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Stinging apparatus of apoid wasps and bees as never seen before

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ABSTRACT: The stinging apparatus is expected to vary depending on the type of prey taken and the way it is carried in apoid wasps and the purpose of defense it serves in bees. To understand the differences in sting morphology, members of two apoid wasp families (Ampulicidae and Crabronidae) and a bee family (Halictidae) were studied. Scanning Electron Microscope images of lancets revealed tooth like projections on dorso-lateral aspect in *Ampulex compressa* (Fabricius, 1781) and blunt barbs on the lancets of *Liris aurulentus* (Fabricius, 1787) and *Tachysphex bengalensis* Cameron, 1889 whereas, in *Halictus fimbriatellus* Vachal, 1894 barbs are arranged in two rows on lancet, which includes four barbs on one side and three barbs on the other side of lancet which are not acutely pointed. The SEM images also indicated the presence of campaniform sensilla on the lancets of *A. compressa*. These findings help us to know the possible relationships of hunting behavior and modification of the sting in accordance.

KEYWORDS: Barbs, Hymenoptera, lancet, prey, sting.

INTRODUCTION

The order Hymenoptera is the third largest order of insects in the world, next to Coleoptera and Lepidoptera (Gaston, 1993; Sharkey, 2007). Hymenoptera is the only endopterygotan order with well-developed ovipositor which is plesiomorphic retention, and is considered as one of the key factors in their diversification (Gauld and Bolton, 1988). Stinging apparatus of Aculeate Hymenoptera evolved under selection in relation with hunting behavior and modification of the sting is expected to vary depending on the prey carrying type (Steiner, 1981).

The Scanning Electron Microscope (SEM) study of certain sclerites, particularly the gonostyli and the lancets shafts has revealed the presence of sensory structures in the former and of different barbs shapes in the latter, which are of considerable interest. The sensory structures vary in shape, density and distribution among the species studied (Gadallah and Assery, 2004). The cast specific ultrastructural specialization of the sting of the worker and queen of *Apis dorsata* was explored by Paliwal and Tembhare (1998) and as the external fine structure of sting apparatus of worker and queen honeybee was illustrated.

In the present study we have made an effort to take a closer look of lancets of *Ampulex compressa, Tachysphex bengalensis*, *Liris aurulentus* and *Halictus fimbriatellus*. All the four studied species nested in the ground. The prey of *A. compressa*

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was cockroaches, while *Tachysphex bengalensis* hunted for grasshoppers and *Liris aurulentus* took crickets. *Halictus fimbriatellus* collected pollen. Thus, there were differences between the species in their behaviour with references to the food stored in the nest cells for their offspring.

MATERIALS AND METHODS

Specimens of apoid wasps and bees were collected from nesting sites in different ecosystems in and around Bangalore and in Gandhi Krishi Vignan Kendra (GKVK) Campus, and representative specimens were preserved in 70% alcohol. In the laboratory, the specimens were relaxed and the sting apparatus was separated using a fine forceps and a pair of hooked minuten pins mounted on a holder, and was transferred to 10% KOH and left for 12-24 hours based on the sclerotization for clearing of soft tissues. After clearing, the sting apparatus and the lancets were separated under a stereo-binocular microscope and washed with distilled water for 5–10 minutes. This was followed by dehydration through a graded series of ethanol (20 min each in 70, 80, 90, and 100%) and then the lancets were dried in a critical point drying apparatus mounted on aluminum stubs.

SEM studies were conducted at Insect Systematic Laboratory, Department of Entomology, Indian Agricultural Research Institute, New Delhi with Zeiss EVOMA 10 Scanning Electron Microscope at 20 KV/EHT and 10 pa at different magnification, after 24 nm palladium gold coating.

A brief outline of the terminologies used for the major components of the sting apparatus is as follows. Each half of the divided terga of the 7th and 8th gastral segment is referred to as the 7th and 8th hemitergite, these have often been termed as spiracular and quadrate plates, respectively (Sollman, 1863; Beyer, 1891; Snodgrass, 1956). General description of ovipositor of Hymenoptera is given in Fig. 1. The first valvifer, which originates from the appendage of the 7th gastral segment, has commonly been referred to as the triangular plate (Cameron, 1882; Snodgrass, 1956) or gonangulum (Scudder, 1961; Kristensen, 1991). Basally gives rise to a long thin process called first valvula. The basal part of the first vavula is the first ramus and the more apical part of the lancet which itself gives rise to the valvilli (lancet). The appendage derived structures if the eight gastral segment called second valvifers. These are termed as oblong plates by same authors (Sollman, 1863; Snodgrass, 1956). Basally the second valvifers give rise to second valvulae. Initially these are narrow, separated and form second rami, but apically they are fused to form the sting shaft. The upper valve (sting shaft–fused second valvulae) is interlocked with each lower valve (lancets or first valvula) by a longitudinal tongue and groove joint referred to as the olistheter. The tongue of rhachis situated ventro-laterally on each side of the upper valve is 'T' shaped in transverse section and these rhachis runs within the 'T' shaped groove or aulax (Fig. 2), which is on the dorso-lateral face of lower valve (Quicke *et al*., 1995).

