. NPA (0.050 ml) and 0.05 M Tris-HCl, pH 8.5 (2.65 ml) were combined to prepare enzyme blank solution. Enzyme solution (0.050 ml) and 0.05 M Tris-HCl, pH 8.5 (2.65 ml) were mixed to prepare substrate blank solution. Recorded the absorbance at 410 nm after 2 hr of mixing the ingredients at room temperature.

Isozymes of esterase were stained by the method of Bruce and Thomas (1977). Poured the ingredients of staining solution [α-naphthyl acetate (40 mg); β-naphthyl acetate (40 mg); acetone 50% v/v (16 ml); 50 mM Tris-HCl buffer pH 7.1 (25 ml) and Fast blue RR 0.2 % salt on the gel in a container with continuous shaking till brown coloured bands appeared.

Hexokinase

The HK activity was determined using the method of Darrow and Colowick (1962). Prepared assay mixture by taking 100 mM triethanolamine HCl, pH 7.5 (5.8 ml); 1 M D-glucose (2.0 ml); 0.1 M ATP (0.3 ml); 0.1 M $MgCl_{2.}$ hexahydrate (6.0 ml); 0.01 M NADP (1.0 ml) and glucose-6-phosphate dehydrogenase (10 U) in a test tube. Added 0.02 ml of enzyme source to 0.98 ml of assay mixture and noted the change in absorption at 340 nm for 3 min at 30 sec interval at 25ºC.

Enzyme hexokinase was stained according to the procedure described by Bergmeyer *et al.* (1983). Ingredients such as Tris-HCl pH 8.0 (30 ml); $MgCl_2$ (50 mg); NAD (10 mg); ATP ((125 mg); NBT (10 mg); PMS (2 mg); glucose (200 mg) and glucose-6-phosphate dehydrogenase (20 units) were poured onto the gel and incubated in dark without disturbing till blue bands appeared.

Statistical analysis

The data were expressed as mean ± S.D. for six populations. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Tukey's significant difference test using SPSS software (version 16). The results were considered statistically significant if the p-values were 0.05 or less.

RESULTS

The results of protein analysis for the six populations of *A. florea* studied are presented in Table 2.

Protein profiling on SDS PAGE

Protein profile of *A. florea* collected from Chandigarh, Gagret, Daulatpur, Parwanoo, Hamirpur and Chintpurni is shown in Fig. 1 in lanes 2-7 respectively. A large number of protein bands were obtained on SDS-PAGE for each sample with overall similarity in number of bands as well as intensities of the bands in different lanes. Therefore, comparative analysis amongst lanes was not done at this stage, though an estimate of the molecular weights is shown by small arrows in the Fig. 1. Results indicated very negligible differences in protein profiles at intraspecific level.

Spectrophotometric determination and substrate specific staining on native PAGE for enzymes

Malate dehydrogenase

Significant difference with highest MDH activity in Parwanoo (5.75 U/mg) population was observed as compared to Hamirpur (4.41 U/mg) and Chintpurni (4.41 U/mg) populations of *A. florea*. There was no significant difference in activity of enzyme from other populations (Table 3).

The samples of *A. florea* from six regions were run on a 7% native gel and stained suitably. Two distinct bands could be observed for MDH (Fig. 2 A). Slow migrating MDH was labelled as MDH-1 the other fast migrating was marked as MDH-2. Both isozyme forms were present in all populations of *A. florea.*

Alcohol dehydrogenase

Specific activity of the enzyme varied from 8.8 to 10 Units/ mg protein in different populations from six regions, however results were statistically non significant (Table 3).

Table 2. Protein concentration and t-value of whole body homogenate of six populations of *Apis florea* (n=5)

S. No.	Region	Protein concentration (mg/ml)	t-value	95% confidence interval of the difference	
				Lower	Upper
1	Chandigarh	0.23±0.016	31.57	0.21	0.25
2	Gagret	0.24±0.015	33.94	0.22	0.25
3	Daulatpur	0.22±0.018	26.29	0.19	0.24
4	Parwanoo	0.25±0.018	29.88	0.22	0.27
5	Hamirpur	0.23±0.021	23.92	0.20	0.25
6	Chintpurni	0.25±0.017	31.50	0.22	0.27

Values represent mean ± SD, *significant at pd 0.001 (n=5)

Table 3. Specific activity of malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), esterase (EST) and hexokinase (HK) in homogenates of *Apis florea* collected from different regions (n=5)

S. No	Region	Protein conc.	Malate dehydrogenase	Alcohol dehydrogenase	Esterase	Hexokinase
		mg ml^{-1}	Umg^{-1}	Umg^{-1}	Umg^{-1}	Umg^{-1}
1	Chandigarh	0.23	5.42±0.23 ns	9.77±0.46 ns	12.59±0.13 ns	13.39±1.34 ns
2	Gagret	0.24	4.68±0.61 ns	9.64±0.40 ns	13.55±1.43 ns	11.38±0.67 ^{d*}
3	Daulatpur	0.22	4.82±0.40 ns	9.64±0.80 ns	12.79±0.74 ^{e*}	12.05±0.67 ^{d*}
4	Parwanoo	0.25	5.75±0.46 $^{e*\&f*}$	9.10±0.61 ns	13.37±0.43 ns	14.73±0.67 $^{b\&c}$
5	Hamirpur	0.23	4.41±0.40 ^{d*}	10.04±0.83 ns	13.37±0.57 ns	12.94±1.02 ns
6	Chintpurni	0.25	4.41±0.40 ^{d*}	8.84±0.23 ns	13.52±0.51 ^{c*}	12.27±1.02 ns

Values are expressed as Mean ± S.D of seven observations. Post hoc test of Tukey HSD was applied to compare the values of different regions with each other. Lower case alphabets represent statistically significant results at pd"0.05* with respect to: 'a' =Chandigarh, 'b'=Gagret, 'c'= Daulatpur,'d'= Parwanoo, 'e' =Hamirpur and 'f'= Chintpurni . Non significant results are shown as ns. The protein content was calculated from BSA calibration curve.

The electrophoretic pattern of ADH isozymes in *A. florea* populations consisted of a single band near the cathode end of resolving gel (Fig. 2 B).

Esterase

Lowest EST activity was obtained in Daulatpur population (12.79 U/mg) and was significantly different from Chintpurni population (13.52 U/mg). No significant difference in enzyme activity was obtained amongst the rest of the populations studied (Table 3).

Two isozymic forms of esterase were observed in *A. florea* populations and were labelled as slow migrating EST-1and fast migrating EST-2 (Fig. 2 C). Slow migrating isozyme form was deeply stained whereas fast migrating form was diffuse in appearance and was faintly stained. The EST-2 showed variation in intensity while EST-1 was similar in all the populations without any particular plain to hill cline. EST was not found to be polymorphic in any population of *A. florea*.

Hexokinase

HK activity in Parwanoo (14.73 U/mg) population of *A. florea* was significantly higher than Gagret (11.38 U/mg) and Daulatpur (12.05 U/mg) populations but no significant difference was found when compared with Chandigarh, Hamirpur and Chintpurni populations (Table 3).

The results of isozyme staining showed that three isoforms of HK were present in all the populations. There were three activity regions on the gel. At the anodal region there were two bands present quite close to each other. These were labelled as HK-1 and HK-2 while another band labelled as HK-3 was observed towards the anodal region (Fig. 2 D). HK-1 was highly intense in Chintpurni and least intense in Parwanoo population, indicating highest activity of HK-1isozyme in populations of Chintpurni and lowest in Parwanoo populations. Pattern of isozymes was same in the six different populations of *A. florea*.

DISCUSSION

Protein bands ranging from 26 kDa to 124 kDa were observed for *A. florea*. The coomassie stained SDS-PAGE (10%) resolved protein bands with molecular weight 26.7, 27.4, 36.5, 37.8, 47, 50.6, 52, 56.4, 66.3, 75.2, 78, 87, 97 and 124.3 kDa in all the populations (Fig. 1). In the present study, the SDS PAGE profile of total proteins from six populations of *A. florea* reflected overall homogeneity.

Working on *Apis mellifera* Hoffman *et al.* (2013) reported that protein concentration in pollen forager haemolymph was significantly higher among multiple drone inseminated (MDI) colonies (M = 257.0 ± 5.6 ¼g/ml) than among single drone inseminated (SDI) colonies (M = 207.9 ± 15.8 ¼g/ml) but for nurse bees it was slightly lower in MDI colonies (M = 314.5 ± 17.3 ¼g/ml) compared to SDI colonies (M = 342.8 ± 23.4 ¼g/ml) showing no significant difference. According to Prakash and Bisht (2010) protein characters are sensitive to selection pressure and some isozymes appear to be specific to a population indicating genetic polymorphism.

Malate dehydrogenase- Enzyme specific staining of the gel revealed two isozymes MDH-1 (slow migrating) and MDH-2 (fast migrating) in the *A. florea* populations obtained from different regions, with apparently no polymorphism at intraspecific level (Fig. 2 A).

In contrast to present results, single MDH isozyme was observed by Nunamaker *et al*. (1984a) using isoelectric focusing for *A. florea*. These results complemented the concept of Soule (1972) that older species were more polymorphic and exhibited significant inter-specific differences with other species. MDH polymorphism was reported in different populations of *A. mellifera* from Greece (Bouga *et al.,* 2005, Ivanova *et al.,* 2010); Poland (Ivanova *et al*., 2011) and India (Kumar and Kumar 2013). Possible cause of clinal variations of MDH-1 locus was associated with difference in thermal stability of the isozyme (Cornuet *et al.,* 1995) and different metabolic rates with the consumption of higher or lower oxygen (Coelho and Mitton, 1988). In case of *A. florea* no reports on electrophoretic profile of MDH are available in the literature, except the isoelectric focusing investigations by Nunamaker *et al.* (1984b). MDH in *A. mellifera* exhibited three enzyme active zones MDH-1, MDH-2 and MDH-3 and was fixed in different castes whereas in *A. cerana* MDH-1 and MDH-3 isozymes were fixed while MDH-2 was found polymorphic (Liu *et al.,* 2002).

Alcohol dehydrogenase- Gel electrophoresis profile of alcohol dehydrogenase exhibited equal number of bands in all the populations and exhibited very little differences in intensity and relative mobility of bands for different populations of *A. florea*, thus no polymorphism was observed (Fig. 2 B). In similar studies Kumar and Kumar (2013) have distinguished the two species of cavity nesting honey bees by electrophoresis and enzyme staining of ADH. They (Kumar and Kumar, 2013) reported one isozyme for thoracic extracts for both *A. cerana* and *A. mellifera* species but differentiated *A. mellifera* having two isozymes as compared to one isozyme in *A. cerana* with abdominal extracts.

Esterase- EST-2 was of low intensity in all the populations and it was much lower in Gagret and Parwanoo populations. Faint band of EST-2 in Chintpurni, Hamirpur and Chandigarh populations indicated lower activity of this enzyme as compared to EST-1, which was found to be further diminished in Gagret and Parwanoo populations. However data is not available for EST polymorphism in *A. florea* at intraspecific level. In previous studies Nunamaker *et al.* (1984a) differentiated three honey bee species *viz*. *A. florea*, *A. dorsata* and *A. cerana* at inter-specific level with iso-electric focusing on polyacrylamide gel electrophoresis by separating isozymes of esterase and malate dehydrogenase. In biodiversity studies EST was found to be polymorphic in Greece (Bouga *et al.,* 2005); Rhodes Mountain of Bulgarian (Ivanova *et al.,* 2004) and Bulgaria and Turkey (Ivanova *et al.*, 2012) populations of *A. mellifera*. Badino *et al.* (1984) correlated slow and fast migrating bands with altitude and reported latitudinal cline in Chile for *A. mellifera* where its frequency increased with increase in latitude. The most frequent allele EST-M was found in all the populations of Greece (Badino *et al.,* 1988) and was fixed in honey bee populations from peninsular Italy, Austria and Romania (Badino *et al.,* 1984, 1988).

Hexokinase- Substrate specific enzyme staining of HK on polyacrylamide gel revealed that three isoforms were present in all the populations of *A. florea* with no intraspecific polymorphism but they differed in their relative mobility in certain populations and it was HK-2 and HK-3 which exhibited hill to plain cline (Fig. 2 D). Nunamaker and McKinnon (1989) through their polymorphism

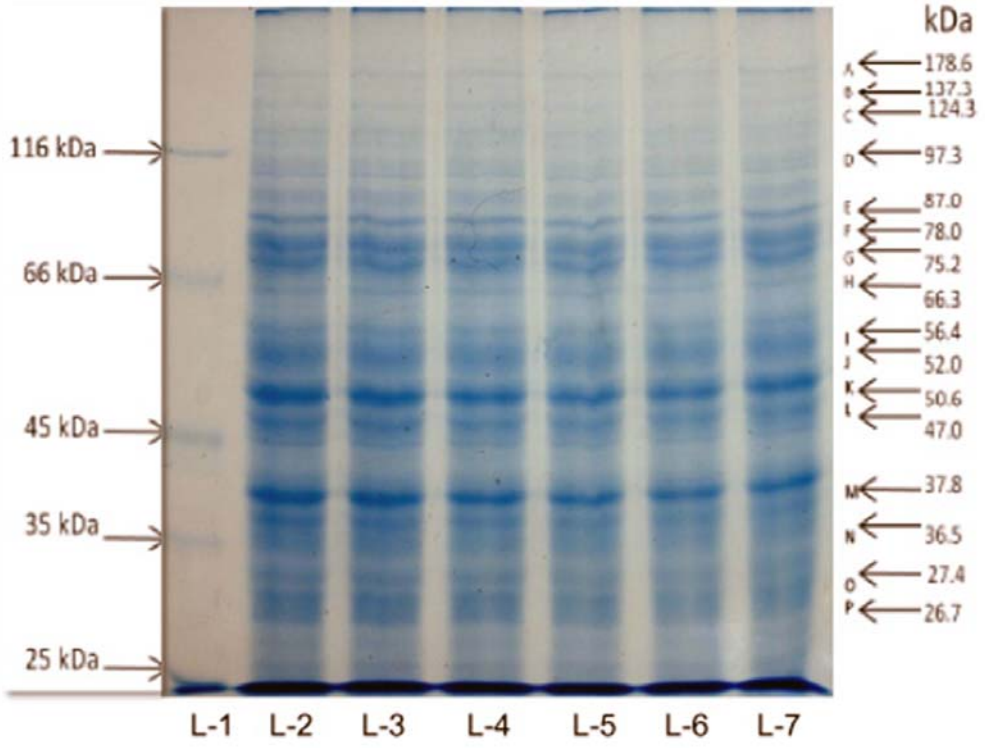

Fig. 1 SDS-PAGE showing the protein profile of *Apis florea* stained with Commassie BB R 250 in 12 % gel. Lane 1- molecular weight markers with their molecular weights are given with arrowheads, respectively. Protein samples of *A. florea* are in L-2 (Chandigarh); L-3 (Gagret); L-4 (Daulatpur); L-5 (Parwanoo); L-6 (Hamirpur); L-7 (Chintpurni)

studies could not find genetic variability for hexokinase in *Culicoides variipennis* and reported three isozymes, with fast migrated band stained intensely than other two bands in each species of fly studied. HK was reported to be polymorphic in Africanized populations of *A. mellifera* from Brazil and central America (Del Lama *et al.,* 1990; Kandemir and Kence 1995) Turkey (Kandemir *et al.,* 2000); Serbia (Ivanova *et al.,* 2010); Bulgaria and Poland (Ivanova *et al.,* 2012) at intraspecific level.

One of the indicators for the degree of genetic similarity, which registers the average frequency of the homozygote in the *A. florea* populations, is homozygosity. Another reason of the least genetic variability is absence of enzyme polymorphism for each of the populations studied.

The data presented here showed that electrophoretic patterns of malate dehydrogenase (MDH), alcohol dehydrogenase (ADH), esterase (EST) and hexokinase (HK) isozymes could not be used to discriminate *Apis florea* populations at intraspecific level. According to Faleiro *et al.* (2005) populations having high level of isozyme polymorphism should be treated as focal points by conservation biologists for capturing much of the genetic variation of biological species. Populations from Hamirpur and Chintpurni studied appeared to have originated from only one population and Chandigarh, Gagret, Daulatpur and Parwanoo populations were founded by another population of *A. florea*. Parker (1979) hypothesized that if mutation, migration and selection were absent in a clonal population, the genotype fixation was supposed to occur in that population.

Since *A. florea* is supposed to be the oldest species of honey bee, no isozyme polymorphism for any enzyme was observed which may be linked to its limited distributional range in terms of area and altitude. The limited distribution of the species is either due to short distance migration or abiotic factors like temperature and wind velocity leading

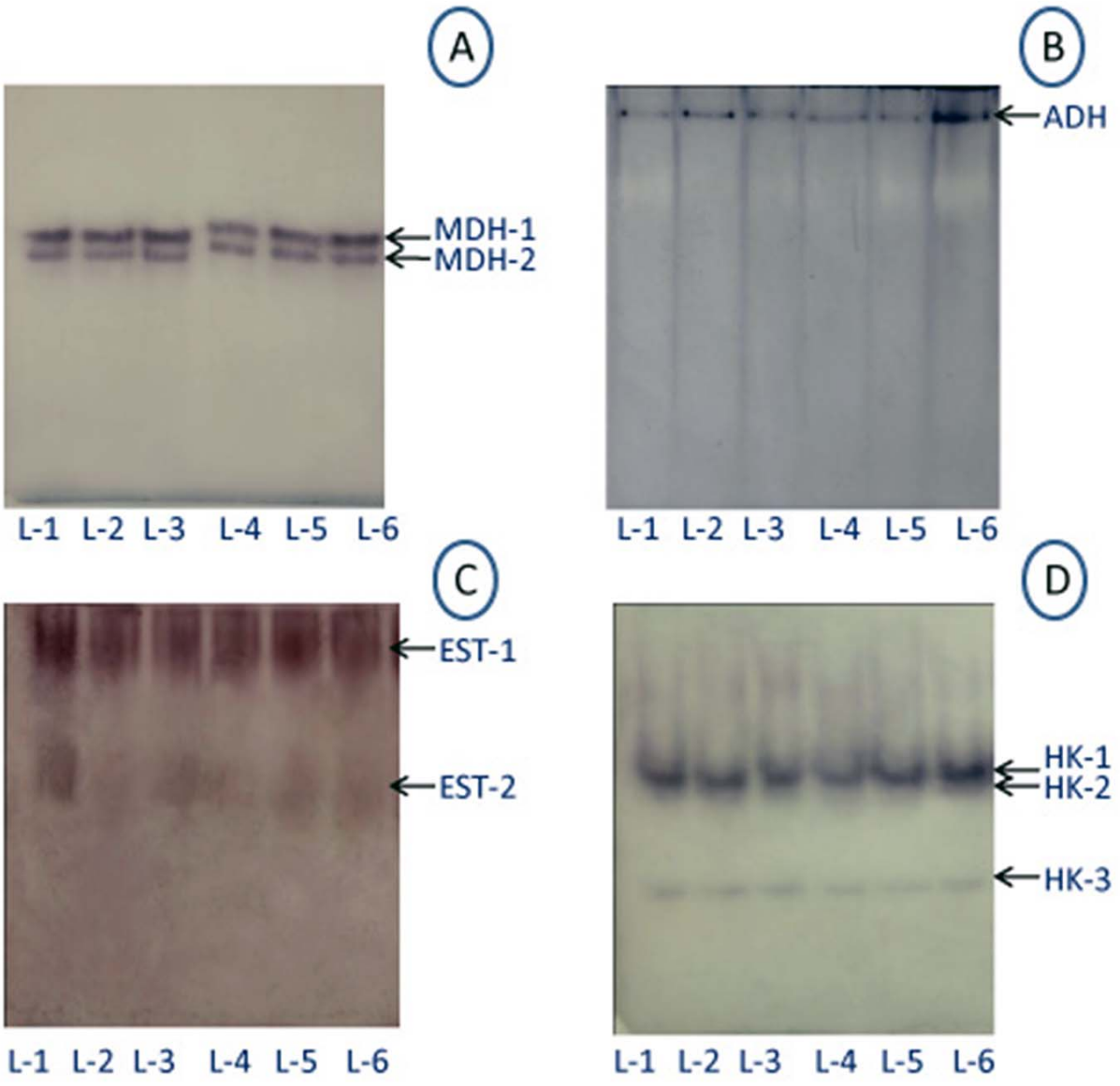

Fig. 2 Gel electrophoresis (Non denaturing/ Native) for enzyme (A) malate dehydrogenase, (B) alcohol dehydrogenase, (C) esterase and (D) hexokinase with Nitro blue tetrazolium staining in *Apis florea.* L1 (Chandigarh), L2 (Gagret), L3 (Daulatpur), L4 (Parwanoo), L5 (Hamirpur) and L6 (Chintpurni)

to a confined adaptability in hot climatic conditions. The other reason for least distribution may be low interest of beekeepers in this species due to least economic gains in comparison to other honey bee species.

Similar studies on genetic variability in three subspecies of *Apis mellifera viz A.m. carnica*, *A.m. caucasica* and *A.m. macedonica* have been conducted by Ivanova *et al.* (2012) using enzyme polymorphism with five enzyme systems namely malate dehydrogenase, malic enzyme, esterase, alkaline phosphatase, phosphoglucomutase and hexokinase. Their (Ivanova *et al.,* 2012) study reported all the enzyme systems to be polymorphic and represented different clades for three populations of *A.m. carnica*, *A.m. caucasica* and *A.m. macedonica* using UPGMA dendrogram at intraspecific level. Levins (1968) reported that genetic variability should be higher in unstable

environments, because they offered a greater variety of conditions. The microclimate of individuals in a bee colony is same leading to least exposure of these insects to environmental fluctuations, at least during their developmental period. Genetic polymorphism tends to accumulate through time so older species exhibit more polymorphism than younger species. Nielsen *et al.* (1994) analyzed populations from Europe and north and south America and reported that *A. mellifera* had no clinal distribution of S allele but they correlated F and M allele with latitude and with temperature extremes of January and July, respectively. Del Lama *et al.* (2004) reported heterogeneity in populations of *A. mellifera* in north-south cline.

According to Soule (1972) relatively stable environments minimized evolutionary change, the older species exhibiting higher levels of protein polymorphism. Nunamaker and Wilson (1980) contradicted the concept that older species were more polymorphic and they reported youngest species of honey bee *A. mellifera* to be more polymorphic for malate dehydrogenase. There seems to be no correlation between the number of isozymes and evolutionary age of the species, since *A. florea* supposed to be the oldest species in the genus *Apis* has more EST isozymes than *A. dorsata* and *A. cerana* though fewer than youngest species *A. mellifera* (Nunamaker *et al.,* 1984 a, b). Bitondi and Mestriner (1983) reported six esterase isozymes in *A. mellifera* and observed that some isozymes were controlled by more than one allele. They (Bitondi and Mestriner, 1983) analyzed the frequency of these genetic variants in four populations of *A. mellifera* from several localities. Esterase 1, 2 and 4 exhibited no developmental changes but esterase 3, 4 and 6 varied during ontogenic development.

Present study proved that gel electrophoresis of proteins and enzymes can be used in qualitative as well as quantitative evaluation of dwarf honey bee biodiversity. Using enzymatic assays of four enzyme systems viz. MDH, ADH, EST, and HK, isozymic polymorphism could not be reported in any of the population at intraspecific level. Biochemical and electrophoretic assay of protein and enzymes confirmed that single species of *A. florea* is distributed throughout the north western India. This is in agreement with the reported homozygosity of *A. florea* which is distributed over a small geographical area encompassing plains and foothills ranging from an altitude of 365 to 900 m above mean sea level. Reason behind the persistence of single species throughout area must be seasonal migration of the bee within the range of altitude and regions studied.