RESULTS AND DISCUSSION

The stinging apparatus includes sclerites like 7th and 8th hemitergite (Spiracular plate and quadrate plate), oblong plate, Triangular plate, and gonostylus, sting shaft lancets all these sclerites are linked with each other and operates effectively when needed. There is direct heavy muscles attachment to quadrate plate and oblong plate. These sclerites further gives rise to ramus which are extended and modified into sting shaft and lancets. Hence lancets are the one which penetrate first in to the victim's skin. In order to penetrate, the lancets which are placed along the sides of semicircular sting shaft are moved forward in alternate strokes, each sliding on its track against the sting shaft. They are either entirely smooth along their lengths or they may be furnished with barbs of week teeth near their tips. This may be correlated with their function during stinging in relation to the kind of body sclerotization of their prey (Radovic, 1985) and it may also be a useful phylogenetic and taxonomic character (Wahl and Sharkey, 1993; Wahl and Gauld, 1998; Gadallah, 2001).

The lancets of *A. compressa* are very slender and long with minute, blunt barbs, with campaniform sensilla and dorsoapical teeth like structure on the aulax of the ridges. The lancets are also hollow

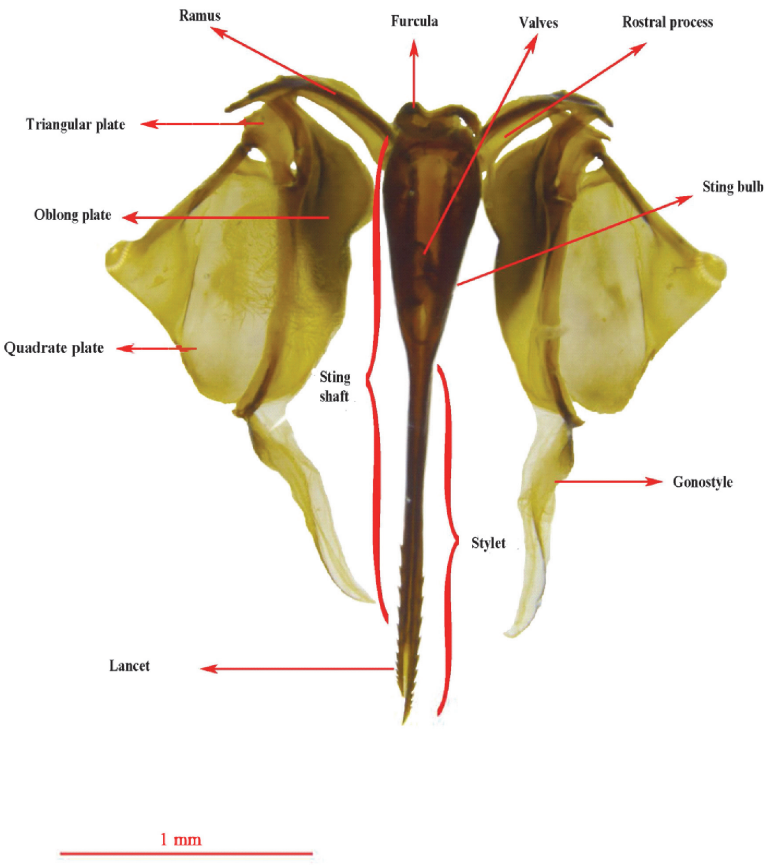

Fig. 1 The detailed description of sting apparatus

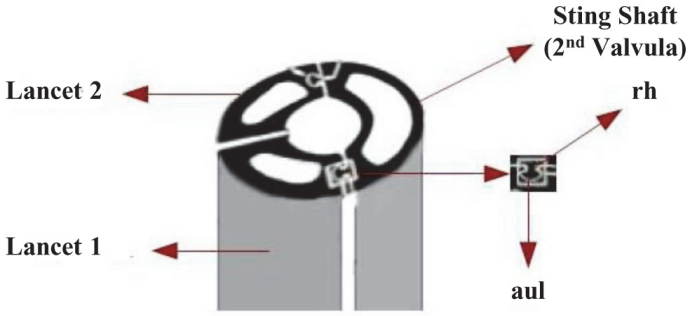

Fig. 2 Diagrammatic representation of transverse section from mid-region of braconid ovipositor (adopted from Quicke et al., 1995) (rh- rhachis, aul-aulax)

(Fig. 3), which probably helps them to be flexible during oviposition. Lancets of *T. bengalensis* and *L. aurulentus* are also slender and thin and equipped with six and five blunt dentate barbs (Figs. 4a, b, c and 5a), respectively. The Barbs are more prominent in *T. bengalensis* (Fig. 4c) compared to *L. aurantalus* (Fig. 5c). Dorso-apical tooth like projections are present (Figs. 4d and 5b) on the ridges of the aulax in both species. Interestingly, punctations on the surface of the lancets are prominent in *L. aurulentus* where the barbs are blunter, while in *T. bengalensis* the punctations are not as prominent but the barbs are more distinctly dentate. This is the first report on the presence of blunt barbs on the lancets of *L. aurulentus* and *T. bengalensis* along with confirmation of teeth like

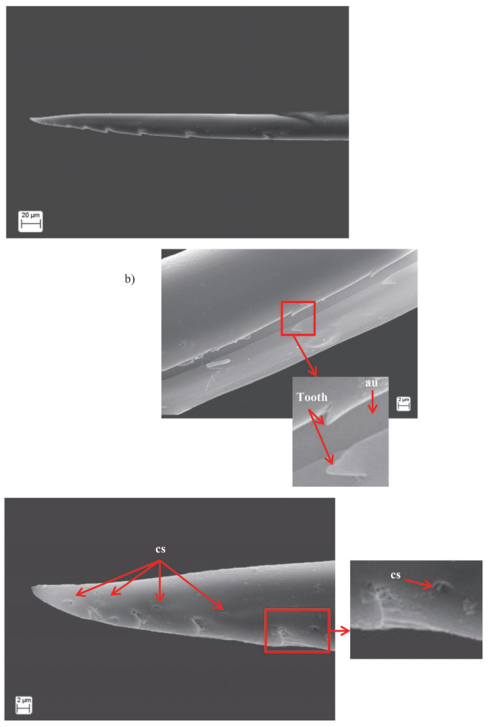

Fig. 3 SEM images of *Ampulex compressa* lancets a) lancet with blunt barbs b) presence of aulax (au) and tooth-like structure dorso-apically c) presence of camponiform sensilla

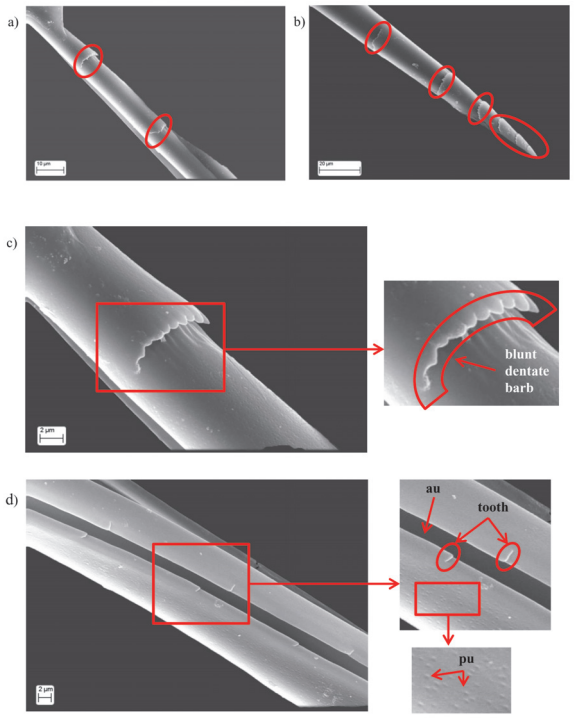

Fig. 4 SEM images of *Tachysphex bengalensis* lancets a) and b) lancet with blunt dentate barbs c) dentate barb d) presence of aulax (au), presence of tooth-like structure and minute punctation (pu).

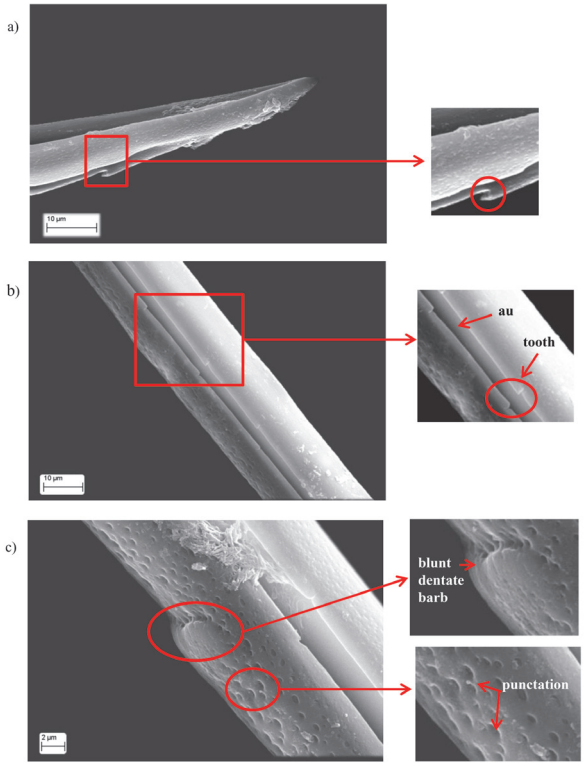

Fig. 5 SEM images of *Liris aurulentus* lancets a) the hooked blunt barb b) presence of aulax (au) and tooth-like structures c) presence of less dentate barb and prominent punctations.