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Screening of rice accessions and newer molecules against lepidopterous pests in Manipur, India

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ABSTRACT: Trials conducted to assess the resistant reaction of some rice accessions and evaluation of newer molecules against the incidence of two key lepidopterous pests viz., *Scirpophaga incertulas* Walker and *Cnaphalocrocis medinalis* Guenee revealed that among the screened varieties, WR-15-6-1, WR-1-9-1-1, CAU-R2, Lamyanbaphou, RC-MANI-PHOU-11, Matamphou, KD-6-18-7-1 and RC-MANIPHOU-6-RC-5 reacted moderately susceptible at vegetative stage and among these eight, four varieties viz., WR-1-9-1-1, CAU-R2, RC-MANI-PHOU-11 and Matamphou showed resistant reaction at heading stage against *S. incertulas* and six accessions *viz*., WR-1-9-1-1, CAU-R3, KD-5-3-14, RC-MANI-PHOU-11, CAU-R4 and KD-6-18-7-1 were categorized as moderately susceptible to *C. medinalis*. The test on efficacy of newer molecule showed that treatment with Fipronil 5 SC @ 100 ml a.i. ha^{1} was found to be most effective in reducing Deadheart (DH) and White Ear Head (WEH) with minimum incidence of 3.85 per cent and 1.87% WEH respectively. Whereas the pooled mean data of two spray indicated that Imidacloprid 17.8 SL @ 250 ml a.i. ha^{1} showed minimum leaf damage of 4.16 % which was at par with Flubendiamide 39.35 SC @ 50 g a.i. ha^{-1} (4.63% LD), Fipronil 5 SC @ 100 ml a.i. ha^{-1}.

KEY WORDS: *Scirpophaga incertulas*, *Cnaphalocrocis medinalis*, fipronil, imidacloprid

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to family Graminae and is considered as the staple food for around one third world's population and occupies almost one fifth of the total land area covered under cereals. India accounts for 43.95 million ha in area of rice cultivation, 103.61 million tonnes of production and productivity of 2462 kg/h (Anon. 2015). It is mainly grown during the *Kharif* season in Manipur covering an area of 2, 44,000 hectares, producing 645 thousand tones with a productivity of 2413.52 kg/ha (Anon. 2016). More than 100 species of insect have been recorded to infest the paddy crop but only about 20 of them are of major economic significance. A significant portion of potential yield of rice is lost primarily due to incidence of the insect pests. Average yield losses inflicted by the various insect pests in rice have been estimated at about 25.0 per cent (Dhaliwal *et al*., 2010). Among the insect pests, the two lepidopterous pest cause significant yield losses posing major constraints for the rice production. Yellow stem borer (*Scirpophaga incertulas* Walker) is ubiquitous pest prevalent in all rice

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ecosystems causing the most damaging symptom at tillering and flowering stage of the crop. during the vegetative stage, YSB larva invades the tillers which eventually leads to drying of the central shoot producing a characteristic symptom 'Deadheart' (DH) and the damage during reproductive stage results in 'White Ear Heads' (WEH) (panicles with chaffy, unfilled grains). Senapati and Panda (1999) reported yield loss cause by YSB can extend from 3 to 93 percent and 50 percent of all insecticides account for use in rice fields (Huesing and English, 2004). The rice leaffolder (RLF), *Cnaphalocrocis medinalis* (Guenee) is of prime importance along with YSB population, the larvae construct longitudinal folded leaves, scraps the leaves into whitish appearance eventually hampers the photosynthetic activity. Extent of yield loss caused by them can range from 63 to 80% (Teng *et al*., 1993; Alvi *et al*., 2003). These are common and important pests in Asian countries, responsible for annual yield losses. Using resistant genotypes constitute an important component of rice IPM. Host plant resistance is identified as the most effective way of stem borer management in various regions. It has been emphasized as a major tactic in IPM for the motive of its monetary and environment friendly benefits (Sarwar, 2012). Varieties with adequate resistance level to pests encourage farmers to less usage of chemicals thus minimizing the environmental hazards. Furthermore, Newer molecules with low residual effect or toxicity towards an ecosystem plays a significant role in reducing the menace cause by the pest, the resistance development is comparatively low on contradictory to insecticides like pyrethroids, carbamates etc. where resistant development are well documented (Aajoud *et al*., 2003). Cole *et al*., (1993) reported that fipronil disrupts the normal nerve influx transmission by targeting the GABA-gated chloride channel leading to neural excitation, paralysis and insect death. The evaluation of the new insecticides must emphasize broadly so as to develop safe and effective alternatives for management of insect pests and minimizing the yield losses. Therefore, a set of two trials was conducted to assess the resistant reaction of some local rice varieties and evaluation of newer molecules against the incidence of two key lepidopterous pests.

MATERIALS AND METHODS

Two sets of field experiments were carried-out during *Kharif* season of 2014 in the Rice research farm of the College of Agriculture, Central Agricultural University, Iroisemba, Imphal to investigate the effect of certain rice accessions and new molecules on the incidence of the yellow stem borer (*Scirpophaga incertulas* Walker) and the leaf folder(*Cnaphalocrocis medinalis* Guenee). The Rice Research Farm, College of Agriculture, Central Agricultural University, Imphal, is situated at 24 ^{0}45' N latitude and 93 ^{0} 56' E longitude with an elevation of 790 m above the mean sea level. The soil type was clay loam in texture and acidic in reaction having pH value of 5.5. For growing the experimental crop the recommended agronomic practices for *Kharif* Rice cultivation were followed and common for all the experiment under investigation. The seedlings were raised in the properly prepared nursery beds for all the experiments Before sowing, the seeds were soaked for two days and kept in shade after treating with Anokhi® (Carbendazim 12% + Mancozeb 63%) 75 WP @ 2 g per kg of seeds in order to make the seeds disinfected from fungal diseases then treated seeds were allowed to sprout by keeping in gunny bag for 24 hours. The sprouted seeds were sown on the prepared seed beds and the seedlings were uprooted when they attained 4-5 leaf stage (30 days old). One month prior to transplanting, the well decomposed Farm Yard Manure @ 10 tonnes per hectare was thoroughly incorporated into the soil. The N, P_2O_5 and K_2O were applied in the form of Urea, Single Super Phosphate and Muriate of Potash @ 60:40:30 kg per hectare, respectively. The thirty days old seedlings were transplanted with inter and intra-row spacing of 15 x 10 cm at the rate of three seedlings per hill.

Screening of certain rice varieties on the incidence of *S. incertulas* and *C. medinalis*

Lay-out of the experiment:

For the field study, the experiment was laid-out in Randomized Block Design (RBD) with three replications. Fifteen rice accessions including one

check (Susceptible) i.e. Leimaphou (KD-2-6-3) were field-tested for their resistance reaction against the pests (Table 1). One-month-old seedlings of each variety were transplanted in the plot size of 3 x 2 m^2 at 15 x 20 cm spacing. No pest control measures were followed in the experimental crop.

Yellow stem borer: Observation on YSB infestation recorded after 30, 50, 70 and 90 DAT (Days after transplanting) from 10 randomly selected hills per plot and at harvest for white ear head. The percentage infestation was determined by using the following formula:

$$\text{Infestination\%} = \frac{\text{Number of dead heart or White head per hill}}{\text{Total number of tillers per hill}} \times 100$$

The damage values (D) was fixed based on the rating scale 0-9 (Heinrichs *et al*., 1985)

Scale	Damage (%)	Level of Resistance
0	None	Highly Resistant (HR)
1	1-20	Resistant (R
3	21-40	Moderately Resistant
5	41-60	Moderately Susceptible (MS)
7	61-80	Susceptible (S)
9	81-100	Highly Susceptible (HS)

Leaf folder: Observation on leaf folder damage was recorded after 30, 50 and 70 DAT from 10 randomly selected hills per plot. The percentage infestation was determined by using the following formula:

$$\text{Infestination (\%)} = \frac{\text{Number of infested leaves per hill}}{\text{Total number of leaves per hill}} \times 100$$

Percentage of damaged leaves was converted to a new figure (D) which corrects the level of infestation. The adjusted damage rating (D) of entry was converted to a scale as described earlier (Heinrichs *et al.,* 1985).

Evaluation of newer molecular insecticides against *S. incertulas* and *C. medinalis* in rice variety "Leimaphou (KD-2-6-3)"

A separate field experiment was laid-out in Randomized Block Design (RBD) replicating thrice with a plot size of 3 x 2 m^2 and spacing of 15 x 20 cm. Eight new insecticides was used and the high yielding susceptible variety 'Leimaphou (KD-2-6-3)' was used as a check for the experiment. There was an untreated control in each replication (Table 4). Two rounds of foliar application of the test insecticides were made, first at 30 DAT (days after transplanting) and second at 50 DAT with a high volume hand compression knapsack sprayer at spray volume of 500 liters per hectare.

Observations recorded:

Yellow stem borer: Observation on stem borer infestation was recorded 5 days and 10 days after each insecticide application from 10 randomly selected hills/plot in each plot. The total number of tillers as well as infested tillers was counted in all the 10 hills under observation. The percentage of the infestation was determined by using the formula as described earlier (Heinrichs *et al.,* 1985).

Leaf folder: Observation on leaf folder and whorl maggot infestation was recorded 5 days and 10 days after each insecticide application from 10 randomly selected hills/plot in each plot. The per cent of infestation was determined by recording total number of leaves as well as infested leaves by using the following formula as described earlier (Heinrichs *et al.,* 1985).

RESULTS AND DISCUSSION

The results of present field investigation on the effect of certain rice varieties and new molecules on the incidence of yellow stem borer (*Scirpophaga incertulas* Walker) and leaf folder (*Cnaphalocrocis medinalis* Guenee) conducted during *Kharif,* 2014 at the research farm of the College of Agriculture, Central Agricultural university, Imphal are briefly outlined in this chapter.

Effect of certain rice varieties on the reaction against *S. incertulas:*

During the experimental period, DH incidence recorded in 15 promising rice varieties varied from 5.30 –12.84 % and 2.93 – 8.70 % WEH at vegetative and reproductive stages of the crop,

Table 1. Effect of certain rice varieties on the incidence of *S. incertulas* during *Kharif,* 2014

No.	Variety	Mean Dead Hearts (%)	D – value	Damage score	Rating
V_1	WR-15-6-1	7.50(2.81)	58.41	5	MS
V_2	WR-1-9-1-1	6.67(2.67)	51.94	5	MS
V_3	CAU-R2	5.30(2.40)	41.27	5	MS
V_4	Lamyanbaphou	7.65(2.84)	59.58	5	MS
V_5	WR-3-2-6-1	8.31(2.97)	64.72	7	S
V_6	CAU-R3	9.33(3.13)	72.66	7	S
V_7	KD-5-3-14	10.37(3.28)	80.76	7	S
V_8	CAU-R1	8.46(2.99)	65.89	7	S
V_9	RC-MANI-PHOU-11	7.06(2.73)	54.98	5	MS
V_{10}	Matamphou	6.85(2.71)	53.35	5	MS
V_{11}	CAU-R4	9.10(3.09)	70.87	7	S
V_{12}	RCM-9	8.86(3.06)	69.00	7	S
V_{13}	KD-6-18-7-1	7.21(2.77)	56.15	5	MS
V_{14}	RC-MANIPHOU-6-RC-5	7.53(2.83)	58.64	5	MS
V_{15}	Leimaphou (KD-2-6-3)	12.84(3.64)	100	9	HS
	CD(P=0.05)	0.39			

Figures in parentheses are $\sqrt{X+0.5}$ transformed values; Mean of three replications

Table 2: Effect of certain rice varieties on the incidence of *S. incertulas* during *Kharif,* 2014

No.	Variety	Mean Dead Ear Head %	D – value	Damage score	Rating
V_1	WR-15-6-1	3.90(2.10)	44.83	3	MR
V_2	WR-1-9-1-1	3.00(1.87)	34.48	1	R
V_3	CAU-R2	2.93(1.84)	33.68	1	R
V_4	Lamyanbaphou	4.20(2.17)	48.27	3	MR
V_5	WR-3-2-6-1	4.27(2.18)	49.08	3	MR
V_6	CAU-R3	5.42(2.43)	62.30	5	MS
V_7	KD-5-3-14	6.13(2.57)	70.45	5	MS
V_8	CAU-R1	4.45(2.22)	51.15	3	MR
V_9	RC-MANI-PHOU-11	3.45(1.99)	39.65	1	R
V_{10}	Matamphou	3.30(1.95)	37.93	1	R
V_{11}	CAU-R4	5.37(2.42)	61.42	5	MS
V_{12}	RCM-9	5.20(2.39)	59.77	3	MR
V_{13}	KD-6-18-7-1	3.72(2.05)	42.76	3	MR
V_{14}	RC-MANIPHOU-6-RC-5	4.15(2.15)	47.70	3	MR
V_{15}	Leimaphou (KD-2-6-3)	8.70(3.03)	100	7	S
	CD(P=0.05)	0.39			

Figures in parentheses are $\sqrt{X+0.5}$ transformed values; Mean of three replications

Table 3: Effect of certain rice varieties on the incidence of *C. medinalis* during *Kharif,* 2014

No.	Variety	Pooled mean	D – value	Damage score	Rating
V_1	WR-15-6-1	3.98 (2.10)	67.80	7	S
V_2	WR-1-9-1-1	3.29 (1.93)	56.05	5	MS
V_3	CAU-R2	4.90 (2.31)	83.47	9	HS
V_4	Lamyanbaphou	5.09 (2.35)	86.71	9	HS
V_5	WR-3-2-6-1	4.10 (2.13)	69.85	7	S
V_6	CAU-R3	3.23 (1.92)	55.02	5	MS
V_7	KD-5-3-14	3.36 (1.95)	57.24	5	MS
V_8	CAU-R1	4.49 (2.22)	76.49	7	S
V_9	RC-MANI-PHOU-11	3.35 (1.93)	57.07	5	MS
V_{10}	Matamphou	3.94 (2.09)	67.12	7	S
V_{11}	CAU-R4	3.16 (1.90)	53.83	5	MS
V_{12}	RCM-9	4.30 (2.16)	73.25	7	S
V_{13}	KD-6-18-7-1	3.08 (1.86)	52.47	5	MS
V_{14}	RC-MANIPHOU-6-RC-5	4.69 (2.26)	79.90	7	S
V_{15}	Leimaphou (KD-2-6-3)	5.87 (2.50)	100	9	HS
	CD(P=0.05)	0.52			

Figures in parentheses are $\sqrt{X+0.5}$ transformed values; Mean of three replications based on three time intervals under observation

Table 4: Effect of different insecticidal treatments on the incidence of *S. incertulas* and *Cnaphalocrocis medinalis* on rice variety 'Leimaphou' (KD-2-6-3)

No.	Insecticides	Dose	Mean percentage		Pooled mean
		a.i. ha^{-1}	DH (%)	WEH (%)	Leaf damage
T_1	Rynaxypyr 18.5 SC	150 ml	6.56(2.65)	3.50(1.99)	5.44(2.40)
T_2	Dinotefuran 20 SG	200 g	7.87(2.89)	4.29(2.18)	5.71(2.46)
T_3	Flubendiamide 39.35 SC	50 g	4.25(2.18)	2.25(1.66)	4.63(2.21)
T_4	Thiamethoxam 25 WG	25 g	8.42(2.98)	4.92(2.33)	6.53(2.62)
T_5	Thiacloprid 21.7 SC	50 g	8.75(3.04)	5.19(2.38)	5.45(2.40)
T_6	Imidacloprid 17.8SL	250 ml	4.39(2.21)	2.45(1.71)	4.16(2.13)
T_7	Imidacloprid 20 SL	100 ml	4.80(2.30)	2.79(1.81)	5.11(2.32)
T_8	Imidacloprid 70 WG	30 g	5.13(2.37)	2.85(1.82)	5.34(2.37)
T_9	Fipronil 5 SC	100 ml	3.85(2.08)	1.87(1.54)	4.82(2.22)
T_{10}	Acephate 75 SP	500 g	9.75(3.20)	6.41(2.63)	6.81(2.66)
T_0	Untreated / control	Water spray	13.20(3.70)	7.50(3.15)	9.16(3.08)
	CD(P=0.05)		0.13	0.36	0.18

Figures in parentheses are $\sqrt{X+0.5}$ transformed values; DH - Dead Heart; WEH - White Ear Head; Mean of three replications based on three time intervals under observation

DBA – Days Before Application
DAA – Days After Application

respectively (Table 1 and 2). However, the variety CAU-R2 recorded the minimum mean dead heart and white ear head incidence of 5.30 and 2.93 per cent, respectively. The lower stem borer incidence was also noticed in the varieties WR-1-9-1-1 (6.67% DH and 3.00% WEH), MATAMPHOU (6.85% DH and 3.30% WEH), RC-MANI-PHOU-11(7.06% DH and 3.45% WEH) and KD-6-18-7-1(7.21% DH and 3.72% WEH) which did not show significant difference among them. While, the maximum yellow stem borer incidence (12.84% DH and 8.70% WEH) were marked in the variety Leimaphou (KD-2-6-3). As per the D-value rating, the eight varieties viz., WR-15-6-1, WR-1-9-1-1, CAU-R2, Lamyanbaphou, RC-MANI-PHOU-11, Matamphou, KD-6-18-7-1 and RC-MANIPHOU-6-RC-5 reacted moderately susceptible at vegetative stage and among these eight the four varieties viz., WR-1-9-1-1, CAU-R2, RC-MANI-PHOU-11 and Matamphou which were showed resistant reaction at heading stage

Reaction against *C. medinalis:*

The pooled data on mean percentage leaf damage due to *C. medinalis* presented in Table 3 indicated that all the varieties of rice screened were not prone to the attack by *C. medinalis* during the period of investigation. However, the mean per cent leaf damage significantly varied among the test varieties. The lowest mean leaf damage of 3.08 per cent was noted in the variety KD-6-18-7-1, followed by CAU-R4, CAU-R3, WR-1-9-1-1, RC-MANI-PHOU-11 and KD-5-3-14 with their corresponding mean leaf damage of 3.16, 3.23, 3.29, 3.35 and 3.36 per cent, respectively. The variety Leimaphou (KD-2-6-3) recorded the highest mean per leaf damage (5.87%), followed by Lamyanbaphou (5.09%) and CAU-R2 (4.90%) varieties which did not differ significantly from one another.

Field efficacy of certain new molecules against *S. incertulas:*

The mean data of two sprays on the incidence of *S. incertulas* (Table 4) revealed that the mean Dead Heart (DH) incidence significantly differed among the treatments throughout the experimental period. The treatment with Fipronil 5 SC @ 100 ml a.i. ha ^{1} was found to be most effective with minimum DH incidence of 3.85 per cent as against 13.20 per cent DH in untreated control plot. It was closely followed by plots treated with Flubendiamide 39.35 SC @ 50 g a.i. ha^{-1}(4.25% DH), Imidacloprid 17.8 SL @ 250 ml a.i. ha^{-1}(4.39% DH), Imidacloprid 20 SL @ 100 ml a.i. ha^{-1} (4.80% DH) and Imidacloprid 70 WG @ 30 g a.i. ha^{-1} (5.13% DH) which did not differ significantly from one another. Also, the mean data of two sprays further indicated that Fipronil 5 SC@100 ml a.i. ha^{-1} proved to be the most effective insecticidal treatments in reducing White Ear Head (WEH) incidence with a record of 1.87% WEH as against 7.50 per cent in untreated control. The lower WEH incidence also found in Flubendiamide 39.35 SC@ 50g a.i. ha^{-1}(2.25% WEH) , Imidacloprid 17.8 SL @ 250 ml a.i. ha^{-1}(2.45% WEH), Imidacloprid 20 SL@ 100 ml a.i. ha^{-1}(2.79 %WEH) and Imidacloprid 70 WG @ 30 g a.i. ha^{-1}(2.85% WEH).

Mean extent leaf damage over two spray based on 5 DAA and 10 DAA:

The pooled mean data of two spray presented in Table 4 indicated that Imidacloprid 17.8 SL @ 250 ml a.i. ha^{-1} showed the least infestation by the insect pest with a record of minimum leaf damage incidence (4.16% LD) which was at par with Flubendiamide 39.35 SC @ 50 g a.i. ha^{-1} (4.63% LD) and Fipronil 5 SC @ 100 ml a.i. ha^{-1} (4.82% LD). And the plot treated with Imidacloprid 20 SL @ 100 ml a.i. ha^{-1} and Imidacloprid 70 WG @ 25 g a.i. ha^{-1} recorded mean leaf damage of 5.11% LD and 5.34% LD respectively. Whereas the plots treated with Acephate 75 SP @ 500 g a.i. ha^{-1} recorded the maximum mean leaf damage of 6.81% LD).

Host plant resistance distinguishes plant varieties exhibiting minimum invasion or damage to pest population. Utilization of insect-resistant crop varieties are economically, ecologically, and environmentally advantageous and contributes towards sustainable agriculture. In this study, we employed 15 local varieties to ascertain the incidence of the two key lepidopterous insect pests in rice. We observed that the varieties did not

performed significantly superior in showing resistance against Deadheart incidence however varieties viz., WR-15-6-1, WR-1-9-1-1, CAU-R2, Lamyanbaphou, RC-MANI-PHOU-11, Matamphou, KD-6-18-7-1 and RC-MANIPHOU-6-RC-5 exhibited moderately susceptible at vegetative stage and varieties viz., WR-1-9-1-1, CAU-R2, RC-MANI-PHOU-11 and Matamphou showed resistant reaction against White Earhead incidence. Pathak and Khan (1994) reported that *S. incertulas* larvae feeding on resistant varieties were smaller, low survival percentage and caused lower percentages of DH/WEH than those feeding on susceptible varieties. Furthermore, Rustamani *et al*. (2002) concluded that differential response of varieties is due to oviposition preference by the adult females of yellow stem borer. Whereas in case of leaf damage caused by *Cnaphalocrocis medinalis* against the local varieties six varieties viz., CAU-R2, CAU-R3, KD-5-3-14, RC-MANI-PHOU-11, CAU-R4 and KD-6-18-7-1 categorized as moderately susceptible to *Cnaphalocrocis medinalis* with mean percent leaf damage of 3.29, 3.23, 3.36, 3.35, 3.16 and 3.08 respectively whereas the other local varieties exhibited highly susceptible towards the pest incidence. However, none of the varieties showed resistant against the insect pest. The susceptibility towards the pest maybe attributed to the morphological structure and biochemical character of the crop. Xu *et al*. (2010) reported that the wider leaves are more susceptible and leaves with high chlorophyll content are suitable for larvae feeding. In summary, the resistance of rice to *C. medinalis* is complex, involving physical and biochemical resistance. Therefore, biochemical mechanisms of resistance need to be further investigated. Among the test insecticides evaluated against the pest, application of fipronil performed significantly superior that the other treatment with a mean DH and WEH incidence of 3.85 % and 1.87 % respectively. Similar trend was reported by Mondal and Chakraborty (2016) whereby Fipronil showed the dominance in reducing DH and WEH of 56.28% and 65.27% respectively. Rath *et al*., (2015) also reported that Imidacloprid 17.8% @ 300g/ha treatment recorded lowest percentage of DH (3.3%) and WEH (3.33%) with the highest grain yield of 5.28 t/ha. In case of *C. medinalis* Imidacloprid 17.8 SL @ 250 ml a.i. ha^{-1} exhibited the superiority as compared to other test insecticides with a mean leaf damage of 4.16%. The present record is in accordance with Kaiwar *et al.* (2017) who reported that leaf infestation by leaffolder after first and second spray revealed Imidacloprid significantly reduced the population with mean percent population of 1.56. Chakraborty and Deb (2011) also reported least number of adult individuals/ trap, larval individuals/hill and incidence of damaged leaf (DL%).in imidacloprid 17.8 SL (100ml/ha) treated field.