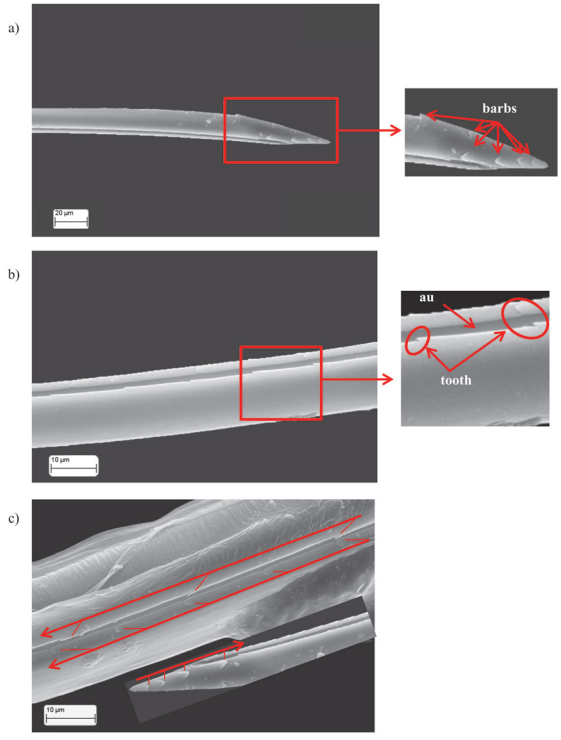

Fig. 6 SEM images of *Halictus fimbriatellus* lancets a) two rows blunt barbs on the lancet b) presence of aulax (au) c) presence of tooth-like projection on dorso-lateral of lancet which are in opposite direction of barbs.

projections on dorso-lateral aspect in *A. compressa* which has significance in understanding the phylogenetic relationships of the group.

The lancets of *H. fimbriatellus* show diverse type of arrangement of barbs. The barbs are arranged in two rows. On a single lancet, four barbs on one side and three barbs on the other side are present and the barbs are blunt (Fig. 6a). The teeth like projections on the ridges of aulax of lancets run in opposite direction of the barbs present on lancets (Figs. 6b and c).

SEM images of *A. compressa*, *L. aurantalus*, *T. bengalensis* and *H. fimbriatellus* indicated the presence of Aulax for the first time, which was previously described in Braconidae (Quicke *et al*., 1995); *Bembix rostrata* (Fabricius) (Matushkina, 2011) and in *Ampulex compressa* (Gal *et al*., 2014). Along with this, teeth-like projections were also noticed dorso-laterally in the three species. Such projections were earlier recorded in Braconidae by Quicke *et al*. (1995) who suspected the structure to enhance the ovipositor steering mechanism.

Apart from bembicine wasps (Crabronidae: Bembicinae), barbed sting has been found in *Sericophorus relucens* F. Smith (Crabronidae: Crabroninae) which possesses spines on the first and second valvulae that may fasten the prey during its transportation on the sting (Radovic and Susic, 1997). Species in which the lancet shaft is smooth are those in which the prey is heavily sclerotized, as in the case of *Gastrosericus waltlii* and *Larra anathema* (Larrinae) which provision their nests exclusively with gryllids and gryllotalpids (Honore, 1942; Bohart and Menke, 1976; Radovic, 1985). On the other hand, many species of apoid wasps which possess barbed stings prey on less sclerotized insects like caterpillars, aphids, cockroach nymphs and mantids (Radovic, 1985), which needs further investigation.

The SEM observation of lancets of the *A. compressa* revealed the presence of barbs; the barbs were progressively present at certain intervals, clustered at the tip (Fig. 3a) and blunt and not pointed as in *Apis* spp. The long and slender lancets may be useful for preying on Cockroaches as reported by Bohart and Menke (1976). SEM images also revealed the presence of campaniform sensilla in *A. compressa* (Fig. 3c) as previously described by the Ogawa *et al*. (2011) in *Apis mellifera*; Matushkina (2011) in *Bembix rostrata* (Fabricius) and Gal *et al*. (2014) in *Ampulex compressa*. According to Gal *et al*. (2014), *A. compressa* uses sensory input from its stinger to differentiate between the brain and other tissues inside the head capsule of its prey cockroach. Scanning Electron Microscope study of species uncovered the presence of various kinds, shapes and numbers of sensory bristles and sensory pore clustered at the tip of the gonostylus which are mechanoreceptors. These appears to be absent in bees (Packer, 2003) except in Megachilidae and some of the sphecids (Blum and Hermann, 1978; Le Relac *et al*., 1996). Comparative morphology of sting barbs of genus *Apis* is presented by Weiss (1978) and various authors have been described some sensory receptors associated with stings of certain Hymenoptera, including *Apis mellifera* (Hermann and Dongles, 1976).