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Life table and economic threshold concept for ecologically sustainable management of *Diacrisia casignetum* Kollar (Lepidoptera: Arctiidae) on jute

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ABSTRACT: Life table and economic threshold (ET) level of *Diacrisia casignetum* Kollar on jute was calculated in West Bengal condition during 2016-2018 for environmentally benign management and better production of jute. The demographic data of *D. casignetum* always construct type-III survivorship curve like most of the insects. Potential fecundity (Pf) and total fertility rate (F_x) were 384.667±5.487 and 30893.60±1063.586, respectively. The average intrinsic rate of natural increase (r_m) and the daily finite rate of increase (ë) were 0.106±0.001 and 1.111±0.003 respectively with the generation time (T_c) of 41.520±0.069 and doubling time (DT) of 6.563±0.021 days. Their mean population momentum factor of increase (PMF) was 19.676±0.605 including vital index (VI) and trend index (TI) of 0.522±0.003 and 92.359±2.648, respectively with different levels of significance. The economic injury level (EIL) and ET based on percent yield loss of jute were 7.119±2.166 and 6.385±1.969 pests per plant, respectively. At single pest observation per plant, the possible time taken to reach EIL (Ti) and ET (Tt) were 6.377±3.292 and 5.377±3.292 days, respectively which will be useful for better cultivation of jute and other subsequent crops.

KEY WORDS: Life table, intrinsic factors, ET, EIL, *Diacrisia casignetum*, Jute

INTRODUCTION

Jute (*Chorchorus capsularis*, cv. JRC-698; Family: Tiliaceae) is the most important natural fibre crop in Asian countries like, India, Bangladesh, Nepal, China, etc. (Kumar *et al.*, 2017; Naik and Karmakar, 2016; Roy, 2014). The jute fibre is one of the most versatile natural fibres that have been used as raw materials in packaging, textiles, nontextile, construction and agricultural sectors (Sarkar and Majumdar, 2016; Kumar *et al.*, 2017). In the context of global awareness about environment, jute and allied fibres are used as eco-friendly sustainable materials against synthetic fibres (Majumdar *et al.*, 2016). The cultivation of jute in India is mainly confined to the eastern region states - West Bengal, Bihar, Assam, Tripura, Meghalaya, Orissa and Uttar Pradesh. Nearly 50% of total raw jute production in India alone figures in West Bengal (Sarkar and Majumdar, 2016). But different climatic factors and occurrence of different pests played a pivotal role in production of jute crop in West Bengal (Rahaman and Khan, 2012; De and Ghorai, 2017). In India, over two dozen different species of pests belonging to insects, mites and nematodes were found feeding on jute (Rahaman and Khan, 2012; Sarkar and Majumdar, 2016). As a result, both the quality and quantity of the crop is affected

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(Rahman and Khan, 2012). In recent decades, spraying different broad spectrum synthetic chemical insecticides or few botanicals are the general practices for the control of such pests in the crop fields without considering their injury level or action threshold (Ndakidemi *et al.*, 2016; Parui and Roy, 2016). So there is a need to explore the possibilities of increasing the productivity through better understanding of different constraints in jute production for getting higher economic returns (De and Ghorai, 2017; Roy *et al*., 2017). Pest population dynamics and yield loss assessment data with economic threshold (ET) are the primary tool to design a module for insect pest management.

Two basic components of decision making in pest management are the economic injury level (EIL) and economic threshold (ET) (Pedigo and Buntin, 1994; Pedigo and Higley, 1992). The ET is a complex value that depends on estimating and predicting several difficult population parameters, EIL variables, pest and host phenology, etc. The ET is more effective than using no ET at all in IPM for minimizing hazards to humans and the environment by judicious pesticide applications (Prokopy *et al.*, 1994; Prokopy and Kogan, 2009). For population parameters, study of life table is the best way to calculate the pest population dynamics including comprehensive description of their survivorship, development, fecundity, mortality and life expectancy (Southwood and Henderson, 2000; Carey, 2001; Ali and Rizvi, 2010). Thus population parameters of any insect pest are widely useful technique in their management (Kakde *et al.*, 2014; Dutta and Roy, 2018; Roy, 2015a, b, 2016, 2017).

Bihar hairy caterpillar (BHC) of *D. casignetum* Kollar is a major predominant jute production constraint in West Bengal by causing direct economic damage (Roy and Barik, 2013; Roy, 2018). Present work was carried out to determine the economic threshold (ET) through the yield loss calculation caused by BHCs in jute ecosystem along with their population dynamics for their ecologically sustainable management (ESM). This population based study ultimately help to develop an alternative method for ecologically sustainable agricultural (ESA) practices by judicious use of any or more suitable control measures other than chemical pesticides under the strategy of GPM/EPM for better production of jute and other such crops in our agro ecosystem.

MATERIALS AND METHODS

Insect mass culture: *D. casignetum* eggs was collected from the jute fields near Chinsurah Rice Research Center(CRRC), Chinsurah, 22°53' N, 88°23' E, 13m above sea level, Hooghly, West Bengal, India during cultivation period of jute during Pre-kharif to Kharif seasons (April to August) in 2016, 2017 and 2018. The eggs were incubated in the laboratory on white jute leaves (cv. JRC-698) in plastic jars (20 cm dia. X 30 cm ht.) until emergence of the caterpillars. The stock culture of *D. casignetum* was initiated on mature jute leaves at 27±1°C, 65±5% RH and a photoperiodism of 12:12 (L:D) hours in a growth chamber for life table study as described previously (Roy, 2014, 2018). In order to obtain the same aged eggs of *D. casignetum* for study of population dynamics, three pairs of moths (male and female) from the stock culture (F_1) were placed in an oviposition cage of fine nylon net (25×25×25 cm) with fresh foliage and replicate three times each year with average of 385, 394 and 375 eggs/cohort in the three consecutive years, 2016, 2017 and 2018, respectively.

Life table parameters: The construction of life table includes several parameters which were calculated with the formulae of Carey (1993), Krebs (1994) and Price (1998). These parameters include probability of survival from birth to age x (l_x), proportion dying each age (d_x), mortality (q_x), survival rate (s_x) per day per age class from egg to adult stages. Using these parameters, the following statistics like, average population alive in each stage (L_x), life expectancy (e_x), exponential mortality or killing power (k_x), total generation mortality (K or GM), generation survival (GS), gross reproductive rate (GRR), net reproductive rate (NRR or R_0), mean generation time (T_c), doubling time (DT), intrinsic rate of population increase (r_m), Euler's corrected r (r_c), finite rate of population increase (λ), weekly multiplication rate (λ^7), increase rate

per generation (λ^{Tc}), were also computed, using Carey's formulae (1993). Some other population parameters like potential fecundity (Pf), total fertility rate (F_x), mortality coefficient (MC), population growth rate (PGR), population momentum factor of increase (PMF), expected population size in 2nd generation (PF_2), expected females in 2nd generation (FF_2), general fertility rate (GFR), crude birth rate (CBR), reproductive value (RV), vital index (VI) and trend index (TI) were also determined by using well defined formulae (Brich, 1948; Southwood, 1995; Roy, 2017).

Field experiment: A field experiment was conducted for consecutive three years from 2016 to 2018by growing the white jute variety (cv. JRC-698) in RDB to determine the population dynamics and ETs of BHC as described by earlier workers with few modifications (Sarkar and Majumdar, 2013; Das *et al.*, 2014; Parui and Roy, 2016). The experiment was done in CRRC, Chinsurah, 22°53' N, 88°23' E, 13m above sea level, West Bengal, India, with 3 replications for both control and treated plots (4×5m) with average plant density 30±2 plants m^{-2} (Sarkar and Majumdar, 2013; Das *et al.*, 2014). The crop production related data from the jute field were collected for determination of ETs of *D. casignetum* (BHC) on jute. The potency of crop damage by BHC infestation was observed over a traditional synthetic pesticide, lambda-cyhalothrin 5% EC @ 1ml $^{-1}$L (Kumar *et al*., 2014), along with control (without pesticide) side by side (Das *et al.*, 2014; Parui and Roy, 2016).

ET calculation: From seed to harvest jute plants and occurrence of Bihar hairy caterpillars of *D. casignetum*were recorded by random quadrat sampling (RQS) from each treated and control plots (Das *et al*., 2014; Parui and Roy, 2016). Calculation of Economic Injury Level (EIL) for *D. casignetum* according to the methodology proposed by Pedigo *et al*. (1986) expressed as numbers or injury equivalents and governed by four primary variables viz. cost of the management tactic per production unit, (*C*), market value per production unit (*V*), $D\hat{E}$ = *per cent yield loss per pest* and the proportional reduction in pest attack (*K*). If the relationship of these variables is linear or roughly so, the EIL can be given as: $EIL = C/VD\hat{E}K$ (Pedigo and Buntin, 1994). The BHC infestation and efficacy of the traditional synthetic pesticide were determined in terms of yield damage reduction (%), proportion of insect controlled (%) and percent yield loss per pest per plant (%) along with the management costs (CC) for the calculation of EIL, ETL and EEIL along with time to reach the EIL(Ti) and ETL (Tt) when a plant is infested by a single pest in the field. The management cost was calculated using the cost of insecticide, lambda-cyhalothrin 5% EC @ 1ml $^{-1}$L (Kumar *et al.*, 2014), and its application which accounted to Rs.1300 $^{-1}$ha. The market value of the produce was considered at Rs.32-36 $^{-1}$kg during 2015-2017 market price prevailed in India.

Statistical Analysis: The data of different parameters were subjected to one-way Analysis of Variance (ANOVA) and correlation analysis (Roy, 2014, 2018). Means of different demographic parameters were compared by Tukey's test (HSD) when significant values were obtained (Zar, 1999). All the statistical analysis was performed using the statistical program SPSS (version 16.0) computer software program.

RESULTS

Life table: The demographic data of *D. casignetum* reared on the mature jute leaves for the three consecutive years (2016, 2017 and 2018) represent a similar pattern of development (Table 1) through the consecutive years ($F_{7,72}$ = 22.854-23.200) with significant variations (P<0.001) in different developmental stages for each year. The proportion of surviving (l_x) and the survivorship (s_x) of *D. casignetum* gradually decreased throughout their developmental stages and they always produced type-III survivorship curve like most of the insects. Whereas, proportion of dying (d_x) and mortality (q_x) in different developmental stages were varied and comparatively higher in egg and pupal stage with a rapid surge during adult stage for the years. The average population alive in each stage (or, age structure) (L_x) and life expectancy (e_x) also followed the same pattern of proportion of survival (l_x) for the years. Whereas, killing power (k_x) was also followed the same pattern always

like d_x and q_x for each year. All the population parameters of *D. casignetum* which derived from the nine cohorts (3 cohorts/ year) calculated over the years (2016-2018) were also varied significantly when compared by Tukey's (HSD) test throughout the developmental stages (Table 1).

Average potential fecundity (Pf) and total fertility rate (F_x) were 384.667±5.487 and 30893.60±1063.586, respectively for the three years. The average gross reproductive rate (GRR) and net reproductive rate (NRR) were 123.215±3.712 and 80.267±1.622, respectively for the three consecutive years. The average intrinsic rate of natural increase (r_m), Euler's corrected r (r_c) and the daily finite rate of increase (λ) were 0.106±0.001, 0.022±0.000, 1.111±0.003 respectively, for the years with the generation time (T_c) of 41.520±0.069 and doubling time (DT) of 6.563±0.021 days (Table 2). The average increase rate per generation for the years was 80.266±1.622 with generation mortality (GM), mortality coefficient (MC) and generation survival (GS) of 0.283±0.003, 0.209±0.001 and 0.699±0.008, respectively. Their mean crude birth rate (CBR) was 1.258±0.013 with general fertility rate (GFR) of 4.794±0.030 for the years (Table 2). Further their average F_2 population (PF_2) can be expected as 3953.282±201.803 and out of them probable females (FF_2) may be 1581.313±80.753 throughout studies of these years (Table 2). Their mean population momentum factor of increase (PMF) was 19.676±0.605 including vital index (VI) and trend index (TI) of 0.522±0.003 and 92.359±2.648, respectively (Table 2). All the 20 population parameters show different level of variance through the three consecutive years with different levels of significance.

Yield loss and ET calculation: Average yield damage without treatment (Yd%) (Control) and after treatment (Ydt%) through the three consecutive years were 8.814±1.699% and 2.362±0.815%, respectively. The mean yield reduction (Yr%) for these years was 6.453±0.921% by average proportional control (PC %) of the pest (75±4.811%) in the field condition. The pest control efficacy of the synthetic pesticide (lambda-cyhalothrin) over the control plots for these years represent mean EIL and ET of 7.119±2.166 and 6.385±1.969, respectively, pests per plant. For a single pest per plant the possible time that can be taken to reach EIL (Ti) and ET (Tt) might be 6.377±3.292 and 5.377±3.292 days, respectively (Table 3). The crop damage and ET associated

Table 1. Stage-specific pooled life table for the nine cohorts (Average of 3 observations each year) of *D. casignetum* on white jute (*C. capsularis*, cv. JRC-698) observed during 2016-2018

Stages	l_x	d_x	q_x	s_x	L_x	e_x	k_x
Egg	1.000±0.000	0.068±0.007	0.068±0.007	0.932±0.007	0.966±0.003	7.944±0.057	0.030±0.003
Inst- I	0.932±0.007	0.033±0.002	0.035±0.001	0.965±0.001	0.916±0.007	7.483±0.044	0.016±0.001
Inst- II	0.899±0.007	0.036±0.004	0.041±0.004	0.959±0.004	0.881±0.006	6.738±0.035	0.018±0.002
Inst- III	0.863±0.006	0.034±0.005	0.039±0.006	0.961±0.006	0.846±0.006	6.003±0.057	0.017±0.002
Inst- IV	0.830±0.007	0.035±0.001	0.042±0.002	0.958±0.002	0.812±0.007	5.226±0.029	0.019±0.001
Inst- V	0.795±0.008	0.031±0.004	0.039±0.005	0.961±0.005	0.779±0.009	4.432±0.033	0.017±0.002
Inst- VI	0.763±0.009	0.032±0.006	0.042±0.008	0.958±0.008	0.748±0.009	3.592±0.019	0.019±0.003
Prepupa	0.732±0.009	0.015±0.002	0.020±0.003	0.980±0.003	0.724±0.010	2.727±0.007	0.009±0.001
Pupa	0.717±0.011	0.065±0.005	0.091±0.005	0.909±0.005	0.684±0.009	1.773±0.013	0.041±0.003
Adult	0.652±0.0.006	0.130±0.010	0.200±0.013	0.800±0.013	0.587±0.001	0.900±0.007	0.097±0.007

Within the columns (Mean ± SE) different developmental stages varied significantly at $P<0.05$ when compared by Tukey (HSD) Test

Table 2. Population dynamics of *D. casignetum* on white jute (*C. capsularis*, cv. JRC-698) of nine cohorts (Average of 3 observations each year) observed during 2016-2018

Population parameters	Mean±SE
Potential fecundity (Pf)	384.667±5.487
Total fertility rate (F_x)	30893.600±1063.586
Gross reproductive rate (GRR)	123.214±3.712
Net reproductive rate (R_0)	80.267±1.622
Generation time (T_c)	41.520±0.069
Doubling time (DT)	6.563±0.021
Intrinsic rate of natural increase (r_m)	0.106±0.000
Innet capacity for increase (r_c)	0.022±0.001
Finite rate of increase (λ)	1.111±0.000
Weelkly multiplication rate (λ^7)	2.094±0.005
Increase rate per generation (λ^{Tc})	80.266±1.622
Generation mortality (GM)	0.283±0.003
Mortality coefficient (MC)	0.209±0.001
Generation survival (GS)	0.699±0.008
Population growth rate (PGR)	21.195±0.493
Population momentum factor (PMF)	19.676±0.605
F_2 population size (PF_2)	3953.28±201.883
Hypothetical F2 females (HFF_2)	6448.000±261.052
Realised F_2 females (RFF_2)	1581.313±80.753
General fertility rate (GFR)	4.794±0.030
Crude birth rate (CBR)	1.258±0.013
Reproductive value (RV)	246.42±7.424
Vital Indwx (VI)	0.522±0.003
Trend index (TI)	92.359±2.648
Annual rate of increase (ARI)	5.585E+16±6.5E+15

values were significantly differed in each year ($F_{7, 16}$ = 76.670, P=0.001) but showed insignificant variations ($F_{2,23}$ = 0.111, P = 0.896) within the years. The yield loss and ET values of *D. casignetum* on white jute (cv. JRC-698) during pre-kharif to kharif seasons showed different level of significant correlations (positive or negative) (Table 4). Thus this study will obviously help farmers for sustainable management of the said pest on jute by applying any appropriate control measures judiciously other than synthetic pesticides if possible within the limit of pest population (ET) and or the time (Tt) span before the ET in the jute agro ecosystem.

DISCUSSION

In ecological research, life table study is a central theme to calculate the vital statistics of pest population dynamics and their management (Carey, 2001; Kakde *et al.*, 2014). There is a range of innate capacity for individual of a population but the variation in available food quality (Rizvi *et al*., 2009; Roy, 2014) along with environmental factors (Ali and Rizvi, 2010) always influence their population dynamics (Schoonhoven *et al*., 2005; Roy and Barik, 2013). The key component of IPM is the maintenance of pest population below economically damaging levels (ET) to minimizing hazards to humans and the environment (Prokopy *et al.*, 1994). But till date there is a gap between long term population study and determination of descriptive ET for a particular pest including *D. casignetum* on jute. Obviously this study somehow fills the gap by judicious use of any control measures for ecologically sustainable agriculture of jute and other such crops.

The stage-specific pooled life table of *D. casignetum* showed four distinct stages with six larval instars (i.e., egg, larva, pupa, and adult) (Roy and Barik, 2013). Survival rate (lx) of *D. casignetum* during the developmental stages (egg to adult) indicated the type III survival curve, with high mortality during the immature stages as found in most insect species (Roy, 2015a). The yield loss assessment data and pest population dynamics are the primary tool to design a module for insect pest management. These data are very important and considered for determining the progressive status of the pest. The percent yield loss increased with increase in larval density of *D. casignetum*. The determined average EIL and ET were 7.119±2.166 and 6.385±1.969 pests per plant, respectively, during the pre-kharif and kharif seasons in three consecutive years (2016-2018). At single pest observation per plant the possible time that can be taken to reach EIL (Ti) and ET (Tt) might be 6.377±3.292 and 5.377±3.292 days, respectively.

Table 3. Yield loss and ET calculation of *D. casignetum* on white jute (*C. capsularis*, cv. JRC-698) during 2016-2018

Crop Parameters	Mean±SE	Mean Square	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Yield damage % before treatment (Yd %)	8.814±1.699	8.657	2.942	1.506	16.123
Yield damage % after treatment (Ydt %)	2.362±0.815	1.992	1.411	1.144	5.868
Yield damage % reduction after treatment (Yr %)	6.453±0.921	2.545	1.596	2.489	10.416
Proportion of insect controlled (PC %)	75.000±4.811	69.444	8.333	54.299	95.701
EIL (pest/plant)	7.119±2.166	14.079	3.752	2.202	16.44
ETL (pest/plant))	6.385±1.969	11.629	3.410	2.086	14.856
Time to reach EIL/pest/ plant (Ti days)	6.377±3.292	32.51	5.702	7.787	20.541
Time to reach ETL/pest/ plant (Tt days)	5.377±3.292	32.51	5.702	8.787	19.541

Table 4. Correlation of yield loss and ET values of *D. casignetum* on white jute (*C. capsularis*, cv. JRC-698) observed during 2016-2018

Correlation	*Yr*	*PC*	*YD*	*Ydt*	*EIL*	*ETL*
PC	-0.900					
Yd	0.981	-0.968				
Ydt	0.915	-0.999	0.976			
EIL	-0.987	0.958	-0.999	-0.968		
ETL	-0.990	0.954	-0.999	-0.963	1.000	
Ti	-0.998	0.870	-0.966	-0.886	0.975	0.978
Tt	-0.998	0.870	-0.966	-0.886	0.975	0.978

Thus population dynamics and ET calculation will be the most important method for the judicious use of different control measures mainly chemical pesticides for environmentally safe management in our agro ecosystem towards better cultivation of jute and other subsequent crops cultivated rotationally. So the knowledge of population parameters of *D. casignetum* and their ET levels will enable jute growers to employ the most appropriate control tactics towards integrated pest management (IPM) for ecologically sustainable agriculture (ESA) in near future of jute as well as other related crops.

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Description of a new species of *Callocleonymus* Masi (Hymenoptera: Chalcidoidea: Pteromalidae) from India

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ABSTRACT: The first Indian species of the genus *Callocleonymus* Masi *viz. C. indiaensis* sp. nov., is formally described from Bengaluru in southern India.

KEY WORDS: Pteromalidae, Cleonyminae, *Callocleonymus*, new species, India

INTRODUCTION

The genus *Callocleonymus* belongs to the subfamily Cleonyminae of Pteromalidae which was established by Masi in 1940 with the type species *C. pulcher* Masi from Somalia which is also known from the Afrotropical regions. The genus is currently known by eight described species and a number of undescribed species worldwide out of which *C.pulcher* belong to the Oriental region (Noyes, 2019; Gibson, 2003, Bou^{a}ek, 1988). The other known species of *Callocleonymus* are - *C. beijingensis* Yang, 1996, *C. xinjiangensis* Yang, 1996, *C. bimaculae* Yang, 1996, *C. chuxiongensis* Yang, 1996, *C. ferrierei* Kerrich, 1957, *C. ianthinus* Yang, 1996 (all from China) and *C. swezeyi* (Yoshimoto and Ishii, 1965) from Australia. *Callocleonymus* species are parasitic on beetles of families Scolytidae, Buprestidae and Curculionidae (Yang, 1996; Bou^{a}ek, 1988). Recently Gupta *et al.* (2015) reported the genus from India for the first time based on a single female specimen collected from Bengaluru, Karnataka. Though having an undescribed status and being singleton, the specimen was not described formally by the authors. Recently another female specimen has been collected from Kerala, which on comparison with the Bengaluru specimen proved conspecific. Both the specimens do not match with any of the earlier known species, hence a new species has been described here based on the Bengaluru specimen as the holotype and the Kerala specimen as the paratype.

MATERIALS AND METHODS

The holotype was collected from the field with an yellow pan trap at Chintamani, Bengaluru, India (13.4020^{0} N. 78.0551^{0} E) and deposited in the collections of ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India (NBAIR). The paratype was collected using sweep net from an abandoned paddy field infested with dry weed grasses located at Kuttichira of Kollam district, Kerala (11.2388° N, 75.7808° E). The specimen is deposited in the National Zoological Collections of

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Zoological Survey of India Western Ghat Regional Centre, Kozhikode (ZSIK). The terminology follows Gibson (2003) and Bou^{a}ek (1988). All measurements are given in millimeters except for the relative measurements. Photographs were taken using a M205FA stereozoom microscope attached with a Leica DC420 camera and syncroscopy automontage (version 3.8). The following abbreviations are used in the text: fu_1-fu_7- funicular segments 1-7; MV- marginal vein; OOL- ocell-ocular distance; PMV- postmarginal vein; POL- post-ocellar distance; SMV- sub marginal vein; STV- stigmal vein; Gt1-6- tergites 1-6.