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Entomological investigations on sporadic Japanese encephalitis sero-positivity in Tamil Nadu, India

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ABSTRACT: In India, Japanese Encephalitis (JE) continues to be a public health issue in some parts of our country. JE surveillance includes early reporting of clinical cases, sentinel sero-surveys and vector surveillance in the endemic areas. In the present study, JE longitudinal vector surveillance and epidemiological investigations were carried out for the first time during two consecutive years in the endemic district of Tamil Nadu. 22,538 mosquitoes were collected, species identified and screened for JE virus by RT-PCR. Predominant was *Culex tritaeniorhynchus* (60%) and followed by it *Anopheles subpictus* (23%), *Culex quinquefasciatus* (8%) and *Culex gelidus* (3%). It suggests that *Culex tritaeniorhynchus* may act as major vector and *An. subpictus* may act as secondary vector. Monsoon and post-monsoon seasons favour breeding of *Cx. tritaeniorhynchus* leading to vector abundance. Preferential resting sites for *Cx. tritaeniorhynchus* were pig and cattle shed. Although clinical cases have been reported seasonally in the three blocks, the presence of virus among field caught mosquitoes could not be established by RT-PCR. It may be due to the low titre value of JE virus in mosquitoes. This is the first report of JE investigations in the endemic district of Tamil Nadu and it helps to formulate the effective control strategies for JE virus transmission.

KEYWORDS: Flavi virus transmission, vector surveillance, *Culex tritaeniorhynchus*, *Anopheles subpictus*, *Culex gelidus*

INTRODUCTION

Globally more than three fourth of the population is exposed to vector borne diseases and among these, mosquito transmitted diseases that are more prevalent in tropical and sub-tropical countries contribute the major burden. Mosquito-borne diseases, especially malaria, dengue, filariasis and Japanese encephalitis, remain endemic in many tropical countries (Poopathi *et al.,* 2014; Franklinos *et al.,* 2019). Japanese encephalitis (JE) caused by Flavivirus is a major public health concern in rural as well as suburban areas of Asian countries and sporadic spread occurs in northern parts of

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Australia and some parts of Western Pacific. Twenty-four JE endemic nations in Western Pacific and South East Asian areas continue to have Japanese Encephalitis Virus (JEV) transmissions, exposing more than three million populations to the risk of infection with an estimated 68,000 clinical cases every year (WHO, 2019). The first JE case in India was reported at Vellore, Tamil Nadu in 1955 (Webb *et al.,* 1956) and JEV was isolated from human brain tissue in 1958 (Carey *et al.,* 1968). Subsequently, many JE epidemics were reported in 1973, 1978, 2005, 2006 and 2007 from all Southern states and some Eastern and North-Eastern states. Widespread epidemics were also reported from large states like Uttar Pradesh, Madhya Pradesh and Maharashtra (Banerjee *et al.,* 1979; Dhanda and Kaul. 1980; Kabilan *et al.,* 2004; Tiwari *et al.,* 2008; Kumari and Joshi, 2012). In the state of Kerala, JE is endemic in the state of Alappuzha, Kottayam, Trivandrum and Thrissur (Tyagi *et al.,* 2014). In 2009, JEV genotype I was first reported in Gorakhpur region (Fulmali *et al.,* 2011). In India, among symptomatic cases, case fatality was reported to be 20 to 30%, and during the last 10 years, it has been drastically reduced due to better case management. However, permanent neurologic or psychiatric sequelae are not reported in India. JE virus is transmitted through zoonotic cycle among mosquitoes (vectors) and vertebrate – amplifying host primarily pigs and birds (carriers). Infection in the human population is incidental and due to poor viral multiplication in human tissues, there is no transmission from human to mosquitoes. *Culex vishnui* groups consisting of *Cx. tritaeniorhynchus* Gilles, *Cx. vishnui* Theobald and *Cx. pseudovishnui* Colless have been associated as principal vectors for JE. Nevertheless, JE virus has been detected from 16 mosquito species belonging to the genera *Culex* (10), *Anopheles* (3) and *Mansonia* (3) (Kanojia *et al.,* 2003).