RESULTS AND DISCUSSION

Callocleonymus Masi

Callocleonymus Masi, 1940: 289-280. Type species: *Callocleonymus pulcher* Masi; by original designation.

Diagnosis: (Based on Gibson, 2003) Body with bright metallic luster and dense sculpture. In female, antenna inserted at least slightly below level of lower margin of eyes but either above or below level of lower margin of eyes in male. Female flagellum with apical margin of preclaval segment extending as slender spine-like process along side of clava and clava with similar spine like process extending subapically from side opposite to that of preclaval process. Males lack similar preclaval and claval processes and rarely flagellum ramose. Propodeum projecting posteriorly between metacoxae, with longitudinal crenulate postspiracular furrow continuous with obliquely angled, crenulate, posterolateral margin; plical region variable in sculpture, uniformly reticulate to smooth and shiny, excluding median carina. Metapleuron with uniform pattern of distinct netlike sculpture defined by impressed lines. Gastral petiole usually distinct and yellowish in female, and often longer than wide in both sexes.

Hosts: Reared as parasitoids of wood-boring beetle larvae of the families Buprestidae and Scolytidae (Gibson, 2003).

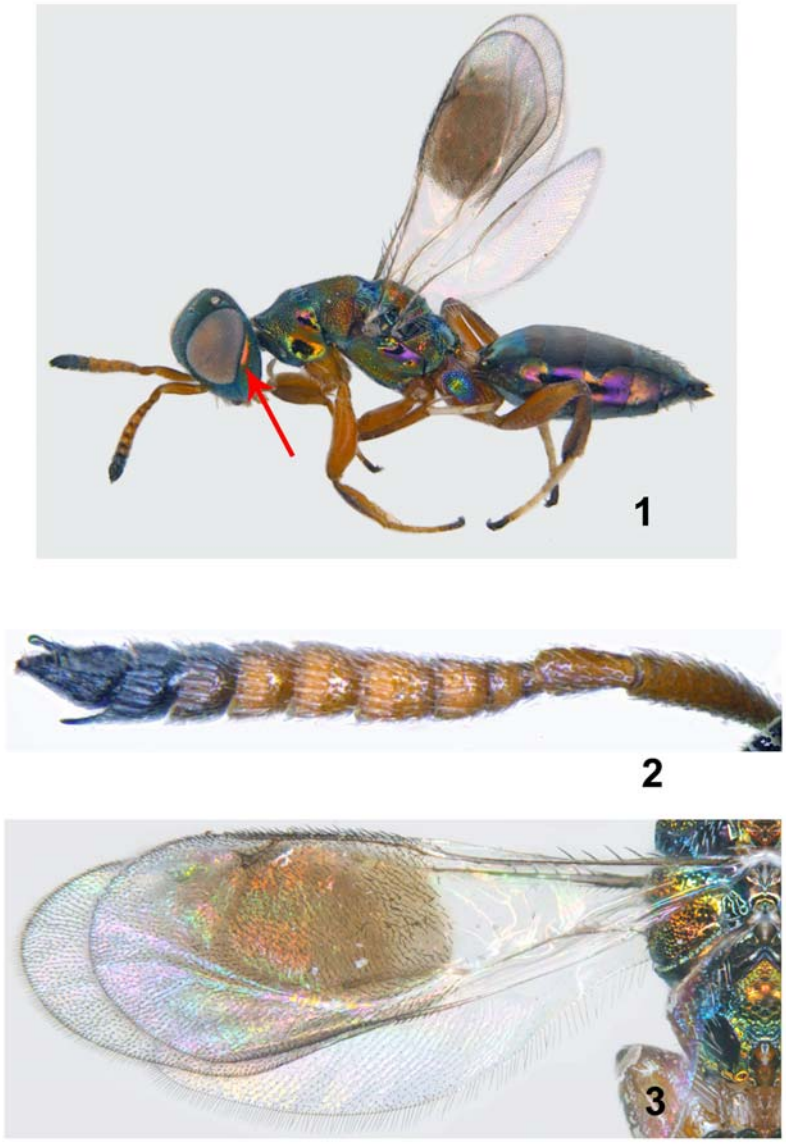
Distribution: Old world.

Callocleonymus indiaensis Gupta & Sureshan sp.nov. (Figs. 1-8)

LSID urn:lsid:zoobank.org:act:16656C32-19C6-487B-8312-3D2A86011F15

Holotype *Female*: Length 2.94 mm (Paratype 1, 2.14 mm). Body with strong metallic luster. Head metallic green, ocelli pale white (brown in paratype), eyes grey. Mandibles brown. Antennae with scape, pedicel, annellus, funicular segments fu1-fu5 yellowish brown; fu6 dark brown; fu7 and clava black (in paratype, antennae with scape and pedicel pale brown; anellus and fu1-fu5 brownish yellow, fu6 pale brown, fu7 and clava black). Pronotum metallic green. Mesoscutum and scutellum metallic green with bronze reflection, base of scutellum and inner margins of axillae with bluish reflection; propodeum with metallic blue shine, with two broad violaceous patches in posterior half. Metasoma brownish black with bluish-purple metallic luster basally and laterally, petiole yellow, ovipositor sheath black. Fore and mid coxae brown, hind coxae yellowish brown with metallic green coloration in middle (in paratype, legs including coxae testaceous except femora and tip of tarsi, brown); trochanters and femur yellowish brown; tibia yellowish brown with dark infuscation dorsally (in paratype, mid and hind tibia except tip, white), tarsi paler testaceous with fifth segment brown. Tegulae dark brown. Wings distinctly infuscate, oval patch starting beneath the MV reaching up to PMV, venation brown, setae dark brown.

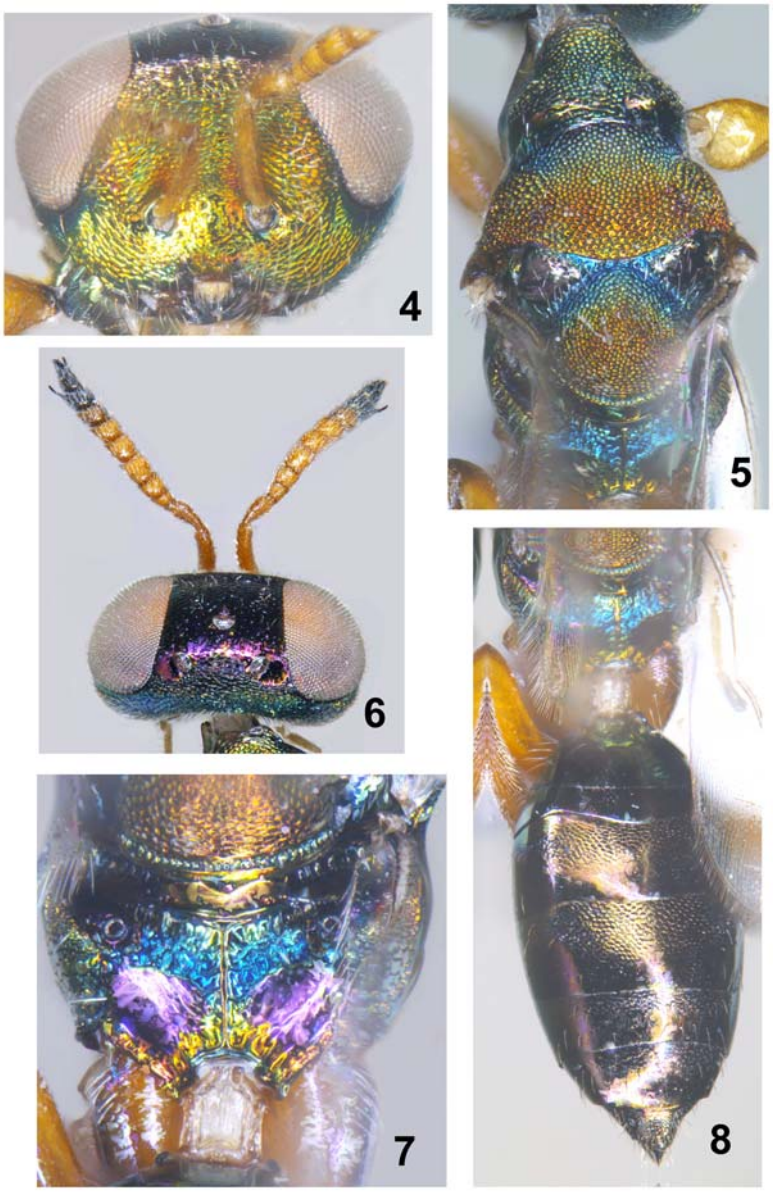
Head (Figs. 1, 4, 6): Reticulate, with scattered white setae; in dorsal view sub rectangular, 1.7× as wide as mesoscutum, width 1.68× length in dorsal view and 1.33× as wide as its median length in frontal view. POL 3.12× OOL. Eyes separated by 1.49× their own length at the level below toruli. Malar suture distinct, malar space length 4.2× eye height. Clypeus not separated by distinct groove from rest of face, with anterior margin shallowly emarginate. Face distinctly reticulate and shiny, reticulations more transverse near the middle of face including scrobal area; gena shiny reticulate. Vertex shiny, smooth shallow with scattered punctuations. Occiput reticulate, more transverse reticulations posteriorly. Scrobal depression slightly indicated, with inter-antennal and lower parascrobal regions



Figs. 1-3: *Callocleonymus indiaensis* Gupta & Sureshan sp. nov.: 1. Female-body in profile view; 2. Antenna; 3. Fore wing

weakly convex. Antenna (Fig. 2) inserted below the lower eye margin, 13 segmented, formula 11713 (if clava counted 3), claval segments not very clear. Toruli separated 2.3× their individual diameter. Scape 0.67× as long as eye length, not reaching median ocellus. Ratio of relative lengths: scape 3.6; pedicel 2.2; annellus 0.03; fu1 0.74; fu2 0.86; fu3 subequal; fu4 transverse 0.98; fu5 transverse 0.78; fu6 transverse 0.82; fu7 transverse 0.82; clava 1.96.

Mesosoma (Figs. 1, 5, 7): 1.85× as long as wide. Pronotum 1.2× as wide as long, bell shaped, collar not carinate anteriorly, distinctly reticulate in the anterior two third and shiny in posterior part, 0.7× as wide as mesoscutum. Mesoscutum 1.89× as wide as long, punctate reticulate with metallic green shine, reticulation larger at apical region, lateral lobes reticulate, notauli incomplete, setation not distinct. Scutellum convex, sub equal to mesoscutum in median length, 1.07× as wide as long, reticulation appears somewhat circular, frenum absent, apex of scutellum rounded. Scuto-scutellar suture raised above axilla. Axillae smooth and shiny with white setae. Dorsellum shiny. Propodeum



Figs. 4-8: *Callocleonymus indiaensis* Gupta & Sureshan sp. nov.: 4. Head in frontal view; 5. Mesosoma in dorsal view; 6. Head in dorsal view; 7. Propodeum and petiole in dorsal view; 8. Metasoma in dorsal view

(Fig.7) coarsely reticulate, 2.55× as wide as median length, 0.50× as long as scutellum medially, median carina distinct and complete, smooth and shiny in the posterior half on either side of median carina; spiracles large, circular, not touching the metanotum, separated by more than their own diameter from the hind margin of metanotum. Anterior half of propodeum coarsely crenulate, plicae absent, costula weakly indicated, callus reticulate with scattered long hairs. Mesopleuron distinctly reticulate posteriorly; mesepimeron shiny. Metapleuron reticulate. Forewing (Fig. 3) 2.62× as long as wide, with a distinct brown patch below MV which is separated from posterior marginal infuscation by a curved hyaline streak, basal hyaline part nearly a little less than half, speculum absent, costal cell narrow, basal hair line not indicated, basal cell bare, discal pubescence as dense setae, patch of thick setae below MV. MV 3.95× as long as PMV and 4.2× as long as STV; stigma moderate-

sized with uncus long. Angle between PMV and STV about 45°. Relative lengths SMV 74.4, MV 36.3, PMV 9.2, STV 8.6. Hind coxae 2.2× as long as wide, with distinct median metallic green reticulate patch, hind tibia with two unequal spurs.

Metasoma (Figs. 1, 8): Petiole 2× as long as wide, testaceous, with a pair of longitudinal rugae laterally. Gaster lanceolate, 2.08× as long as wide in dorsal view, non collapsing dorsally. Metasoma slightly shorter than head plus mesosoma combined (0.91×). Gt1 with few hairs on dorso-lateral sides; Gt2 smallest; Gt3 and Gt4 largest. Gt4 > Gt3, Gt3 onwards finely reticulate in basal half. Posterior margin of all tergites straight except Gt2 slightly projecting. Hypopygium not reaching beyond middle of gaster.

Male: Unknown.

Biology: Probably occurring in dry grasses infested with beetles.

Material Examined: Holotype, female, INDIA: Karnataka: Bengaluru: Chintamani; yellow pan trap; coll. A. Ramesh Kumar, 3.ix.2014, Reg. No. NBAIR/Ptero/Callo/3914. Paratype female, INDIA: Kerala, Kollam district, Kuttichira, 9.xii.18, Ayisha; Reg. No. ZSI/ WGRC/ IR/ INV/12180.

Etymology: The species name is derived from its region of discovery, India.

Remarks: This is the first described species of *Callocleonymus* from the Indian subcontinent which closely resembles *C. pulcher* Masi distributed in the Afrotropical regions. The new species differs from *C. pulcher* as follows: head in side view more sharply angled above middle with eye almost straight behind (Fig.1), in facial view head shorter with cheeks strongly rounded, toruli separated from oral margin by about twice their own diameter (Fig.4), median length of mesoscutum half of its maximum width (Fig.5) and eyes without pubescence (in *C. pulcher* head in side view more rounded above middle with eyes more deeply emarginate in lower half (Masi, 1940: fig.XI b), in facial view longer and with cheeks straighter (Masi, 1940: fig.XI a); toruli separated from oral margin by about their own diameter (Masi, 1940: fig.XI a), median length of mesoscutum two fifth of its maximum width (Masi, 1940: fig.XI e)) and eyes with distinct pubescence). In having the same combination of characters, the new species also resembles *C. ferrieri* Kerrich but differs from it in having forewing without double row of hairs on the cubital hair line. The new species also resembles the Chinese species in general morphology and coloration but differs from them as follows: *C. chuxiongensis* Yang has 2 rows of setae on the cubital hair line and basal cell with few hairs basally and STV arises at an angle of about 60^0 with the PMV (*C. indiaensis* has forewing without any hairs on the basal half, STV arises at an angle of 45^0 with PMV); *C. bimaculae* Yang differs from all the other species in having forewing with two smoky spots and also differs from the new species in having 2 complete rows of hairs on the cubital hair line and surface of scutellum quiet plane (*C. indianesis* has cubital hairline without rows of hairs and scutellum highly convex); *C. beijingensis* Yang has forewing with MV 3.5x as long as STV and antennae with fu_1 2× as wide as long (in *C. indianesis* MV 4.2× STV and fu_1 1.35× as wide as long); *C. xinjiangensis* Yang has reticulation on occiput expanding to POL and OOL and forewing with PMV 1.4× STV (in *C. indianensis* POL and OOL area shiny and PMV 1.06× STV); *C. ianthinus* Yang has antenna with fu_1 and fu_2 as long as wide and eyes hairy (in *C. indianensis* fu_1 and fu_2 sub equal and pilosity on eyes not distinct). *C.swezeyi* Yoshimoto differs from the new species in having eyes hairy, antennae rather short with scape considerably dilated below, pedicel nearly twice as long as wide (in *C. indiaensis* eyes not hairy and antennae longer with scape not much dilated below and pedicel more than twice as long as wide).

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Phytochemical screening for identification of bioactive compounds of *Lantana camara* Linn. (Verbenaceae) responsible for larvicidal action against *Aedes triseriatus* Say, 1823 (Diptera : Culicidae)

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ABSTRACT: Study to assess the larvicidal property of *Lantana camara* leaves against *Aedes triseriatus* larvae found that the ethyl acetate extract had profound larvicidal action with the crude extract having a LC_{50} value of 409.831ppm. GC-MS analysis of the ethyl acetate extract confirmed the presence of twenty-one compounds out of which beta-caryophyllene covered the highest percentage of the chromatogram area. Further tests with beta-caryophyllene against the mosquito larvae proved it to be the active ingredient of *L. Camara* with a LC_{50} value of 104.243ppm.

KEY WORDS: *Aedes triseriatus*, *Lantana camara,* bioactive molecules, beta-caryophyllene, insecticide

INTRODUCTION

Mosquitoes act as vectors of chikungunya fever, filariasis, malaria, yellow fever, dengue fever, and JE. Mosquito borne diseases are prevalent in more than hundred countries affecting mainly the population residing in the tropical ones (Chakraborti and Bandyopadhyay, 2017). *Aedes triseriatus* Say, 1823 (Diptera : Culicidae) is an invasive mosquito species which has been reported for the first time in India (Chakraborti and Bandyopadhyay, 2017). Adults are terrestrial and are commonly found in forested regions with high canopies. (Obenauer *et al.* 2009). Larvae breed in tree holes, tyres, artificial containers (Borucki *et al.,* 2002) etc. in the urban areas. *Ae. triseriatus* is a known vector of La Crosse virus which commonly causes paediatric arboviral encephalitis (Borucki *et al.* 2002). Apart from this, *Ae. triseriatus* has been found to be a competent vector of West Nile virus (Styer *et al.,* 2007), Venezuelan equine encephalitis (Davis *et al.,* 1966), Eastern equine encephalitis, Dengue (type I), Western equine encephalitis etc.

Mosquitoes borne diseases can be controlled either by killing the adults, preventing mosquito bites by use of repellents or by promoting larval mortality in a large scale at the breeding sites of the vector. There are several synthetic insecticides for these purposes. For example organophosphates and insect growth regulators are used worldwide to control mosquito larvae (Rahuman *et al.,* 2009). However,

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attempts have failed as mosquitoes are efficient in attaining physiological resistance. Repeated use of synthetic insecticides has disrupted the natural biological control system causing resurgence in the mosquito population and creating a subsequent human health concern (Das *et al.,* 2007, Pedro *et al.,* 2014). This has initiated a search for cheaper and safer alternatives for insect pest control, including plant based products (Dayan *et al*., 2009). *Lantana camara* Linn. (Verbenaceae) grows widely throughout the tropical, sub tropical and temperate parts of the earth. The leaves of *L. camara* are a rich source of bioactive molecules having insecticidal property (Rajshekhar *et al.,* 2014). They display antibacterial, antimutagenic, antioxidant, hemolytic and fungicidal properties (Arunachalam *et al.,* 2016).

The work embodied in this paper explores the efficacy of *L. camara* leaves against *Ae. triseriatus* larvae with the objective of identifying the active component responsible for killing the larvae through GC-MS analysis.

MATERIALS AND METHODS

Phytochemical screening:

Leaves of *L. camara* were collected from the campus of University of Kalyani, Kalyani, West Bengal, India. A specimen was authenticated by the taxonomist of the Department of Botany, University of Kalyani, Kalyani. The leaves were washed with tap water and dried on paper towels in shade at room temperature. After drying they were blended in a commercially available electric blender to obtain the powder.

Leaf powder was sieved and prepared for extraction using Soxhlet apparatus. For extraction the powder was mixed with the solvents (methanol, ethyl acetate and ethanol) individually in 1:10 ratio (weight : volume) and extracted in a Soxhlet apparatus for 6 hours at 50-60°C. The extracts were filtered using Whatman no.1 filter paper placed in a Buchner funnel. 1ml of the filtered extract of each solvent was stored at 4° C for GC-MS analysis. The remaining filtrates were then concentrated using Rota evaporator at 50-55°C, followed by drying in air or incubator (Ghosh *et al.,* 2016). Stock solution 1500 ppm was prepared in the different solvents for the qualitative tests.

Phytochemical screening was carried out by subjecting 1ml of the respective extracts to the following tests as described below:-

Test for flavonoids : Alkaline reagent test -

1ml of extract solution was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned colourless by adding few drops of dilute acid, indicating the presence of flavonoids. (Saha and Bandyopadhyay, 2017).

Test for saponins: Frothtest -

2.0 g of the powdered plant material was boiled in distilled water in a test tube and filtered. Filtrate was mixed with distilled water (2:1 ratio) and was shaken vigorously till formation of stable persistent froth. The frothing was mixed with few drops of olive oil and shaken vigorously for the formation of emulsion; a characteristic of saponins.

Test for carbohydrates: Molisch's test -

1ml of extract was treated with few drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicated the presence of carbohydrates.

Test for steroids and terpenoids: Salkowskitest

Few drops of conc. Sulphuric acid along with chloroform were mixed with the crude extract, shaken well and allowed to stand for some time. Appearance of red colour in the lower layer indicated the presence of steroids and formation of a yellow coloured layer indicated the presence of terpenoids (Saha and Bandyopadhyay, 2017).

Test for glycosides -

Five ml of the different extracts were hydrolysed individually with 5 ml each of conc. Hydrochloric acid and boiled for few hours on a water bath. Hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10%

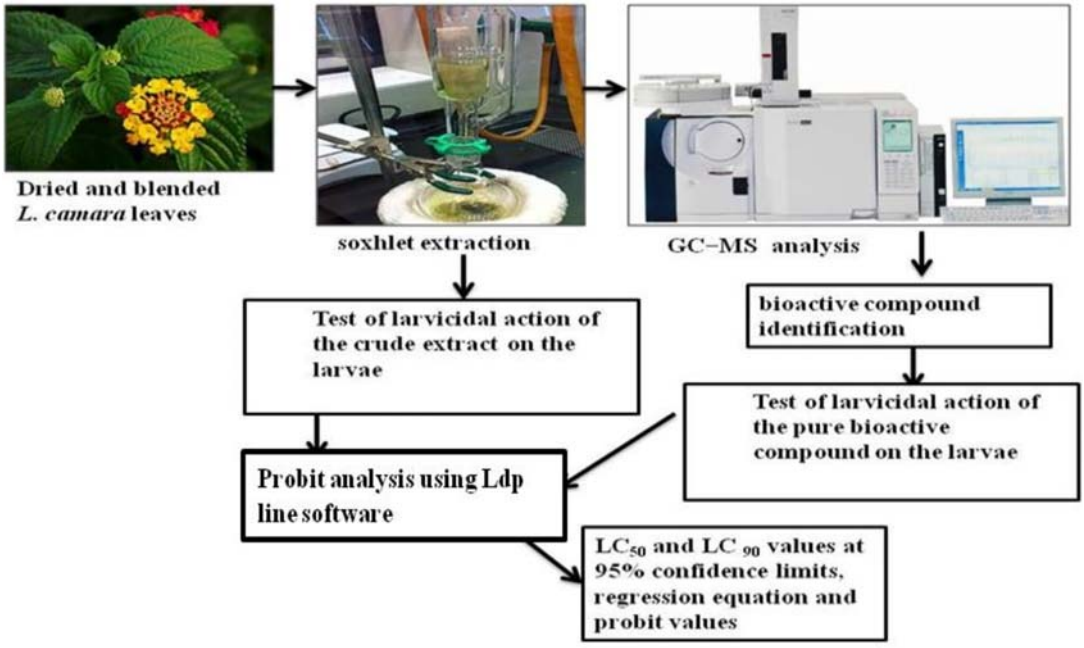

Fig. 1: Showing the methodology undertaken for the study

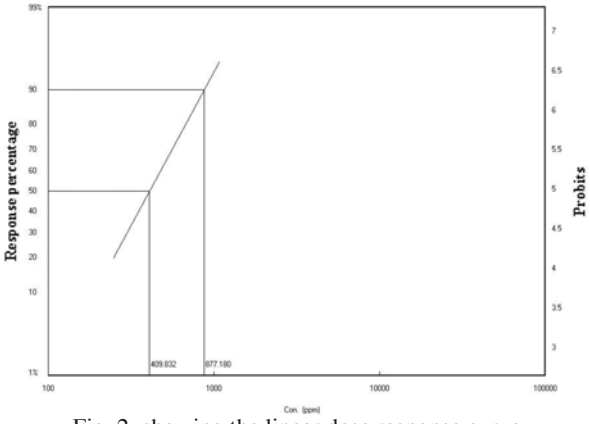

Fig. 2: showing the linear dose response curve for the crude extract

sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

Test for coumarins -

0.5 g of the moistened extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N Sodium hydroxide solution and placed for few minutes in boiling water. The filter paper was removed and examined under the UV light for yellow fluorescence indicating the presence of coumarins.