In India, twenty four states still are considered as endemic zones. In 2007, a health education programme was conducted to improve the hygiene of population at risk in India. In Tamil Nadu state, an extensive epidemic was reported in 1981 in Cuddalore district, since then, the disease were reduced until 2013 with no death. Latest JE case death was reported during the year 2014 in Tamil Nadu and since then JE occurrence was increasing (NVBDCP, India). Though the Department of Public Health and Preventive Medicine, Tamil Nadu introduced SA 14-14-2 type of JE vaccine more than 10 years back for the children aged one to fifteen years, 12 districts continued to be listed as endemic in the State of Tamil Nadu (NVBDCP, Tamil Nadu). One among the state is the Thiruvallur district where entomological and epidemiological investigations are reported in the present study. Concurrently vector control activities are also being implemented in three JE control units Perambalur, Villupuram, and Cuddalore under the supervision of the monitoring unit at Chennai. However, sero-positive cases of JE are being reported almost every year in these districts indicating JE virus circulation in Tamil Nadu. There has been a change in the epidemiological trend in JE during last decade. JE cases in adult were reported from several districts where JE vaccination programme has been in operational (TAG, 2017). The Government of India introduced E adult vaccination in three states viz: Assam, Uttar Pradesh and West Bengal (Vipin and Ramachandran, 2015). Changing epidemiological trends warranted vector surveillance at several parts of the country. Considering the above seriousness on JE incidence entomological and epidemiological investigation were carried out in the JE endemic district of Tami Nadu to find out the seasonal abundance, adult density, vector infection rate which was not reported earlier to suggest effective strategies for vector control.

MATERIALS AND METHODS

Study area: Thiruvallur district, Tamil Nadu is geographically located between the Latitude of 13°08'37.54" N and Longitude of 79°54'32.00" E. The elevation of the area ranges from 183 m Above Mean Sea Level (AMSL). Being at the North end of the Tamil Nadu State, it is bordered by the Southern end of Andhra Pradesh in North, Kancheepuram district in the South, Bay of Bengal in East and Vellore district in West. The most common occupation of the population is agriculture and the district has more than 131.17 thousand hectares of cropped areas. Seven seasonal rivers

are the major sources of water for cultivation through North-East monsoon (52%) as well as South-West monsoon rain (41%) respectively. The annual average rainfall through both monsoons is 1104 mm. The incidence of JE cases was obtained from the collaborating agency of Department of Public Health and Preventive Medicine and King Institute of Preventive Medicine and Research, Chennai. Based on the JE clinical cases reported in the Thiruvallur district from 2011 to 2016, the villages of Ellapuram, Sholavaram and Thiruvelangadu were selected for this study. It is important to emphasize that, so far entomological surveillance was not carried out in these villages, even though these villages were reported with larger number of JE cases for the past several years.

Mosquito collection: Mosquito collections were carried out indoor and outdoor resting sites during throughout the study period in dusk hours (between 18.00 and 19.30 hours) by using manual and mechanical aspirator. Each selected village was sampled fortnightly for entomological study from January 2017 to December 2018. With the aid of the torch, mosquitoes were collected by manual and mechanical aspirator from walls, ceiling, under furniture, hangings, and curtains. Search for mosquitoes was carried out systematically starting from the main door and moving clockwise inside the house room by room. While collecting mosquitoes from indoor, attention was given to the preferential resting locations for mosquitoes. Resting places were recorded in the standard proforma to determine the preferential indoor resting sites. Collections were carried out for at least 30 minutes depending upon the size of the house and crowding of the domestic items and utensils. New standard CDC miniature light trap was fixed near the pigsties, bushes and cattle sheds, two meters above ground level set before sunset and collected after sunrise in the next morning. The mosquitoes caught during dusk hours and light traps in the field were stored in liquid Nitrogen and transported in labelled containers to the laboratory for species identification.

Species identification and storage: Standard taxonomic key was adopted for mosquito species identification (Reuben *et al.,* 1994). The wild caught mosquitoes were segregated species wise and pooled (25 mosquitoes per pool) for virological assay.

Seasonal abundance: Information in terms of seasonal abundance was collected in four seasons *viz*: winter - cool and dry (December to February), summer - hot and dry (March to June), monsoon - cool and wet (July to September) and finally post-monsoon cool and wet (October to December) seasons. The abundance of JE vector was calculated for different seasons. Vector density was recorded as the number of female mosquitoes per man hour (PMH) spent during collection. The relative density of female mosquito was estimated as the number of females collected PMH and it was denoted as per man hour density (PMD).