Rearing of mosquitoes:

Mosquito larvae were collected from abandoned tyres, artificial containers and tree holes and were identified as those of *Ae. triseriatus* as per the identification keys (Chakraborti and Bandyopadhyay, 2017). They were identified as per the identification keys by Farajollahi and Price (2013). Larvae were fed on larval food; dog biscuit and yeast extract in the ratio 3:1 to obtain adults. Cyclic generations of vector mosquitoes were maintained at 25–29 °C insectariums and adult mosquitoes were fed on 10% glucose solution (Arivoli and Samuel, 2011) and blood of mice to obtain larvae. The larvae obtained by rearing the adults were used in the study.

Test of larvicidal action of the crude extract on the larvae:

The larval susceptibility tests were carried out according to the WHO procedure (WHO, 2005). A total of five trials were carried out with three

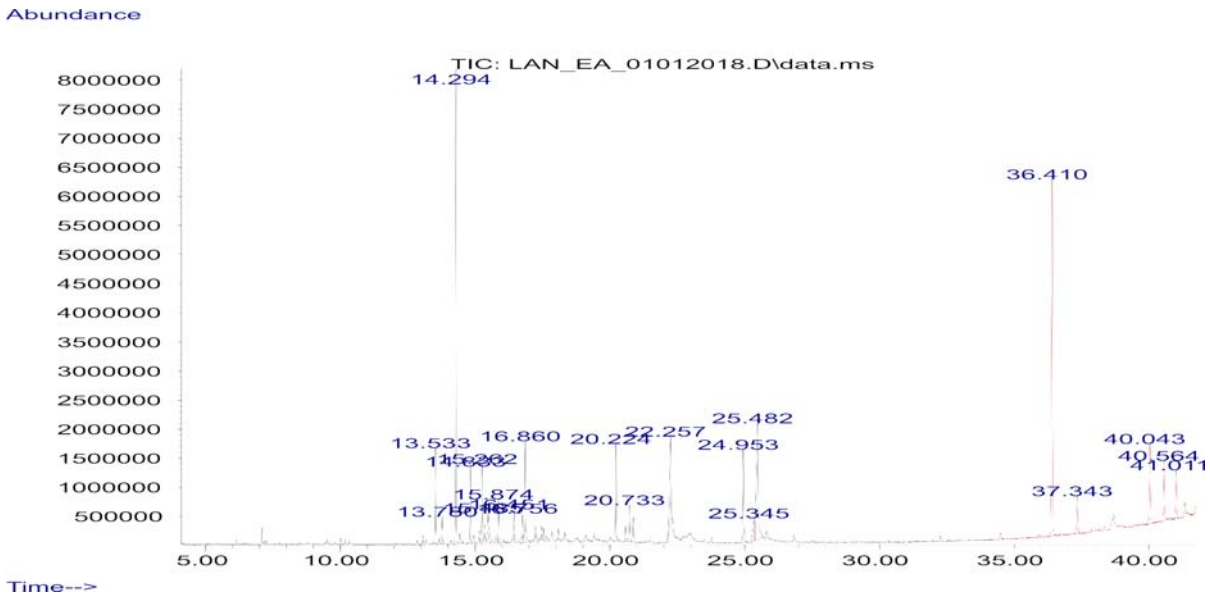

Fig 3. Showing the GC-MS chromatogram of the ethyl acetate extract of *L. camara* leaves

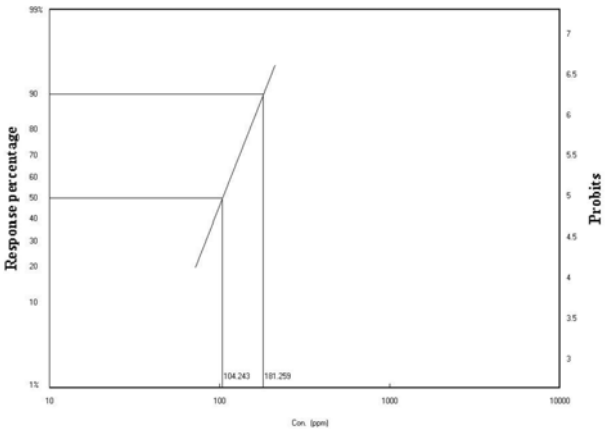

Fig. 4: Showing dose response curve for the pure bioactive compound

replicates per trial against the larvae. The stock solution (1500 ppm) was prepared by dissolving 600 mg of crude extract in 100 ml of the solvent separately with the final volume made up to 400 ml with distilled water. From the stock solution five test concentrations (150, 300, 650, 700, 1000 ppm) were prepared in 200 ml distilled water placed in a beaker. 25 fourth instar larvae were released and the beakers were kept at room temperature. Larvae exposed to 0.1 ml of ethyl acetate in distilled water served as control (Tonk *et al*., 2006).The dead larvae were counted after 24 h and the mortality was reported from the average of five trials. When control mortality ranged from 5% to 20%, mortality values were corrected by Abbott's formula using the Ldp line software.

Statistical analysis

Based on the mortality values, LC_{50} and LC_{90} values of ethyl acetate extract of *L. camara* against *Ae. triseriatus* larvae were recorded by employing probit analysis of Finney, 1971. For probit analysis Ldp line software was used. Results with $P < 0.05$ were considered statistically significant.

GC-MS analysis of the ethyl acetate extract and identification of the compounds

Ethyl acetate extract of *L.camara* leaves was subjected to GC-MS analysis. The experiment was carried out in the instrument from Agilent Technologies; GC-6860N Network GC System with 5973 inert Mass Selective Detector hosted at the National Test House Kolkata-91, West Bengal, India. It was fitted with HP- 1MS (19091S-602) column with Length x I.D. x Film Thickness = 25 x 0.2 x 0.33. The carrier gas was Helium and the flow rate was 1 mL/min. The oven program was

50 °C, 1 min, 6 °C/min to 220 °C, 5 °C/min to 300 °C and again at 220 °C, 2 min. Interpretation of mass spectrum of GC-MS was conducted following the database and using the NBS75K.L library. The spectrum of the unknown components was compared with that of known components in the Database/NBS75K.L library attached to the GC-MS instrument and documented. Using the website https://webbook.nist.gov/chemistry/cas-ser/, the name, molecular weight, molecular formula and structure of the components were ascertained by using their CAS numbers.

Determination of larvicidal property of the pure bioactive compound

The compound that occupied the highest area percentage of the GC-MS chromatogram (beta-caryophyllene) of the ethyl acetate extract of *L. camara* leaves was purchased from Sigma Aldrich company. A stock solution of 1500ppm of the pure compound was made in ethyl acetate as described above. From stock five concentrations 40, 80, 100, 150 and 200 ppm were made. 0.1 ml of ethyl acetate in distilled water was used in the control experiment. These were tested against the larvae in the same way as already mentioned. Mortality (mean of individual trials) was recorded after 24h exposure. LC_{50} and LC_{90} values and the linear dose response curve were generated by probit analysis using Ldp line software. Results with $P < 0.05$ were considered statistically significant.

Table 1. Qualitative tests performed using the solvent extracts

Phytochemical group	Methanol	Ethanol	Ethyl acetate
Flavonoids	-	-	+
Saponins	+	+	+
Carbohydrates	-	-	+
Steroids	-	+	+
Glycosides	+	-	-
Coumarins	+	-	+

RESULTS AND DISCUSSION

Suitability of the extraction solvent as proved by qualitative screening of plant extracts:

Screening of the three solvent (methanol, ethanol and ethyl acetate) extracts of *L. camara* leaves for presence of phytochemicals showed ethyl acetate to be the most effective solvent. The ethyl acetate extract showed the presence of steroids, saponins, terpenes, flavonoids, carbohydrates and coumarins (Table 1). Therefore ethyl acetate was chosen as the solvent for phytochemical extraction for performing the studies thereafter.

Test of larvicidal action of the crude extract on the larvae

The efficacy of the ethyl acetate extract against the fourth instar larvae was recorded as per the

Table 2. Dose, total number of larvae, mean mortality (M ± SD), percentage mortality, linear probit value, χ^2 value, p value, LC_{50} and LC_{90} values at 95% confidence limits for the crude plant extract

Dose (ppm)	Total larvae	Mean Mortality (M±S.D.)	Percentage mortality	Probit	χ^2	p	LC_{50} (ppm)	LC_{90} (ppm)
0	25	nil	nil	-	13.14	0.004	409.831	877.179
150	25	2.3±1.19	9.2	3.307				
300	25	5.8±2.18	23.2	4.474				
650	25	14.26±3.56	57.04	5.766				
700	25	24.2±2.24	96.8	5.901				
1000	25	24.6±3.2	98.4	6.502				

Table 3 showing the compounds identified by GC-MS analysis

Sl. no.	Retention time	Area %	Name of the compound	CAS number	Mol. formula	Mol. weight (g/mol)
1	13.535	3.06	Copane	003856-25-5	$C_{15}H_{24}$	204.3511
2	13.779	1.05	Elemene	011029-06-4	$C_{15}H_{24}$	204.357
3	14.293	22.39	Beta-caryophyllene	000087-44-5	$C_{15}H_{24}$	204.36
4	14.833	2.53	Alpha-caryophyllene	006753-98-6	$C_{15}H_{24}$	204.35
5	15.263	2.80	Germacrene D	023986-74-5	$C_{15}H_{24}$	204.357
6	15.486	1.83	Naphthalene, 1,2,3,4, 4a,5,6,8a-octahydro- 4a,8-dimethyl-2- (1-methylethenyl) -, [2R-(2α,4aα,8aβ)]-	000473-13-2	$C_{15}H_{24}$	204.3511
7	15.875	1.47	Naphthalene, 1,2,4a,5,8, 8a-hexahydro-4,7- dimethyl-1- (1-methylethyl)-, [1S-(1α,4aβ,8aβ)]-	000523-47-7	$C_{15}H_{24}$	204.3511
8	16.451	1.43	Cyclohexane, 1-ethenyl -1-methyl-2- (1methylethenyl) -4-(1-methylethylidene)-	003242-08-8	$C_{15}H_{24}$	204.3511
9	16.758	1.19	1H-Cycloprop[e]azulen -7-ol, decahydro-1,1,7- trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	006750-60-3	$C_{15}H_{24}O$	220.3505
10	16.861	4.08	Caryophyllene oxide	001139-30-6	$C_{15}H_{24}O$	220.3505
11	20.224	3.20	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	000473-55-2	$C_{10}H_{18}$	138.2499
12	20.732	1.43	Dibutyl phthalate	000084-74-2	$C_{16}H_{22}O_4$	278.3435
13	22.258	5.72	Tetradecanoic acid	000544-63-8	$C_{14}H_{28}O_2$	228.3709
14	24.950	4.17	Phytol	000150-86-7	$C_{20}H_{40}O$	296.5310
15	25.345	1.84	9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	$C_{18}H_{32}O_2$	280.4455
16	25.480	10.56	11,14,17-Eicosatrienoic acid, methyl ester	055682-88-7	$C_{21}H_{36}O_2$	320.5093
17	36.412	19.04	Squalene	007683-64-9	$C_{30}H_{50}$	410.7180
18	37.341	1.87	Heptadecane	000629-78-7	$C_{17}H_{36}$	240.4677
19	40.044	4.45	Octadecane	000593-45-3	$C_{18}H_{38}$	254.4943
20	40.563	3.28	Vitamin E	000059-02-9	$C_{29}H_{50}O_2$	430.7061
21	41.009	2.59	Pentatriacontane	000630-07-9	$C_{35}H_{72}$	492.9462

Table 4 showing dose, total number of larvae, mean mortality (M ± SD), percentage mortality, linear probit value, χ^2 value, p value, LC_{50} and LC_{90} values at 95% confidence limits for the pure bioactive compound.

Dose (ppm)	Total larvae	Mean Mortality (M±S.D.)	Percentage mortality	Probit	χ^2	p	LC_{50} (ppm)	LC_{90} (ppm)
0	25	nil	nil	-	9.259	0.026	104.243	181.259
40	25	1.6±1.6	6.4	2.781				
80	25	2.2±1.17	8.8	4.386				
100	25	12.8±3.48	51.2	4.903				
150	25	20.6±5.26	82.4	5.843				
200	25	24±3.45	96	6.509				

WHO directives. No larval mortality was recorded in the control experiment. Results indicated that the plant extract was effective against the larvae with mortality (%) values of 9.2, 23.2, 57.04, 96.8, 98.4 recorded for the five concentrations (150, 300, 650, 700, 1000 ppm) used. LC_{50} and LC_{90} values of the ethyl acetate extract of *L. camara* against *Ae. triseriatus* larvae were recorded by probit analysis. The crude extract had LC_{50} and LC_{90} values of 409.831ppm and 877.179 ppm respectively. Results with $p < 0.05$ were considered statistically significant. Dose, total number of larvae, mean mortality (M ± SD), percentage mortality, linear probit value, χ^2 value, p value, LC_{50} and LC_{90} values at 95% confidence limits for the crude plant extract are given in the table 2. Figure 2 shows the linear dose response curve for the crude extract.

GC-MS analysis of the ethyl acetate extract and identification of the compounds

GC-MS analysis of the plant extract confirmed the presence of twenty-one compounds in it. Out of these major components were beta-caryophyllene (CAS number 000087-44-5) which occupied 22.39% of the chromatogram area followed by squalene (CAS number 007683-64-9) which occupied 19.04% of the chromatogram area and lastly 11,14,17-Eicosatrienoic acid, methyl ester (CAS number 055682-88-7) occupying 10.56% of the chromatogram area. Apart from these there were other phytochemicals that have proven anti-bacterial, anti-fungal, anti-parasitic activities like di-butyl phthalate (CAS number 000084-74-2) (Saha and Bandyopadhyay, 2017), phytol (CAS number 000150-86-7) and 9,12-Octadecadienoic acid (Z,Z) (linoleic acid) (CAS number 000060-33-3) and some alkanes. Table 3 showing the compounds identified by GC-MS analysis. Figure 3 showing the GC-MS chromatogram of the ethyl acetate extract of *L. camara* leaves.

Determination of larvicidal property of the pure bioactive compound

Five concentrations of 40, 80, 100, 150 and 200 ppm were made from the stock solution of beta-caryophyllene (CAS number 000087-44-5) in ethyl acetate. These were tested against the larvae and mortality (mean of individual trials) was recorded after 24h exposure. 0.1 ml of ethyl acetate in distilled water was used in the control experiment. No mortality was recorded in control. Probit analysis using Ldp line software showed that pure beta-caryophyllene had LC_{50} and LC_{90} values of 104.243 ppm and 181.259 ppm against *Ae. triseriatus* larvae respectively. Results with $P < 0.05$ were considered statistically significant. Table 4 showing dose, total number of larvae, mean mortality (M ± SD), percentage mortality, linear probit value, χ^2 value, P value, LC_{50} and LC_{90} values at 95% confidence limits for the pure bioactive compound. Figure 4 showing the linear dose response curve for the pure bioactive compound.

Mosquito larval control using larvicidal agents plays a major role in the control of vector borne diseases.

Plants are a storehouse of several chemicals of which some can serve as potential larvicides. Repeated use of synthetic insecticides has promoted acquisition of resistance in mosquitoes thereby causing resurgence in their population. This is becoming a severe human health concern (Das *et al.,* 2007 and Pedro *et al*., 2014). Thus a search for cheaper and safer alternatives for insect pest control has become necessary (Dayan *et al*., 2009). Plant based phytochemicals are considered viable and preferred as an alternative to synthetic insecticides in the control of the mosquito species at the community level. These phytochemicals can be generally toxic to adults as well as the larvae with some acting as growth inhibitors, chemosterilant or just repellants. Although a large number of plant extracts have been reported to have larvicidal or repellent activities against mosquitoes but only few of them have practical utility for large scale application (Sun *et al.,* 2006). Beta-caryophyllene is an oxygenated sesquiterpene. It has been proven that this sesquiterpene is both larvicidal and adulticidal to *Culex quinquefasciatus* within 24h of treatment (Gleiser and Zygadlo, 2007). Beta-caryophyllene has been found to inhibit growth of *Pseudomonas syringae* pv. *tomato* DC3000; a bacterial pathogen of brassicaceous plants on the stigmas of the flowers of *Arabidopsis thaliana* (Huang *et al.,* 2011). Apart from these this sesquierpene is a major component of the oil of *Piper marginatum* leaves from Brazilian Amazon and exhibits potent activity against the 4th instar larvae of *Ae. aegypti* (Autran *et al.,* 2008). These evidences support our findings that beta-caryophyllene is the principle component responsible for the larvicidal property of *L. camara* leaves against *Ae. triseriatus* larvae.

It is evident from the present study that ethyl acetate extract of *L. camara* can be used as a promising larvicide. The crude extract of the leaves has many bio-active components. Some of these have proven larvicidal and anti-parasitic functions like beta-caryophyllene, phytol, linoleic acid etc. Purification of these compounds from the plant and spraying them in stagnant water bodies which are known to be the breeding grounds for the mosquitoes may bring down the vector population and will certainly reduce the dependence on chemical insecticides.

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Negative cross resistance of *Spodoptera litura* Fabricius population of tomato to newer molecule spinetoram

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ABSTRACT: Field population of *Spodoptera litura* from tomato (resistant to the majority of the conventional insecticide molecules) were subjected to the *in vivo* toxicity of spinetoram 12 SC to assess whether cross resistance exists or not. Untreated larvae of both field and laboratory strains showed no mortality during 48 hours of feeding. After 48 hours of feeding on spinetoram 12 SC treated leaves, LC_{50}s of field larvae were 0.28, 0.93, 3.71 and 7.11 ppm for the 2nd, 3rd, 4th and 5th instars of *S. litura* respectively. However, in the laboratory strain these values were 1.12, 5.86, 36.72 and 91.55 ppm for 2nd, 3rd, 4th and 5th instars of *S. litura* respectively. Resistance ratio was 0.25, 0.16, 0.10 and 0.08 for the 2nd instar up to the 5th instar of *S. litura.* © 2019 Association for Advancement of Entomology

KEY WORDS: Spinetoram 12 SC, tomato, negative cross resistance, *Spodoptera litura*, probit analysis, resistance ratio

INTRODUCTION

Generally in vegetable ecosystem due to poor natural enemy complex and the concealed nature of the pests, need based insecticide application along with other IPM strategies were developed and used to mitigate pests especially on tomato (Sardana *et al.,* 2004). Insecticides have been used extensively for the control of these insect pests for quicker remedy. But these chemicals with varied mode of action due to indiscriminate use carry the danger of resistance development, pest resurgence, outbreaks of secondary pests, reduction in biodiversity of natural enemies, and bio-concentrations of residues in consumable produce at harvest (Krishnamurthy, 1999 and Mitra *et al.,* 1999). Available reports reveal that repeated applications of synthetic chemical insecticides dominate the other means for the control of the pest and their indiscriminate use has led to the resurgence of whitefly, aphid and mite (Rahman, 1999).

Muggleton (1984) used the term "resistant" (1) to describe a resistant population which contains a single individual capable of surviving a laboratory dose of insecticide which would kill almost all (at least 99.9%) of a laboratory strain and (2) to describe only a field population which could not be controlled by field dose properly applied. For resistance management, it is important that resistance is detected at the earliest stage. For effective implementation of resistance management practices, it is essential to have some estimate of changes of resistance level throughout the area when a particular chemical is applied (Taylor, 1993). These estimates rely on initial baseline levels of susceptibility and variability in population so that

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future comparisons can be made (Georghiou and Mellon, 1983). Spinetoram is effective against insect pest, *P. xylostella* which is resistant to the existing insecticides (organophosphates, chitin biosynthesis inhibitor and synthetic pyrethroids) in crop protection (Anonymous, 2012). However, there are no reports on negative cross resistance of *Spodoptera litura* Fabricius for spinetoram. Therefore, this study was undertaken with the objective to investigate the negative cross resistance of the *Spodoptera litura* population of tomato to spinetoram 12 SC in the laboratory.

MATERIALS AND METHODS

Mass culturing of *Spodoptera litura*: The egg masses or larvae collected from field were maintained on castor leaves until pupation. The pupae were transferred to adult emergence cage. The emerging adult moths were confined to oviposition cage and provided with 10 per cent honey solution as adult food following the method of Britto (1980). Nerium leaves were placed as substrate for egg laying to avoid desiccation of eggs. Egg masses laid on the leaves were collected and surface sterilized with 10 per cent formaldehyde and transferred to castor leaves after shade drying. The whole set up was kept in a plastic bucket and the mouth was secured with khada cloth. After hatching, fresh castor leaves were provided every day as larval feed. The larvae were maintained in the rearing buckets at 300, 100, 50 and 25 numbers during the first, second, third and fourth instar stages respectively to avoid overcrowding. In order to prevent larval escape the mouth of the trough was secured with khada cloth. Adequate care was taken to avoid disease incidence. During pre-pupal stage, the larvae were transferred to a container provided with sawdust and placed in adult emergence cage. The culturing was carried out at 26±1°C, RH 75±5% and photoperiod 16:8 h scoto/photo regimes. The emerging adults were used for the maintenance of subsequent cultures (Fig. 1).

Negative cross resistance of *S. litura* population of tomato to spinetoram 12 SC:

Tomato growing farmers often use a number of insecticide molecules with varied chemical nature and mode of action. This situation leads to the development of resistance to the pests like *S. litura*. There is also a hypothesis that new chemistry molecules are desired not to show any cross resistance with the existing insecticides used against crop pests. In order to test and verify the hypothesis, conventional insecticides resistant field populations of *S. litura* (from tomato) were collected and assessed for their susceptibility to various concentrations of spinetoram 12 SC.

S. litura larvae were reared for five generations in the laboratory without any exposure to insecticides, and used as standard reference. Field population of *S. litura* were collected from farmer's holding (Mr. Kaaranam) at Perumalpatti, Melur Block, Madurai District, which might had been subjected selection pressure due to continuous application of varied categories of insecticide molecules. These larvae were reared and maintained in the laboratory separately. Acute toxicity experiment of spinetoram 12 SC against *S. litura* was done by leaf dip technique. Spinetoram 12 SC was diluted with water to obtain a range of seven different concentrations (2.4 ppm, 4.8 ppm, 7.2 ppm, 9.6 ppm, 12.0 ppm, 14.4 ppm and 16.8 ppm).