Virus detection tests: Virus detection in mosquitoes was carried out by standard Reverse Transcriptase PCR assay. First, wild caught mosquito pools (25 mosquitoes in each pool) were homogenized in a Remi motar (Remi Elektrotechnik Ltd, India) by using separate pestle for each pool. Following centrifugation at 12,000 rpm for 30 minutes, the supernatants were separated and utilized for extraction of viral RNA by Trizol method. The Reverse Transcriptase-PCR reaction was accomplished by Superscript-III one step RT-PCR with Platinum Taq High Fidelity (Invitrogen, Life Technologies, California, USA). Primer pairs JEV-Ef (5'-TGYTGGTCGCTTCCACAYCTC -3') and JEV-Er 5'-AAGATGCCACTTCCACAYCTC-3' were used to amplify the JEV. Following parameters were applied to carry out RT-PCR: 55ÚC for 30 minutes; 94ÚC for 2 minutes; 40 cycles of 94ÚC for 15 seconds, 57ÚC for 30 seconds, and 68ÚC for 1 minute; and a prolonged elongation at 68ÚC for 5 minutes. Purification of RT-PCR products was done following the manufacturer's instructions using the quick Gel Extraction kit (QIAGEN). JEV was confirmed in 1.5% gel electrophoresis along with positive control which was received from NIV, Pune, India. Mosquito virus infection rate (MIR) was expressed using the formula:

$$MIR = \frac{\text{No. of positive pools}}{\text{Total no. of mosquito mosquitoes tested}} \times 1000$$

Data analysis: All field collected information was entered in Microsoft Office 2013 Excel, cross-checked independently. Wherever required, the data were checked with original field data sheets and appropriate corrections were done in digital data before analysis. Data from Excel was transported to statistical software SPSS for analysis. Descriptive statistics mean, standard deviation and proportion were calculated on version 16.0 IBM SPSS statistics for windows.

RESULTS

Preferential resting sites: It was observed that pig and cattle sheds were found to be the most preferential resting sites for the JE vectors such as *Cx. tritaeniorhynchus* and *An. subpictus* respectively. Whereas, the preferential resting site for *Cx. gelidus* was found in vegetative bushes around the domestic houses (Table1). As reported earlier *Cx. tritaeniorhynchus* prefers more on pig sheds whereas *An. subpictus* prefers cattle sheds.

Vector abundance: The seasonal abundance of JE vectors in the endemic district of Tamil Nadu is presented in table 2. It was observed that only the JE vectors of *Cx. tritaeniorhynchus, An. subpictus, Cx. quinquefasciatus* and *Cx. gelidus* were found spreading throughout the four seasons. Comparative density analysis for the high, moderate and less densities indicates the pattern *Cx. tritaeniorhynchus* > *An. subpictus* > *Cx. quinquefasciatus* > *Cx. gelidus*. *Ar. subalbatus* a rare species on the JE transmission was also collected from these study sites (0.2 to 5.6%) (Fig. 8).

The vector species composition in the entire study period revealed fourteen mosquito species from four genera namely *Culex, Anopheles, Aedes* and *Armigeres* were collected from the study blocks of the Thiruvallur district and these comprised of *Culex* (6 species), *Anopheles* (5 species), *Aedes* (2 species) and *Armigeres* (1 species). Though *Cx. tritaeniorhynchus* (60%) and *An. subpictus* (23%) continue to be the major and secondary JE vectors respectively, the collection of *Cx. quinquefasciatus* (8%) *Cx. gelidus* (3%) *Ar. subalbatus* (2%), *An. barbirostris* (*1%)* were also high in these areas and other eight JE vectors such as, *Cx. fuscocephala* (0.4%), *An. vagus* (0.3%), *Cx. bitaeniorhynchus* (0.3%), *An. culicifacies* (0.06%), *Ae. linneatopennis* (0.013%), *Ae. vexans* (0.09%), *Cx. infula* (0.004%) and *An. minimus* (0.004%) were also recorded (Fig. 2). The mosquitoes reported from Ellapuram block includes *Cx. tritaeniorhynchus* (53%), *An. subpictus* (29%), *Cx. quinquefasciatus* (10%), *Cx. gelidus* (3%), *Ar. subalbatus* (2%) *An. barbirostris* (1%) and *An. vagus* (1%) (Fig. 3). In Sholavaram block, *Cx. tritaeniorhynchus* (69%), *Cx. quinquefasciatus* (12%), *Cx. gelidus* (8%), *An. subpictus* (6%), *Ar. subalbatus* (2%), *Cx. bitaeniorhynchus* (1%), *An. barbirostris* (1%) and *Cx. fuscocephala* (1%) were recorded (Fig. 4). Mosquitoes reported in Thiruvelangadu block include *Cx. tritaeniorhynchus* (65%), *An. subpictus* (30%), *Cx. quinquefasciatus* (2%), *An. barbirostris* (2%) and *Cx. fuscocephala* (1%) (Fig. 5).

Consolidated analysis on seasonal variation with respect to vector density showed that the PMD increased during March - June and steadily increased upto post monsoon season of October – December. Thereafter the density declined in the winter season (December – February) (Fig. 6). There was increase in vector abundance during monsoon and post-monsoon for all the four JE vectors though abundance was much higher for *Cx. tritaeniorhynchus* and *An. subpictus* vectors in Ellapuram and Tiruvelangadu blocks. In addition, there was a perceptible increase in vector abundance during summer months and that may be responsible for maintenance of the virus in the environment (Fig. 7 a,b,c). RT-PCR assay results on the detection of JE virus infection in the mosquito pools collected from these three blocks. It was observed that JE positives were not traceable though 902 pools with 22538 mosquitoes were examined (Table 3).