Fresh leaves of potted tomato plants were collected and dipped for 10 seconds in different concentrations of spinetoram 12 SC and left dry under laboratory condition for one hour. Leaves of untreated control were dipped in water. All the treated leaves were placed in large plastic containers separately and covered with muslin cloth. Laboratory reared *S. litura* larvae (20) were released on treated leaves. Similarly, 20 numbers of field collected *S. litura* larvae were also placed on another set of treated leaves (Temerak, 2003). All the two experiments were replicated three times. The larvae were considered dead if they became desiccated with shortened body and dark cuticle, and/or unable to move in a coordinated manner when disturbed with a needle.

Statistical analysis:

For the above three negative cross resistance experiments data on larval mortality after 48 hours was subjected to Abbott's formula for correction

Fig. 1. Mass culturing of *Spodoptera litura* Fabricius

wherever required. Probit analysis was determined to calculate LC_{50} and LC_{95} (Finney, 1971) and resistance ratio was calculated by dividing LC_{50} of field population with LC_{50} of laboratory reared population.

RESULTS AND DISCUSSION

Due to recurrent application of insecticides on vegetables like tomato most of the lepidopteran pests may have developed resistance. Generally, newly synthesized insecticide compounds are preferred not to show cross resistance with existing insecticide molecules. With this background, field population of *Spodoptera litura* from tomato which was known to be resistant to most of the conventional insecticides were collected and subjected to the *in vivo* toxicity of spinetoram 12 SC to assess whether cross resistance exists.

Probit analysis criteria of field and laboratory strain of all *S. litura* instars revealed that (Table 1) untreated larvae of both field and laboratory strains showed no mortality during 48 hours of feeding. After 48 hours of feeding on spinetoram 12 SC treated leaves, LC_{50}s of field larvae were 0.28, 0.93, 3.71 and 7.11 ppm for the 2nd, 3rd, 4th and 5th instars of *S. litura* respectively. However, in the laboratory strain these values were 1.12, 5.86, 36.72 and 91.55 ppm for 2nd, 3rd, 4th and 5th instars of *S. litura* respectively. The results confirmed the greater LC_{50} of the laboratory strain than of the field strain. The laboratory strain of the 2nd instar showed higher LC_{50} than 2nd and 3rd instars of field strain. Resistance ratio was 0.25, 0.16, 0.10 and 0.08 for the 2nd instar up to the 5th instar of *S. litura* (Table 2).

Insecticide molecules with varied mode of action due to indiscriminate use carry the danger of development of resistance, resurgence of pests, secondary pest outbreak, reduction in biodiversity of natural enemies and bio - concentrations of residues in consumable produces at harvest have opened the new era of green insecticides having a novel mode of action with higher activity against target insects. Traditional resistance management plans have often used a "use and discard" approach, changing the chemical to target a different mode of action in the pest species once resistance becomes a problem in the field. An alternative strategy is to identify compounds that confer negative cross - resistance (NCR), where the NCR compound is more toxic to pesticide resistant insects as compared to their pesticide susceptible counterparts. Such an insecticide spinetoram 12 SC shows results for laboratory strains of 2nd to 5th instar of *S. litura* registered higher LC_{50}s from 1.12 to 91.55 ppm. Thus field collected larvae of *S. litura* from tomato showed higher sensitivity towards spinetoram 12 SC. This finding may offer a great advantage to spinetoram 12 SC for being used in the integrated pest management programme in tomato.

Table 1. Probit analysis of larval instars of field *S. litura* versus laboratory strain

Larval instars	LC_{50} (ppm)	Lower limit	Upper limit	Folds	LC_{95} (ppm)
Field 2nd instar	0.28	0.16	0.43	1.00	1.98
Field 3rd instar	0.93	0.67	1.19	3.32	4.07
Field 4th instar	3.71	2.49	5.01	13.24	53.12
Field 5th instar	7.11	6.13	8.09	25.38	94.37
Laboratory 2nd instar	1.12	0.87	2.09	4.00	9.54
Laboratory 3rd instar	5.86	4.98	6.91	20.92	21.69
Laboratory 4th instar	36.72	25.14	59.11	131.09	198.23
Laboratory 5th instar	91.55	79.83	113.27	326.83	329.45

Table 2. Resistance ratio between field strain of *S. litura* and laboratory strain

Larval instars	LC_{50} of field strain	LC_{50} of laboratory strain	Resistance ratio
2nd instar	0.28	1.12	0.25
3rd instar	0.93	5.86	0.16
4th instar	3.71	36.72	0.10
5th instar	7.11	91.55	0.08

Present results indicate that field strains could exhibit more susceptibility to the unconventional insecticides such as spinetoram 12 SC. This may be due to the differences in the mode and site of action (Salgado, 1997). These results also substantiate with the findings of scientist who concluded that spinosad selected strain of *Plutella xylostella* (L.) did not show any cross resistance to certain conventional insecticides (Arora, 2002). Similar results were found by Temerak (2003) between field and laboratory strains of cotton leaf worm, *S. littoralis* when tested spinosad. According to Hamouda and Dahi (2008), spinetoram 12 SC was a fairly toxic compound to 4th larval instar of *S. littoralis* larvae treated with LC_{50} (1.11 ppm). It has a neurotoxic effect manifested as well defined histopathological changes in nerve and neurosceretory cells. Also, neurotoxic effects were manifested as paralysis of some *S. littoralis* larvae after treatment with low concentrations and lethality at the high concentrations of spinetoram 12 SC.

Mascarenhas *et al.* (1998) indicated that LC_{50} of chlorpyriphos and thiodicarb of field strain of beet army worm *S. exigua* had significantly high LC_{50} than reference strain. Spinosad and spinetoram 12 SC has both contact and stomach toxicity, appears to be unique with primary site of attack being the nicotinic acetyl choline receptor and a secondary site of attack being GABA receptors (Salgado, 1997 and Watson, 2001). This mechanism of action suggests that resistance due to changes in the target sites of many other insecticides might not result in cross resistance to spinetoram 12 SC as well (Toshio and Scott, 2003).

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Description of a new species of *Lathromeris* Foerster (Hymenoptera: Trichogrammatidae) from India

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ABSTRACT: A new species of *Lathromeris* Foerster (Hymenoptera: Trichogrammatidae), *L. indica* sp. nov., is described from India (Haryana). A key to the Indian species of *Lathromeris* also provided.

KEY WORDS: *Lathromeris*, new species, Trichogrammatidae, India

The species of the genus *Lathromeris* Foerster (Hymenoptera: Trichogrammatidae) are an important group of egg parasitoids, and are of potential use in the biological control of mainly lepidopteran pests. Sankaran (1974) introduced for the first time in India *Lathromeris ovicida* (Risbec 1956) for biological control of sugarcane borers (*Chilo infuscatellus, C. sacchariphagus indicus, C. auricilius, Scirpophaga excerptalis* and *Acigona steniellus*). Viggianii & Laudonia (1994) recorded *L. cecidomyiiae* as a larval parasitoid of *Lasioptera* sp. (Diptera: Cecidomyiidae). The genus *Lathromeris* contains 27 species throughout the world, and three species from India *L. viggianii* Yousuf & Shafee, (1988), *L. yousufi* Hayat (2009) and *L. nainitalensis* Khan& Anis (2017). In the present paper one new species, *Lathromeris indica* **sp.nov.**, is described and a key to the four Indian species is provided.

The specimen of the new species was recovered along with other material collected with sweep net from Novgavan; Jhajjar, Haryana, India, and preserved in 70% ethanol. After dehydration, the specimen was dissected in clove oil under microscope and dissected body parts were mounted on a slide in Canada balsam. All photographs were taken with Nikon Digital sight camera attached with opti-phot microscope and relative measurements were taken at different magnification, 20x and 40x (Objective lens) under Leica compound microscope (Leitz labor luxs). The holotype of the new species is deposited in the National Forest Insect Collection, Forest Entomology Discipline, Forest Protection Division of the Forest Research Institute, Dehradun, India (NFIC-FRI-22174).

Key to Indian species of *Lathromeris* (females)

1. Antennal club less than 3× as long as wide…2

- Antennal club 3.2× as long as wide. (Scape 4× as long as wide; pedicel 2.2× as long as wide; fore wing with length of marginal fringe one-fifth of wing width) ……1. ***L. indica* sp. nov**.

2. Antenna with scape 3× or less as long as wide 3

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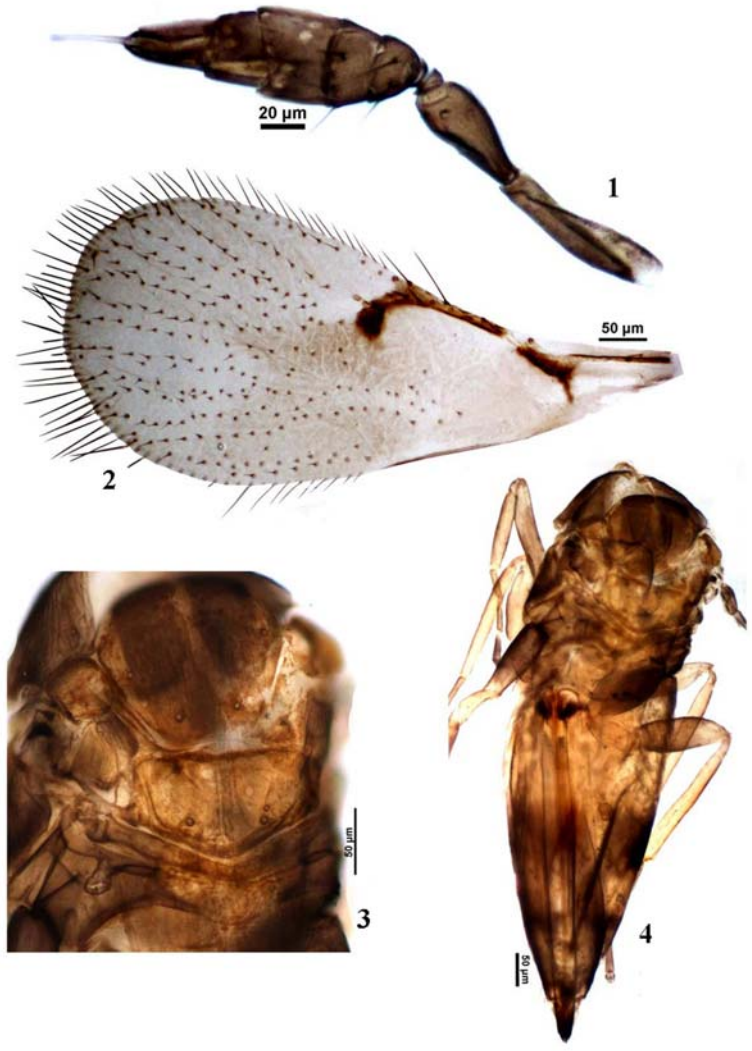

Figs. 1-4. *Lathromeris indica* sp. nov. Mohd. Yousuf, Mohsin Ikram & Neha Rajwar (Female).
1. antenna; 2. fore wing; 3. mesosoma; 4. meso+metasoma & ovipositor

- Antenna with scape more than 3.45× as long as wide. (Fore wing slightly less than 2.5× as long as wide; ovipositor 2.48× as long as hind tibia) .....2. ***L. viggianii*** Yousuf & Shafee

3. Antenna with scape less than 2.8× as long as wide; pedicel 1.5× as long as wide; fore wing with marginal fringe about half of wing width..….....3. ***L. yousufi*** Hayat

- Antenna with scape 3× as long as wide; pedicel more than 1.69× as long as wide; fore wing with marginal fringe about one-third of fore wing width.…....4. ***L. nainitalensis*** **Khan &Anis**

Lathromeris indica **sp. nov. Mohd. Yousuf, Mohsin Ikram & Neha Rajwar (Figs. 1–4)**

LSID urn:lsid:zoobank.org:act:C10C60C7-AB7F-4E24-B90F-F40C6E7FE073

Description

Female: Body length 0.36 mm. Head pale brown except fronto-vertex pale yellow; eyes and ocelli dark; mandibles dark brown. Antenna with scape, pedicel and club pale brown. Pronotum short and dark brown; mesosoma and metasoma brown; axillae and mesopleuron brown. Fore wing hyaline with slight infuscation beneath the stigmal vein. Legs pale yellow except fore and hind femur, coxae and trochanters dark brown. Gaster brown.

Head, in frontal view, slightly broader than long; mandilbles tridentate. Antenna (Fig. 1) with scape 4× as long as wide (77: 19); pedicel 2.2× as long as wide (55: 24); two anelli present; funicle absent; club five-segmented (C1, C2, C3, C4 and C5), 3.2× as long as wide (135: 42), C5 with rod like apical projection slightly less than C5 length (32: 37).

Mesosoma (Fig. 3). Midlobe of mesoscutum having 2 pairs of setae and sculpture as shown in Fig.3, slightly wider than long (138:123); scutellum with one pair of setae, 1.6× as wide as long (127:77); propodeum 1.8× as long as metanotum (29:16) medially. Fore wing (Fig. 2) 2.2× as long as wide (623:276), all discal setae arranged in rows; marginal fringe about one-fifth of wing width (57: 276); stigmal vein well-developed and less than half length of marginal vein.

Metasoma (Fig. 4): Gaster 1.8× as long as mesosoma (520:278); ovipositor 2.3×as long as hind tibia length (538:228).

Male: Unknown.

Host: Unknown.

Type specimen examined: Holotype (NFIC-FRI-22174), ♀ (on slide). INDIA: HARYANA: Jhajjar, Nogavan, 26.i.2019, coll. A. K. Mishra. Type specimen has been deposited in NFIC (National Forest Insect Collection), Forest Entomology Discipline, Forest Protection Division, Forest Research Institute, Dehra Dun, Uttarakhand.

Distribution: India: Haryana.

Etymology: The species name is derived from the name of the country of origin of the type specimen,India.

Discussion: *Lathromeris indica* sp. nov.is very similar to *L. balcanica* (Nowicki, 1940) but differ by having antenna with scape 4× as long as wide, pedicel more than 2.2× as long as wide. Fore wing 2.2× as long as wide, marginal fringe one-fifth of fore wing width, stigmal vein slightly less than half of marginal vein. In *L. balcanica* (Nowicki, 1940) scape 3× as long as wide, pedicel 2× as long as wide. Fore wing slightly more than 2.5× as long as wide, marginal fringe less than one-third of fore wing width and stigmal vein one-third of marginal vein.

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Eco-friendly management of leaf eating caterpillar *Noorda blitealis* Walker in moringa

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ABSTRACT: The study revealed that regardless of seasons two sprays with Meeno amilam @ 2.5 lit. at monthly interval was consistently and significantly effective against *Noorda blitealis*. It recorded 75.67 per cent reduction over untreated check. Amirtha karaisal @ 5.0 lit. ranked next with 73.48 per cent reduction, followed by Arappu mor karaisal and raking up of the soil. Further application of Meeno amilam and Amirtha karaisal have recorded higher yield and ICBR. Hence, sprays of meeno amilam or Amirtha karaisal may be suggested as an eco-friendly pest management of. *N. blitealis*.

KEY WORDS: *Moringa oleifera, Noorda blitealis,* meeno amilam, amirtha karaisal and arappu mor karaisal, efficacy, ICBR

Moringa, *Moringa oleifera* (Lamk.), belongs to the family Moringaceae, which is consisting of only one genus with 13 species of deciduous trees (Keay, 1989). India is the largest producer of moringa with an annual production of 1.3 million tons of tender fruits from an area of 38,000 ha. Among different states, Andhra Pradesh leads in area of production (15,665 ha), followed by Karnataka (10,258 ha) and Tamil Nadu (7,408 ha) (Singh, 2011). *M. oleifera* leaves, tender pods, fruits, flowers, *etc*. are edible and possess tremendous nutritional and medicinal values. The leaves are rich in protein, carotene, iron and ascorbic acid and are used as leafy vegetable. With all of its many quality attributes, ability to thrive under difficult conditions and as an income generating crop to farmer, it is considered as a "miracle tree". Moringa is susceptible to many insect pests *viz.,* leaf eating caterpillar, *Noorda blitealis* Walker, bud worm, *Noorda moringae* Tams., pod fly, *Gitona distigma* Meigen., hairy caterpillar, *Eupterote mollifera* Walker, stem borer, *Indarbela quadrinotata* Walker, ash weevils, *Myllocerus* sp., Tea mosquito bug, *Helopeltis antonii* (Sign.) (Butani and Verma, 1981; Parrota, 2009). Among these pests, leaf-eating caterpillar, *N. blitealis* and its early instars feed on the leaves by scrapping the chlorophyll content, resulting in the papery appearance of leaves and later instars feed on entire leaves by leaving only veins behind. In severe infestation, the trees are almost without leaves resulting in cent per cent damage to foliage (Munj *et al*., 2001; Honnalingappa, 2001). Keeping this in view, the present studies are under taken to evaluate the efficacy of eco- friendly treatments against leaf eating caterpillar.

Ecofriendly organic treatments such as Arappu mor karaisal were prepared by using two kgs of

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grounded Arappu (Usil) leaves, 5 litres of sour butter milk and one litre tender coconut were mixed and fermented for seven days; Meeno amilam @ 1:1 were prepared by using fish waste and nattu vellam (jaggery) each at 1 kg were mixed well and allowed to ferment for 40 days. Later, the filtrates were used for field spraying; Amirtha karaisal extract were prepared with cow dung 5 kg, cow urine 5 litres, nattu vellam (jaggery) 0.250 kg were mixed with 10 litres of water and allowed to ferment for 24 hours. Then, the supernatant liquids of all organics were collected and the volume was made up to another 10 litres. Besides, racking up of soil was also done once in a week to expose of pupae of leaf eating caterpillar.

Two field experiments were conducted against leaf eating caterpillar, *N. blitealis* one at Sathankulam (Sep 2016- Dec 2016) and another at Ayyanarkulam (Jan 2017- April 2017) of Thoothukudi District, Tamil Nadu. A randomized block design was adopted. Each treatment was replicated four times. The plot size was 5 x 4 m comprising of 20 moringa trees of PKM 1 varieties in farmers holding under irrigated condition. Two rounds of foliar spray were given at monthly interval. One at new flush formation period and the second at 30 days after the first spray, using a high volume knapsack sprayer @ 500 litres of spray fluid per ha. The population of leaf eating caterpillar was recorded in randomly selected three branches in three directions of selected 5 trees and expressed as number of larvae per tree. At harvest the leaf yield was recorded from each plot, replication wise and treatment wise, the same was weighted and recorded to work out the ICBR. The mean yield was computed in terms of kg ha-1 and the per cent increase in yield in treated plot over control was computed. Cost of protection includes cost of treatment with cost of application. Data on number of larvae per tree were analyzed statistically after subjecting to square root transformations. The treatment mean values were compared by DMRT at 5 per cent probability to assess the effective treatment.

The larval population of leaf eating caterpillar differed significantly in various treatments in all periods of observation (Table 1). Computation of overall mean population throughout the periods in field experiment I revealed that application of Meeno amilam 1:1 @ 5 liters recorded the lowest population of 3.78/tree followed by Amirtha karaisal 4.82/tree and Arappu mor karaisal 5.55/tree as compared to 21.47 in untreated check. In the second field experiment also, Meeno amilam 1:1 @ 5 lit. has significantly reduced the leaf eating caterpillar population (69.63 %), followed by Amirtha karaisal (62.37 %) and Arappu mor karaisal (55.63) respectively.

The results of the pooled mean data on the population of leaf eating caterpillar revealed that Meeno amilam 1:1 @ 5 liters treatment recorded significantly less population (6.53 larvae/tree) which was followed by Amirtha karaisal (7.12 larvae/tree) compared with control (26.85 larvae/ tree) with a reduction over control of 75.67 and 73.48 respectively (Table 1).

Meeno amilam has recorded the highest leaf yield (13.98 t/ha) as against 8.63 t/ha in untreated check, registering 62.00 per cent increase in yield over untreated check. Next in the order of efficacy was Amirtha karaisal (36.03 %) and Arappu mor karaisal (21.23 %). With regard to moringa leaf yield again Meeno amilam as recorded the highest ICBR 21.95. Amirtha karaisal ranked second an ICBR of 14.24 respectively (Table 2).

In the recent past, the moringa has gained importance among human beings due to its innumerable medicinal properties (Lynch and Spafford, 2014; Parrota, 2009). Accordingly, reviews on the insect and non-insect pests associated with annual moringa have been increasing (Kotikal and Math 2016; Joshi *et al*., 2016). Integrated pest management for only few insect pests of annual moringa has been developed (Saha *et al*., 2014; Selvi and Muthukrishnan, 2009). (Brunda Kumari, 2014) indicated that the lowest *N. blitealis* population was noticed in plots sprayed with *B. bassiana* 1.15SP @ 2g $^{-1}$l, NSKE @ 5%, azadirachtin 1000ppm @3ml^{-1}l were at par with each other by recording the larval population of 1.67, 2.34 and 3.00 larvae per branch respectively. In the present study, Meeno amilam and Amirtha karaisal were found to be effective against *N. blitealis*. It is concluded that each two rounds of

Table 1. Effect of various treatments against *N. blitealis* (Field experiment I & II)

Treatments	Dosage	Field experiment I									Field experiment II									Pooled mean	Percent Reduction over control
		Number of larvae/ tree*								Percent Reduction over control	Number of larvae/ tree*								Percent Reduction over control		
		PTC	I spray			II spray			Mean		PTC	I spray			II spray			Mean			
			7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT				7 DAT	14 DAT	21 DAT	7 DAT	14 DAT	21 DAT				
T_{1}- Rake up the soil	Once in a week	14.37 (3.79)	8.20 (2.86)^{c}	7.50 (2.74)^{c}	6.70 (2.59)^{c}	5.60 (2.37)^{c}	4.80 (2.19)^{d}	3.31 (1.82)^{d}	6.02 (2.45)^{b}	71.96	17.77 (4.22)	15.67 (3.96)^{c}	14.67 (3.83)^{d}	13.33 (3.65)^{d}	20.67 (4.55)^{d}	17.33 (4.16)^{d}	13.67 (3.70)^{d}	15.89 (3.98)^{e}	48.00	10.96 (3.31)^{b}	59.18
T_{2}- Meen amilam	5 l ha^{-1}	15.26 (3.91)	6.37 (2.52)^{a}	4.70 (2.17)^{a}	4.10 (2.02)^{a}	3.10 (1.76)^{a}	2.51 (1.58)^{a}	1.91 (1.38)^{a}	3.78 (1.94)^{a}	82.39	18.00 (4.24)	11.33 (3.37)^{a}	9.33 (3.05)^{a}	8.33 (2.89)^{a}	12.67 (3.56)^{a}	7.67 (2.77)^{a}	6.33 (2.51)^{a}	9.28 (3.03)^{a}	69.63	6.53 (2.55)^{a}	75.67
T_{3} - Amirtha karaisal	50 l ha^{-1}	14.30 (3.78)	7.10 (2.66)^{b}	6.53 (2.56)^{b}	5.50 (2.35)^{b}	4.42 (2.10)^{b}	3.07 (1.75)^{b}	2.28 (1.51)^{b}	4.82 (2.19)^{ab}	77.55	17.87 (4.23)	13.33 (3.65)^{b}	11.33 (3.37)^{b}	9.67 (3.11)^{b}	15.67 (3.96)^{b}	10.33 (3.21)^{b}	8.67 (2.94)^{b}	11.50 (3.38)^{ab}	62.37	7.12 (2.66)^{ab}	73.48
T_{4} - Arappu mor karaisal	50 l ha^{-1}	14.70 (3.83)	7.67 (2.77)^{bc}	7.00 (2.64)^{bc}	6.37 (2.52)^{c}	5.21 (2.28)^{bc}	4.22 (2.05)^{c}	2.82 (1.68)^{c}	5.55 (2.35)^{ab}	74.15	18.13 (4.26)	14.67 (3.83)^{c}	12.67 (3.56)^{c}	12.00 (3.46)^{c}	17.33 (4.16)^{c}	13.33 (3.65)^{c}	11.33 (3.37)^{c}	13.56 (3.67)^{bc}	55.63	9.79 (3.12)^{b}	63.53
T_{5}- Untreated check	-	14.60 (3.82)	15.50 (3.94)^{d}	18.17 (4.26)^{d}	24.57 (4.96)^{d}	25.13 (5.01)^{d}	27.27 (5.22)^{e}	28.17 (5.31)^{e}	(4.63)^{c}	21.47 -	17.80 (4.22)	20.33 (4.51)^{d}	24.67 (4.97)^{e}	27.33 (5.23)^{e}	33.33 (5.77)^{e}	36.33 (6.03)^{e}	41.33 (6.43)^{e}	30.56 (5.52)^{d}	-	26.51 (5.14)^{c}	-
SEd	-	NS	0.0516	0.0680	0.0400	0.1140	0.0271	0.0346	0.2070	-	NS	0.0617	0.0812	0.0676	0.0581	0.0960	0.0739	0.2538	-	0.20	
CD (0.05)	-	NS	0.1191	0.1569	0.0922	0.2628	0.0625	0.0798	0.4772	-	NS	0.1422	0.1872	0.1559	0.1340	0.2213	0.1704	0.5852	-	0.48	

PTC=Pre Treatment Count, DAT= Day(s) after treatment, NS – Non significant

*Each value is the mean of four replications

Figures in parenthesis are square root transformed values

In a column, means followed by same letter (s) are not significantly different by DMRT (p= 0.05)

Table 2. Effect of various treatments on leaf yield and ICBR, (Field Experiment-I & II)

Treatments	Field Experiment - I								Field Experiment - II							
	Leaf yield (t/ ha)	Percent increase over control	Yield (kg/ ha)	Incremental yield over control (kg)/ ha	Incremental benefit over control (Rs/ ha)	Cost of Protection	Additional net profit	ICBR	Leaf yield (t/ ha)	Percent increase over control	Yield (kg/ha)	Incremental yield over control (kg)/ ha	Incremental benefit over control (Rs/ ha)	Cost of Protection	Additional net profit	ICBR
T_1 - Rake up the soil - Once in a weekt	10.02	16.10	10020	1390	11120	840	10280	12.24	9.11	18.01	9110	1390	11120	840	10280	12.24
T_2 - Meen amilam 5 L /ha	13.98	62.00	13980	5350	42800	1865	40935	21.95	12.80	65.80	12800	5080	40640	1865	38775	20.80
T_3 - Amirtha karaisal 50 L/ha	11.74	36.03	11740	3110	24880	1633	23247	14.24	10.57	36.92	10570	2850	22800	1633	21167	12.96
T_4 - Arappu+curd extract 50 L/ha	10.47	21.32	10470	1840	14720	1375	13345	9.70	9.86	27.72	9860	2140	17120	1375	15745	11.45
T_5 - Untreated check	8.63	-	8630	-	-	-	-	-	7.72	-	7720	-	-			

Market Price of leaves was Rs. 8/kg

* Cost of treatment with insecticide + Cost of application

ICBR= Incremental Cost Benefit Ratio

foliar application of Meeno amilam and Amirtha karaisal at monthly interval starting from new flush period can be recommend for the management of moringa leaf eating caterpillar.

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Effect of coriander *Coriandrum sativum* L. on parasitization behaviour of *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) in brinjal ecosystem

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ABSTRACT: For the management of the brin-jal shoot and fruit borer augmentatory biocontrol using *Trichogramma chilonis* Ishii egg parasitoid is a viable option. Experiments conducted in the net house (pro tray technique) and field to study the effect of coriander on the parasitization, revealed that under free choice test, maximum numbers of parasitized eggs were observed in brinjal grown with coriander at 1:1 ratio (29.48 eggs/ card). Under no-choice condition the mean number of parasitized eggs recorded was 20.88 eggs per card observed in brinjal. In the field experiment using sentinel egg card technique no statistical difference in parasitoid performance between the sole crop and intercropped plot was observed. In this studies (field experiments) clearly indicate that there is no significant effect of coriander on parasitisation of *T. chilonis*, though the results were promising in the green house. The probable reason for this may be due to influence of climate factors like wind direction, temperature and RH.

KEY WORD: Brinjal, *Leucinodes orbonalis,* coriander, *Trichogramma chilonis*, parasitization behaviour

Brinjal, *Solanum melongena* (L.) is an eco-nomically important vegetable crop cultivated throughout the India and is grown in more than 729 thousand hectares with an annual production of 12.62MT (Anon, 2018). According to Ahmad *et al.* (2009) more than 150 insect pests infest the brinjal crop, among which brin-jal shoot and fruit borer (BFSB), *Leucinodes orbonalis* Guenee (Crambidae: Lepidoptera) is the most serious and destructive. A wide range of insecticides including synthetic pyrethroids are regularly being used for the control of *L. orbonalis* leading to resurgence of sucking pests like whitefly, aphid and mite (Srinivasan, 2009). Because of these reasons biological control using *Trichogramma chilonis* Ishiiis an viable option. Crop diversification viz., mixed cropping and intercropping also provides several significant advantages over monoculture as it improves the microclimate of agroecosystems which may be unfavorable for pests and favourable for natural enemies (Rachappa and Krishna Nalik, 2004). However, information on the performance of *T. chilonis* in coriander intercropped brinjal crop is not available. Keeping the facts, the present

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investigation on effect of coriander *Coriandrum sativum* L. on parasitization behaviour of *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) in brinjal ecosystem was undertaken and results are presented.

Both net house and filed experiments were conducted at Agricultural College and Research Institute, Killikulam, Vallandau In Thoothukudi District during rabi 2017 and summer 2018 to study the performance of *T. chilonis* in coriander intercropped brinjal crop.

Influence of coriander crop on parasitization behaviour of *T. chilonis* under net house condition:

Free choice test: Thirty day old brinjal seedlings (var. KKM1) raised in protray (60 cm × 30 cm) with or without coriander plant was used for comparison. In this experiment, there was four number of portray was used with three replications. In a portray minimum of 25 brinjal seedlings were maintained it may differ in treatments. The portray having brinjal seedling alone (T1), coriander alone in a protray (T2), brinjal seedlings surrounded by one row of coriander along the border in a protray (T3) and coriander seedling in alternate rows in a protray (T4) were compared for their influence of *T. chilonis* parasitization. For the parasitoid performance, the extent of parasitization was assessed by sentinel egg card technique. The unparasitized UV sterilized 0.1 cc *Corcyra* egg cards (5 numbers) were placed in random in an each protray. The protrays having the unparasitized *Corcyra* egg were placed inside a field cage of 120 × 120 cm fixed with nylon net (100 micron)fixed in all sides. The parasitoid *T. chilonis* egg card (0.05 cc) was placed in the middle of the experimental cage and the experimental setup was labeled and kept undisturbed for further three days. After 3 days of release, the *Corcyra* cards from each protray were collect and observed for the number of parasitized egg differentiated by their black color. Subsequently the individual cards were kept in a labeled zip lock cover and kept undisturbed in laboratory for making further observations on parasitoid emergence. Experiment was repeated four times**.**

No-choice test: The influence of coriander on *T. chilonis* parasitization was assessed through no-choice tests also. There were four treatment sets which included brinjal seedling alone in a protray (T1), coriander alone in a protray (T2), brinjal seedlings surrounded by one row of coriander along the border in a protray (T3) and brinjal seedling along with coriander seedling in alternate rows in a protray (T4). In this experiment, there was a four portray with three replications. In a portray minimum of 25 brinjal seedlings were maintained. The protray was placed separately inside the cage (60 cm × 60 cm). The age of the brinjal variety KKM 1 seedling used in the protray experiment was 30 days. The parasitoid performance of *T. chilonis* and the extent of parasitization was studied by sentinel egg card technique. In each protray five unparasitized *Corcyra* egg cards (0.0625 cc approximately) were placed. The quantity of parasitoid release was 0.1 cc per cage. Experiment was repeated four times**.**

Influence of coriander crop on parasitization behaviour of *T. chilonis* under field condition:

The influence of intercrop coriander in brinjal crop on *T. chilonis* egg parasitoid was studied through on-station experiment during *rabi* 2017 and summer 2018. The brinjal crop (var. KKM1) of one acre was divided into four equal quarters in which coriander (var. Co1) was sown as intercrop at 1:1 ratio in diagonal quarters. Remaining two diagonal quarters having brinjal crop alone formed the control plot. The parasitoid *T. chilonis* was released at the rate of 2 cc per acre. A total of 80 tricho cards having approximately 0.025 cc were released. In a 0.25 acre sub plot, the required 0.5 cc trichocard was cut in 20 pieces and were distributed at a uniform distance across the entire plot. The interaction effect of intercrop on *T. chilonis* was assessed by bait card technique (sentinel egg card technique) using laboratory host *Corcyra* egg (Niranjana, 2015; Dominic *et al.,* 2018). In this study, field experiments were conducted in three different field trials. The parasitoids were released at 30 days interval and the observation was taken from third day of parasitoid release to 8th day of parasitoid release.

The experimental data were analysed by using CRD (Completely Randomized Design) with DMRT (Duncan Multiple Range Test).

Influence of coriander crop on parasitization behaviour of *T. chilonis* under net house condition:

Free choice test: Based on overall mean data, the maximum level of parasitization (29.48 eggs/ card) was observed in brinjal and coriander raised in alternate row at 1:1 ratio followed by brinjal raised with coriander at 4:1 ratio (19.48 eggs/ card), brinjal (14.56 eggs/card) and coriander (14.14 eggs/ card) (Table 1).

No-choice test: In overall mean basis, the maximum level of parasitization observed in brinjal + coriander raised at 1:1ratio (31.08 eggs/ card) was found more favourable for *T. chilonis* parasitizationfollowed by brinjal + coriander raised in 4:1 ratio (22.99 eggs/ card), brinjal (20.88 eggs/ card) and coriander (20.16 eggs/ card) (Table 2).

Influence of coriander crop on parasitization behaviour of *T. chilonis* under field condition:

In terms of overall mean data from the field trials, a maximum level of parasitization were recorded in brinjal sole crop plot (15.38 eggs/ card) and a minimum level of parasitization was observed in

Table 1. Influence of coriander crop on parasitization behaviour of *T. chilonis* in brinjal crop - Free choice test

Treatments	Number of parasitized eggs/ card (Mean of 20 observation)				Mean
	Exp 1	Exp 2	Exp 3	Exp 4	
Brinjal alone	14.70^{bc}(3.86)	11.15^{bc}(3.39)	7.32^{c}(2.79)	25.07^{b}(5.05)	14.56
Coriander alone	6.33^{c}(4.68)	16.25^{b}(4.07)	12.24^{bc}(3.57)	21.73^{bc}(4.63)	14.14
Brinjal + Coriander (4:1)	16.83^{b}(4.15)	10.24^{c}(3.26)	24.26^{b}(4.97)	26.58^{b}(5.19)	19.48
Brinjal+ Coriander (1:1)	21.52^{a}(2.57)	22.62^{a}(4.80)	37.45^{a}(6.13)	34.43^{a}(5.90)	29.01
SEm	0.237	0.221	0.346	0.126	
CD (0.01)	1.961	1.824	2.776	1.041	

Figures in parentheses are square root transformed values, DMRT.

Table 2. Influence of coriander crop on parasitization behaviour of *T. chilonis* on brinjal crop - No-choice test

Treatments	Number of parasitized eggs (Mean of 20 observation)				Mean
	Exp 1	Exp 2	Exp 3	Exp 4	
Brinjal alone	24.66^{b}(5.01)	18.62^{bc}(4.35)	14.70^{bc}(3.87)	25.54^{b}(5.02)	20.88
Coriander alone	21.31^{b}(4.57)	15.04^{bc}(3.92)	23.21^{b}(4.87)	21.08^{b}(4.65)	20.16
Brinjal + Coriander (4:1)	24.62^{b}(4.99)	28.57^{b}(5.39)	15.70^{bc}(4.01)	23.09^{b}(4.86)	22.99
Brinjal+ Coriander (1:1)	33.26^{a}(5.80)	34.34^{a}(5.89)	32.98^{a}(5.78)	23.76^{a}(4.92)	31.08
SEm	0.278	0.215	0.330	1.090	
CD (0.01)	2.297	1.773	2.725	9.001	

Figures in parentheses are square root transformed values, DMRT.

brinjal intercropped with coriander (11.00 eggs/ card). Statistically the mean data indicated no significant difference among the treatments (Table 3).

In the subsequent experiment (II) conducted during Summer 2018 season, in terms of overall mean data, maximum level of parasitization was noticed in brinjal sole cropped plot (7.9 eggs/ card) and in coriander intercropped plot a minimum level of parasitization was observed (5.9 eggs/ card) and there was no significant difference noticed between the plots (Table 4).

In the another experiment (III) validating the influence of coriander intercropping, indicated a higher level of parasitization in brinjal sole cropped plot (9.2 eggs/ card) and was 7.1 eggs in coriander intercropped plot. However both the treatments were found statistically on par (Table 5).

The egg parasitoid *T. chilonis* is a widely used in augmentatory biological control for *L. orbonalis* management in brinjal (Niranjana, 2015). According to Krishnamoorthy (2012), crop plants which have high palatability or ovipositional suitability have positive influence on *Trichogramma* and is important aspect in biological control. In the attempt made in the present study to understand host acceptance behaviour of *T. chilonis* under coriander intercropping system, it is observed that the *T. chilonis* egg parasitoid was compatible with the intercrop coriander. The study conducted at net house condition revealed a synergy in *T. chilonis* performance due to the intercrop coriander but in the field condition this synergy was not seen because of climate conditions like wind direction, wind speed, temperature and others. The present findings are accordance with Rachappa and Nalik, (2004) who reported, the presence of coriander

Table 3. Influence of coriander crop on parasitization behaviour of *T. chilonis* under field condition - Experiment I

Experiment	Number of parasitized eggs/ card (Mean of four replication)		
	Brinjal sole crop plot	Brinjal + Coriander plot	t (P < 0.05)
1	13 ± 2.5 (3.7)	9.7 ± 2.9 (3.2)	NS
2	15.5 ± 4.9 (4.0)	10.4 ± 3.2 (3.3)	NS
3	16.3 ± 5.0 (4.1)	11.9 ± 3.5 (3.5)	NS
4	16.7 ± 4.3 (4.1)	12.1 ± 3.6 (3.6)	NS
Mean	15.38 ± 4.2 (4.0)	11.0 ± 3.3 (3.4)	

Mean ± SE, NS = Non-significant, figures in parentheses are square root transformed values

Table 4. Influence of coriander crop on parasitization behaviour of *T. chilonis* under field condition - Experiment II

Experiment	Number of parasitized eggs/ card (Mean of four replication)		
	Brinjal sole crop plot	Brinjal + Coriander plot	t (P < 0.05)
1	9.5 ± 2.7 (3.2)	6.8 ± 2.6 (2.7)	NS
2	3.9 ± 1.9 (2.1)	2.5 ± 1.2 (1.8)	NS
3	8.3 ± 2.8 (3.0)	6.6 ± 3.6 (2.7)	NS
4	9.7 ± 3.3 (3.2)	7.8 ± 3.0 (2.9)	NS
Mean	7.9 ± 2.7 (2.9)	5.9 ± 2.6 (2.5)	

Mean ± SE, NS = Non-significant, figures in parentheses are square root transformed values

Table 5. Influence of coriander crop on parasitization behaviour of *T. chilonis* under field condition - Experiment III

Experiment	Mean Number of parasitized eggs/ card (Mean of four replication)		
	Brinjal sole crop plot	Brinjal + Coriander plot	t (P < 0.05)
1	11.1 ± 2.5 (3.4)	9.2 ± 2.1 (3.1)	NS
2	4.7 ± 1.7 (2.3)	3.1 ± 1.1 (2.0)	NS
3	9.5 ± 2.2 (3.2)	7.1 ± 2.2 (2.8)	NS
4	11.4 ± 3.9 (3.5)	8.9 ± 2.6 (3.1)	NS
Mean	9.2 ± 2.6 (3.1)	7.1 ± 2.0 (2.8)	

Mean ± SE, NS = Non-significant, figures in parentheses are square root transformed values

intercrop registered maximum level of parasitization in sugarcane ecosystem. According to Kemp and Simmons (1978) inmixed cropping systems more alternative host eggs may be available, so *Trichogramma* populations can be maintained at higher densities. The chemical components present in the companion crop may also attract or stimulate the female wasps leading to higher parasitism rates (Altieri *et al.,* 1981; Nordlund *et al.,* 1984). The intercrop may also act as an alternative food sources, e.g. honeydew, pollen, or nectar to *Trichogramma* (Andow and Risch, 1987).

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Invasion and establishment of Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae) in Indian mainland and Andaman and Nicobar Islands

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ABSTRACT: Invasion and establishment of Bondar's nesting whitefly, *Paraleyrodes bondari* Peracchi in Indian mainland and Andaman & Nicobar Islands is reported. This forms the first report of *P. bondari* from Andaman & Nicobar Islands. Host range of Bondar's nesting whitefly in India is discussed. Partial sequence of cytochrome *c* oxidase I gene for *P. bondari* is submitted to GenBank (MK333262). © 2019 Association for Advancement of Entomology

KEYWORDS: Bondar's nesting whitefly, *Paraleyrodes bondari, India, invasive species*

Plants, animals, and microbes are introduced more frequently into regions that had never hosted them in an increasingly globalized world (Venkataraman *et al*., 2016). The agricultural economy in India is vulnerable to threat from exotic pests and there are 116 alien insect species (Mandal, 2011). Among the insect pests, globally over the past 25 years, exotic whiteflies invaded several countries causing direct losses in agriculture, horticulture and forestry. Such reported invasive whiteflies in India are the spiraling whitefly, *Aleurodicus dispersus* Russell, which is known to breed on 320 host plants belonging to 225 genera and 73 families (Sundararaj and Pushpa, 2012); solanum whitefly, *Aleurothrixus trachoides* (Back) reported to breed on 24 plant species including medicinal plants (Sundararaj *et al*., 2018) and rugose spiraling whitefly (RSW), *Aleurodicus rugioperculatus* Martin that invaded in 2016 (Shanas *et al*., 2016; Sundararaj and Selvaraj, 2017) and found breeding on more than 20 host plants including coconut. In December 2018, Central Plantation Crops Research Institute (CPCRI) recorded two exotic whitefly species, *Paraleyrodes bondari* Peracchi and *P. minei* Iaccarino on coconut palms of Kerala and issued a pest alert (CPCRI, 2019).

The present study is based on the whiteflies collected from Karnataka during April 2018, Kerala during October-November 2018 and Andaman and Nicobar Islands during May 2017 and November 2018 on different host plants (Table 1). Both

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conventional taxonomy as well as cytochrome *c* oxidase I (COI) based molecular method were followed for species identification. Mounted puparia and male adults were studied for morphological identification. Observations, micro-measurements and photomicrographs were made by using Nikon Optiphot T-2 EFD (Japan) and Labomed LX400 microscope at Andaman and Nicobar Regional Centre, Zoological Survey of India, Port Blair and the identity of the whitefly was confirmed. Molecular identification was done after the extraction of DNA from adult insect using Qiagen DNeasy® Kit, following the manufacturer's protocols. PCR amplification of 5' end of the COI gene was carried out following standard protocols. The amplified product was sequenced by Sanger sequencing method at AgriGenome Labs. Pvt. Ltd., Kochi, Kerala. Based on the morphological and molecular methods, the species was identified as *P. bondari*.

Diagnosis: Genus *Paraleyrodes* is physically much smaller than most other aleurodicines; puparium with 5 or 6 compound pores in which the anterior 1 or 2 pairs much smaller than the remaining 4 abdominal pairs and the cephalic pair; thorax with two pairs of cicatrices and a pair of submedian setae; outer submargin with a row of 14 pairs of hair-like setae. Adults with all wing veins unbranched; females have 4 articulated antennal segments; males have only 3 articulated antennal segments and complex aedeagal apices. The larvae and puparia secrete long waxy filaments that often form an annulus surrounding the feeding insects. Adults remain inside a nest like mealy wax and females usually secrete so much wax around them while ovipositing and hence the members of this genus are appropriately known as "nesting whiteflies" (Martin, 2004).

The puparia of *P. bondari* are characterized by the presence of 14 pairs of submarginal setae and

Table 1. Host plants of *Paraleyrodes bondari* in India

Plant species	Host family	Economic importance	Distribution	Whiteflies intermingled on infested leaf
Annona sp.	Annonaceae	Fruit crop	Kerala	*Pealius nagerkoilensis* Jesudasan & David
Artocarpus heterophyllus	Moraceae	Fruit crop	Kerala	*Dialeuropora decempuncta* Quaintance & Baker
Bridelia retusa	Euphorbiaceae	Medicinal and Timber	Kerala	-
Capsicum annum	Solanaceae	Spice crop	Kerala	*Aleurodicus dispersus* Russell
Cinnamomum verum	Lauraceae	Spice crop	Andaman & Nicobar Islands	-
Cocos nucifera	Arecaceae	Plantation crop	Kerala	*Aleurodicus rugioperculatus* Martin
Leucaena leucocephala	Fabaceae	Fodder and Paper pulp	Karnataka	*Tetraleurodes acaciae* (Quaintance)
Macaranga peltata	Euphorbiaceae	Timber	Kerala	*Dialeuropora decempuncta* Quaintance & Baker *Martiniella fletcheri* Sundararaj & David
Mangifera indica	Anacardiaceae	Fruit crop	Kerala	-
Morinda citrifolia	Rubiaceae	Medicinal plant	Karnataka	*Dialeurodes kirkaldyi* (Kotinsky)
Musa sp.	Musaceae	Fruit crop	Kerala	*Aleurodicus dispersus* Russell
Psidium guajava	Myrtaceae	Fruit crop	Kerala	*Aleurodicus dispersus* Russell
Tectona grandis	Verbenaceae	Timber	Kerala	*Martiniella fletcheri* Sundararaj & David

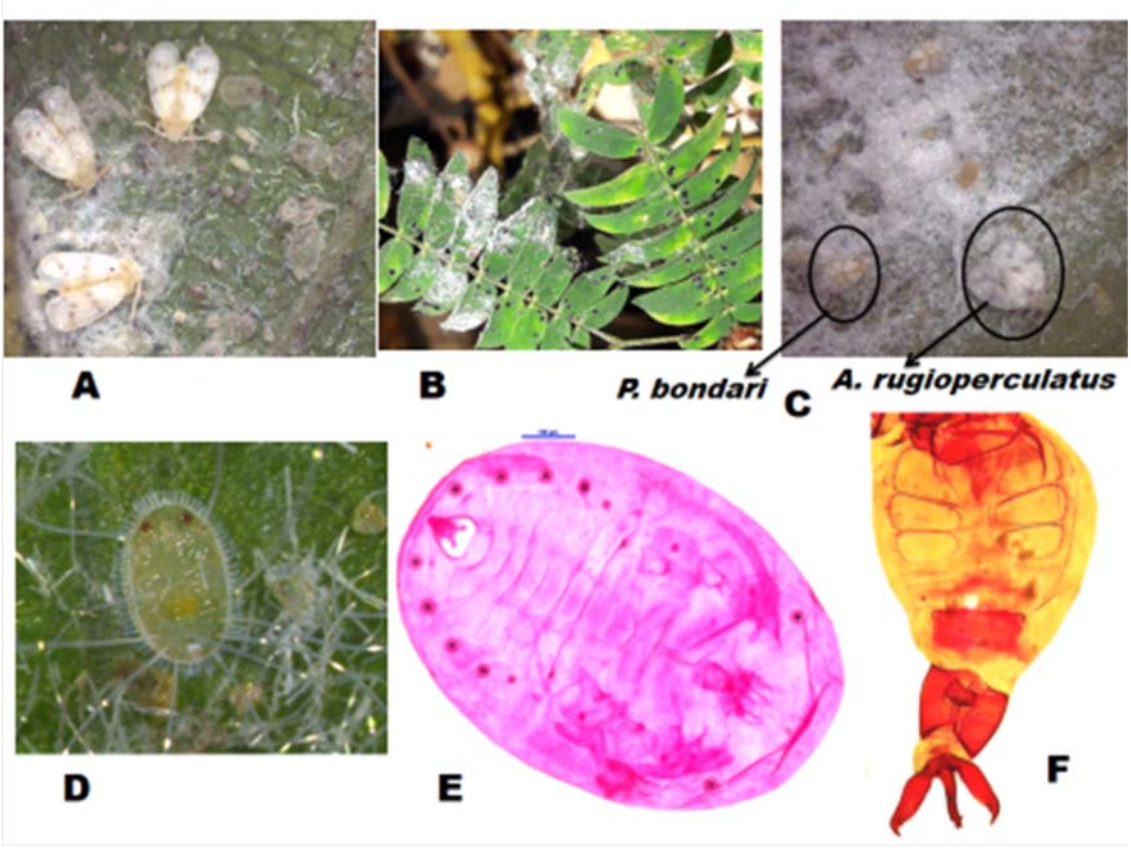

Fig. 1. A-D. Infestation of *P. bondari* on different host plants, A. on *Annona* sp.; B. on *Leucaena leucocephala along with Tetraleurodes acaciae;* C. on *C. nucifera* along with *A. rugioperculatus,* D. III instar nymph on *M. citrifolia,* E. Mounted puparium, F. Abdomen with claspers and aedeagus

anterior two of six abdominal compound pores reduced in size and with a rim of chitinous splines (Fig. 1E). In life, the adult fore-wings are with two rows of mottelings. The aedeagus of *P. bondari* is unique and easily distinguishable from other *Paraleyrodes* species in having three subapical aedeagal process of which two are in one direction and one opposite to them, and one process near mid-length of it (Fig. 1F).

The partial COI sequence of *P. bondari* generated was submitted to GenBank and received the accession number (MK333262). Homology search of sequence generated with NCBI database showed 100 per cent similarity with *P. bondari* accessions from Uganda (MH178372) and Florida (KP032215), which confirmed the identity of the species.

Paraleyrodes bondari was found to infest both monocot and dicot plants and host range from India include 13 plants (Fig.1A-D). The whitefly usually infests the lower surface of leaves, but occasionally occurs on upper surfaces. Sooty mould was found in association with the infested plant leaves. In Kerala, *P. bondari* was found infesting economically important plants like coconut, banana, guava, teak etc. On guava and banana, it was found infesting along with another invasive spiraling whitefly, *A. dispersus* whereas on coconut, it was found with the invasive whitefly, *A. rugioperculatus*. In many instances, population of *P. bondari* was more (8.04 nymphs per 30 cm leaflet) than that of *A. rugioperculatus* (4.4 nymphs per 30 cm leaflet) on coconut. In Karnataka, it was found infesting subabul (*Leucaena leucocephala*) along with *Tetraleurodes acacia* (Quaintance), and *Morinda citrifolia* along with *Dialeurodes kirkaldyi* (Kotinsky). However, in Nicobar Islands *P. bondari* was found to infest alone on *Cinnamomum verum*.

The whitefly fauna of India comprises 455 species under 66 genera in which the subfamily Aleyrodinae Westwood is represented by 64 genera and the subfamily Aleurodicinae Quaintance & Baker by 2 genera *viz*., *Aleurodicus* Douglas and *Palaealeurodicus* Martin. The genus *Aleurodicus* is represented by *A. dispersus* and *A. rugioperculatus*, both invasive to India. The other genus *Palaealeurodicus* is represented by three species. David and Dubey (2006) reported 20 species of whiteflies from Andaman Islands, but no whitefly records from the Nicobar Islands. Thus, it is the first report of a whitefly from the Nicobar Island, the Great Nicobar Biosphere Reserve. The occurrence of nesting whitefly was noticed in Bhati Basti, Port Blair, Andaman during May 2017. This indicates possible route of invasion of *P. bondari* to India. Geographically, the Andaman & Nicobar Islands are placed close to Indonesia than mainland India. Occurrence of *P. bondari* in these islands indicates possible availability of this species in neighboring countries. The invasion and establishment of *P. bondari* in India brings the total number of genus under the subfamily Aleurodicinae known from India to three and the number of species to six.

Paraleyrodes Quaintance is a Neotropical genus which comprises 17 species in the world (Martin and Mound, 2007). *Paraleyrodes bondari* was first described in 1971 on *Citrus* species from Brazil (Peracchi, 1971). It may be assumed that this species occurred in the Neotropical region only till 1990s. Later, it was recorded from Madeira during 1995 (Martin, 1996), then spread to Oriental region, and in 1998 reported as a pest of fruit trees from Taiwan on host trees of 17 families (Wen and Chen, 2001). This species had spread to Hawaii by around 2003 (Stocks, 2012). Florida Department of Agriculture and Consumer Science gave a 'pest alert' for *P. bondari* as a new pest of *Ficus* species in 2011 (Stocks, 2012), though it was known earlier from Florida and California (Martin, 2004). Omango *et al*. (2018) reported that *P. bondari* was present in Uganda on cassava from 2006. *Paraleyrodes* present in the Old World may be introductions of New World species into this region (Evans, 2007).

Paraleyrodes bondari is establishing invasive pest status in India as it infests on economically important fruit/ medicinal crops and timber yielding trees and also on many alternative hosts. Unlike *A. rugioperculatus*, where 50 to 60 per cent natural parasitisation by *Encarsia guadeloupae* Viggiani (Hymenoptera: Aphelinidae) was reported (Shanas *et al*., 2016), no parasitisation was noticed on *P. bondari*. The invasion of this species to India corroborate the comment of Martin (2004) that within the genus *Paraleyrodes*, *P. minei* and *P. bondari* are most geographically mobile species. Its breeding in association with other whiteflies on many hosts confirms the fact that members of this genus have a marked prospensity for ovipositing amongst the puparia or colony remains of other whitefly species (Martin, 2004). *Paraleyrodes bondari* may cause economic loss for horticulture farmers and tree growers due to its polyphagous nature coupled with unexplored native parasitoids. Further, there is a spurt in the invasion of exotic whiteflies to India. Hence, efforts must be undertaken for the prevention /management of alien whitefly species on a long-term basis and strengthening of the plant quarantine measures.

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First record of body colour polymorphism in giant African snail *Achatina fulica* (Bowdich, 1822) - a comparative study using mitochondrial cytochrome oxidase subunit I (COI) gene

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ABSTRACT: The presence of the body colour polymorphism in the tropical invasive pest giant African snail is reported for the first time from South India. Three different body colour polymorphs were recognised *viz*. grey, black and white. The grey body colour is the most common polymorph. The black and white colour polymorphs are found to be in almost equal proportions in the reported localities with the grey counterparts. The cytochrome oxidase subunit I (COI) sequences of the three colour polymorphs are found to be identical. The presence of the body colour polymorphism in south India may be attributed to the avian predation and other selection pressures.

KEYWORDS: Polymorphism, giant African snail, COI gene

Colour polymorphism is the co-occurrence of two or more different colour patterns within a population. The physical structures which reflects light or the pigments which are present in the body of an organism is the major cause of different colours in nature. Both these are extremely sensitive to genetic variation (White and Kemp, 2016). Generally, colour polymorphism patterns in animal groups influence the predator-prey relationships. That is while considering the predators which consumes polymorphic preys, suffers from a lesser performance as compared with the others who consumes only monomorphic preys (Karpestam *et al*., 2016). Colour polymorphism also hinders the competence or efficiency of the predators which focuses on specific coloured preys and this increases the survival of the prey species (Karpestam *et al*., 2014). This is demonstrated by apostatic selection in which the common colour polymorph of a species is memorised by the predator and the rare colour polymorph is maintained in the population. The typical case of this is the polymorphic grove snail *Cepaea nemoralis*. Here the rare colour morph is rarely recognised by the avian predators and they become a common variety. Functionally, body colour polymorphism is used for phenomena such as sexual signalling, crypsis, thermoregulation, mutualism, aposematism and sometimes in Batesian and Mullerian mimicry (White and Kemp, 2016). Another aspect of colour polymorphism is the melanin induced body colour change due to climate change in which pale body coloured organisms were

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present in those areas where more desertification occurred and black body coloured ones in those areas with high humidity (Roulin, 2014).

Shell colour polymorphism has been studied intensely in many species of land snails like *Thais* (or *Nucella*) *emarginata* of North America (Palmer, 1985). In *Cepaea nemoralis* snails, those snails living in shaded habitats are having dark coloured shells and those living in open habitats are having light coloured shells (Schilthuizen, 2013). Shell colour is also associated with the substrate colour in the species *Littorina saxatilis* which varies from grey to brown. The grey coloured shells were found on the grey coloured rocks and brown coloured shells on red coloured rocks (Byers, 1990). Dark to light shell colours were exhibited in the snail *Bradybaena similaris* along with presence and absence of bands. The colours of the shell and banding pattern was controlled by two different alleles B and C which are linked (Asami and Asami, 2008). Homozygous recessive alleles make the shells of the African Achatinidae un-streaked and the dominant alleles provide streaked shells (Allen, 1985). Shell banding pattern and chirality of the shells of the Giant African snails revealed dextral and considerable variation in the banding pattern in the Bengaluru region of India (Jayasankar *et al*., 2014). Very few studies have described body colour polymorphism in land snails. The body colour polymorphism in the snail *Pomaceae canaliculata* exhibited two different body colours- yellow and brown- and their inheritance followed simple Mendelian inheritance pattern with yellow recessive to Brown (Yusa, 2004). The black and white skinned snails of *Archachatina marginata* has shown significant difference in the genetic level when examined using RAPD markers (Etukudo *et al*., 2016). The Giant African land snails *Achatina achatina* and *Archachatina marginata,* showed marked body colour polymorphism with black and white snails, while white in urban and black in the forest zones (Owen and Reid, 1986). Fresh water snails also exhibit body colour polymorphism (Tiecher *et al*., 2017). No reports are available regarding the body colour polymorphism in the Giant African Snail *Achatina fulica* (Bowdich, 1822), an invasive alien pest in the tropical region.

The Giant African Snail *Achatina fulica* (Bowdich, 1822) native of East Africa is one of the worst of 100 invasive species in the world. The snail was introduced to India from Mauritius in 1847 and spread to many parts. In South India, the snails were first reported around 1900s (Mead, 1961). In this study we present three different body colour polymorphs of the Giant African Snail from south India and have used a partial sequence of cytochrome oxidase subunit I (COI) gene to identify the extent of genetic variation in the colour polymorphic snails.

Field surveys were conducted in the states of Kerala and Tamil Nadu during the years 2016 to 2018 and collected samples of giant African snails from the infested areas. The geo-coordinates of all the collected localities were recorded in a hand-held Global Positioning System (GPS). We also recorded the body colour in the field and the collected snails were reared in a glass house. The dead snails were preserved in 70% ethanol for molecular analysis.

DNA isolation

DNA isolation was achieved from the foot muscle tissue of ethanol preserved snail samples. The tissue parts were soaked in sterile water for an hour or two to remove ethanol. The general Cetyl Trimethyl Ammonium Bromide CTAB method modified from Winnepenninckx *et al*., (1993) was used. The washing step using Phenol: Chloroform: Isoamyl alcohol (25:24:1) solution was repeated several times to remove the mucopolysaccharides present in the snail tissues. The DNA was precipitated by adding iso-propyl alcohol and overnight incubation. The isolated DNA was stored at -20°C for further use.

PCR Amplification and Sequencing

The isolated DNA was subjected to PCR amplification using the Cytochrome Oxidase Subunit I (COI) gene. The universal COI primers developed by Folmer *et al*., (1994) LCO-1490: 5' GGTCAACAAATCATAAAGATATTGG 3' HCO-2198: 5' AAACTTCAGGGTGACCAAAAAA TCA 3' was used for gene amplification. PCR amplification was standardised with the following

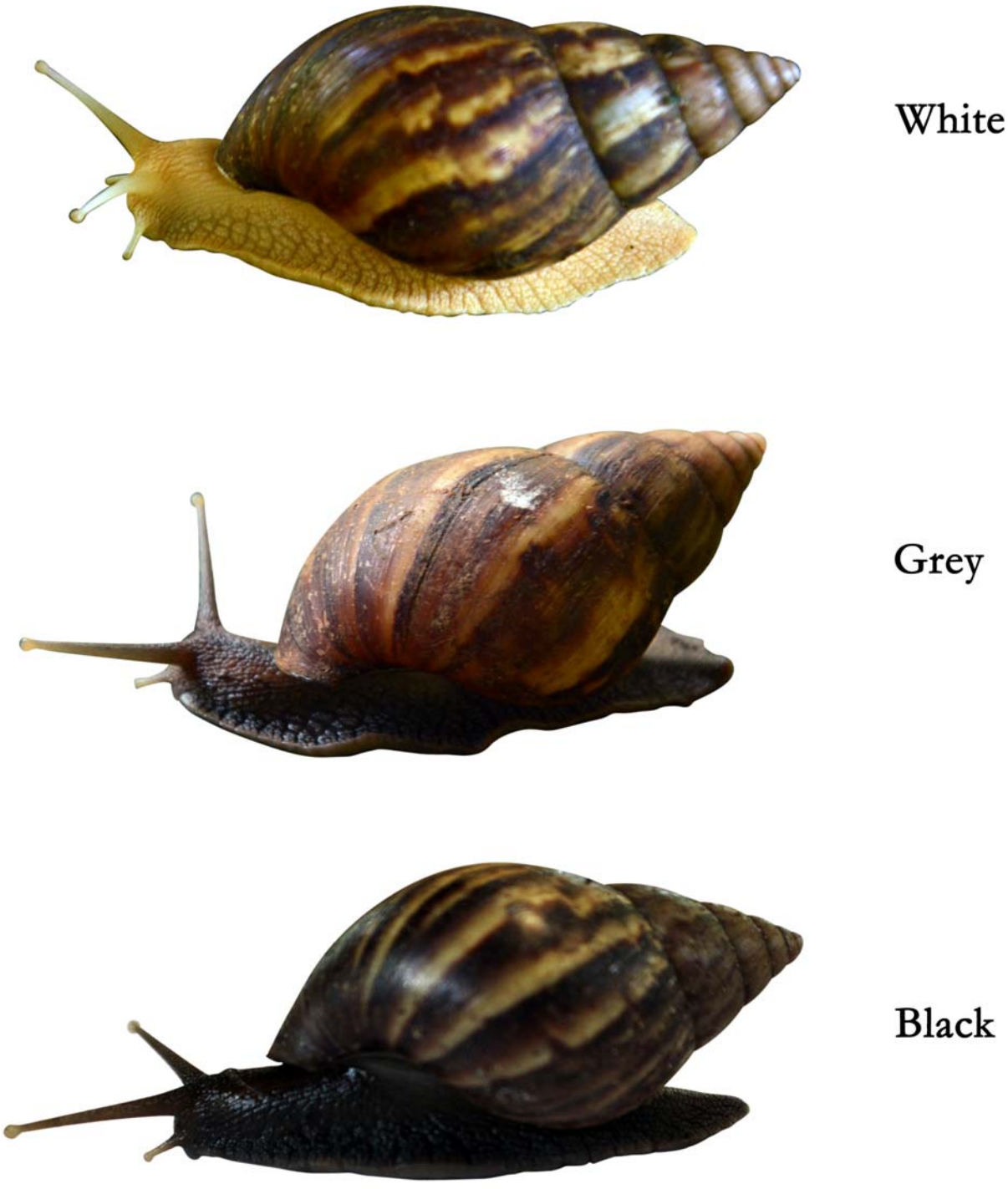

Fig 1. Body colour polymorphism in Giant African Snail *Achatina fulica*

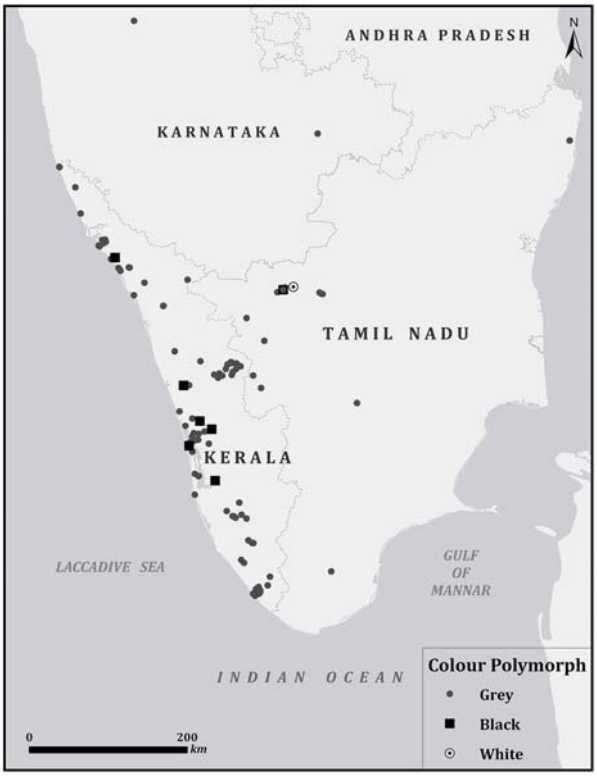

Fig 2. Localities of the three different colour polymorphs of *Achatina fulica*

conditions: initial denaturation at 94°C for 2'; 38 cycles of 94°C for 30", 48°C for 30" and 65°C for 1'; followed by final elongation of 65°C for 10'. The PCR reaction yielded 406 base pair sequence of the COI gene of the snails. The PCR products were visualised on a 1.2% agarose gel and the sequencing of the PCR products was done through service providers.

Matching of the sequences was done using NCBI BLAST search tool. The sequences obtained from the study have shown similarity with the complete mitochondrial sequence and the Cytochrome Oxidase Subunit I gene sequences of *Achatina fulica* available in the NCBI. The raw sequences were trimmed using Bioedit and aligned using ClustalW and assembled manually in Molecular Evolutionary Genetic Analysis (MEGA) version 7.0.26 (Kumar *et al*., 2016).

A total of 263 Giant African Snail infested localities were surveyed in south India of which 246 were from Kerala and 17 were from Tamil Nadu. Three different colour polymorphs, grey, black and white were distinguished from the surveys and the samples collected (Figure 1). Grey is the common body colour and are thoroughly distributed all along the snail distribution localities. The black body colour polymorph was reported from seven localities in Kerala and Tamil Nadu. They were from Willington Island, Angamaly, Kottayam, Perumbavoor, Poonkunnam and Pinarayi in Kerala, and in Sathyamangalam from Tamil Nadu. The white bodied snails were seen in two localities *viz*., Konamoolai and Kenjanur near Sathyamangalam in the Erode district of Tamil Nadu. The three body colour polymorphs were seen together in a single locality from Kenjanur near Sathyamangalam in Tamil Nadu. In all other places either grey and black or grey and white (from one locality Konamoolai in Tamil Nadu) were seen together. In the populations from two localities in Tamil Nadu, the white and black polymorphs were seen almost in equal proportion with the grey polymorphs. The black snails in Kerala where it was reported from six localities, they are found to be in almost equal numbers with that of the grey polymorphs (Figure 2). The young ones of the white bodied snails and black bodied snails were checked to know whether the snails are leached out due to the manure applied in the fields or due to some other natural phenomenon. But the young ones of these snails which were collected from the field are also having the same body colour like their adults. But the eggs laid by the white bodied snails and the black bodied snails failed to hatch in the laboratory conditions. The grey, black and white polymorphs of three snails from Kenjanur near Sathyamangalam in Tamil Nadu, where the three exists together were subjected to molecular analysis using the partial sequence of the 406 base pair long COI. The sequence was deposited in the National Centre for Biotechnology Information (NCBI) GenBank database and the accession number is LC440023.1.

The alignment and assembly of the sequences obtained from the colour polymorphic snails showed no genetic variation in their sequences. The sequences were found to be completely similar. This shows that in the Giant African snails are exhibiting colour polymorphism. All the colour polymorphic

snails are of the same species and not even a different variety. The complete similarity in the genetic makeup of the organism confirms this. Also, the presence of the colour polymorphism in the snail is not due to any external factors like application of manure, extremely harsh climates or some other environmental factors. Because, the colour polymorphic snails were found in equal proportion with that of its grey counter parts.

Along with high reproductive potential, ability to withstand harsh climatic conditions and adaptability in many environments of the snail in South India and the presence of colour polymorphism makes *Achatina fulica* an excellent invasive species. The black snails of Kerala are very useful ecotype to study the movement of snails within the state. Generally the snails which were captive bred are found to show a phenomenon of white body colour and this occurs due to continuous inbreeding. The white polymorph is available from only one report outside India from a single source in Venezuela (http://caracol.cbm.usb.ve/). The Giant African snails *Archachatina marginata* and *Achatina achatina* are found to be showing remarkable body colour polymorphism from black to white in Africa. The local people are consuming the snails in that region but due to cultural and medicinal beliefs they avoid collection of the white bodied snails. This helps in maintaining the polymorphism and species (Owen and Reid, 1986). Avian predators also play very important in maintaining the colour polymorphism in snails. The cryptic behaviour of the snails based on the background is helpful for the snails to maintain its population (Surmacki *et al*., 2013). The black and the white skinned snails are showing genetic differences in Africa also. This proves high heterogeneity in the Giant African land snails in Africa (Etukudo *et al*., 2016). In one sampled locality in Tamil Nadu, the snails are seen to be predated in large numbers by the open billed stork *Anastomus oscitans*, which could be a reason for the presence of white polymorphs in an equal proportion. Similarly, in Kerala the black polymorphs are seen in equal proportions which might supports the phenomenon of dark colour which is adapted to live in the high rainfall regions. This is the first report of the body colour polymorphism in giant African snails. The presence of body colour polymorphism in *A. fulica* and its huge adaptability to different environments makes the species a very successful invasive species. This adaptation makes the snail to escape from different avian and other predators. More molecular studies have to be attempted in this regard using suitable nuclear markers to understand the hereditary mechanism behind the adaptation and the movement of the body colour polymorphism in the species.

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