DISCUSSION

Several studies have established that *Cx. tritaeniorhynchus* is the primary vector of Japanese Encephalitis in different locations of India

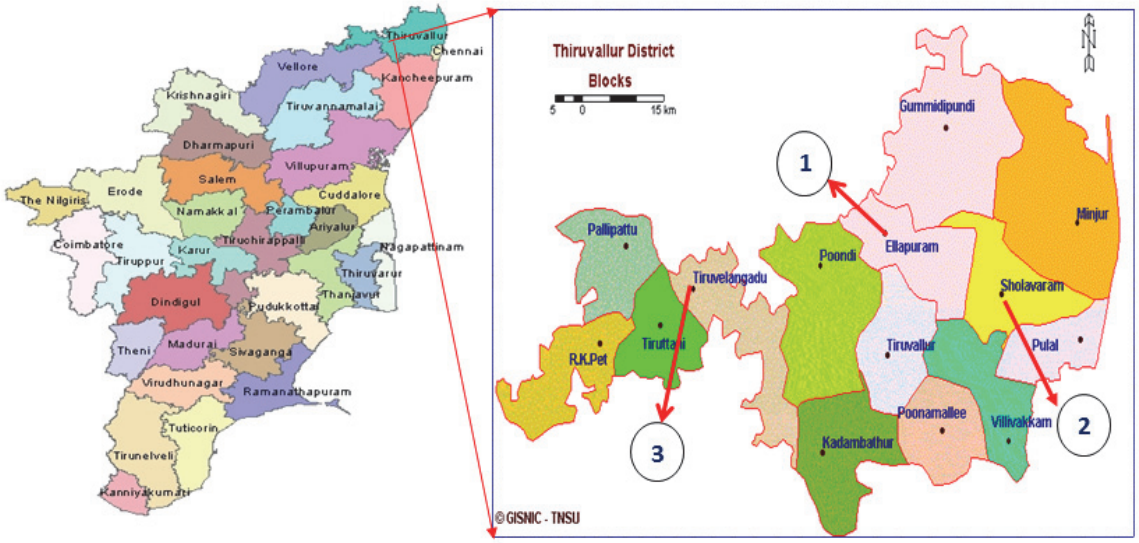

Fig. 1 Study area – Thiruvallur district, Tamil Nadu, India

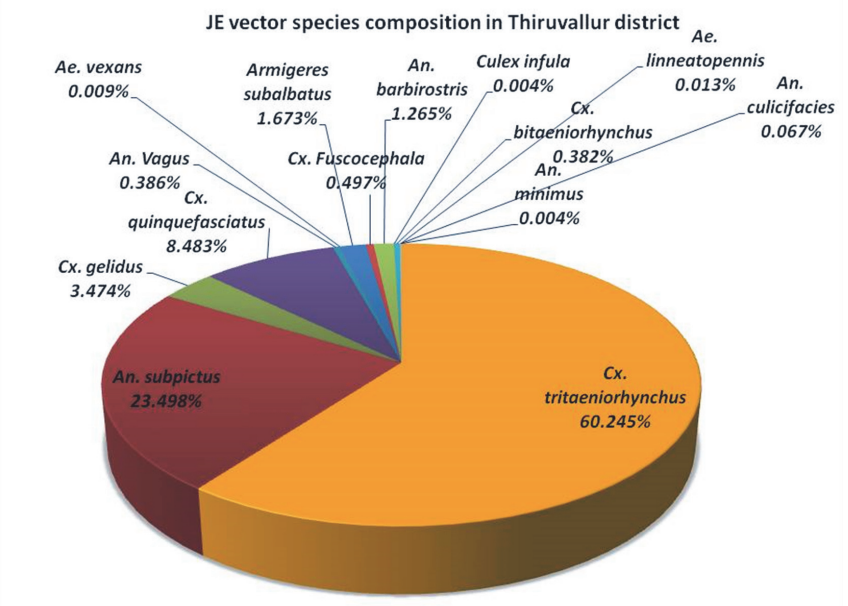

Fig. 2 Mosquito fauna of Thiruvallur district, Tamil Nadu, India

(Dhanda *et al.,* 1997; Burke and Monath 2001; Murthy *et al.,* 2002; Kanojia, 2007; Arunachalam *et al.,* 2009; Ramesh *et al.,* 2015; Samuel *et al.,* 2018). In the present study, although JE confirmed cases were reported in the study areas, so far no JE-vector surveillance was carried out. Thiruvallur district is an adjacent location and has a close proximity the metropolitan areas of Chennai and transmission potential to urban areas is also to be considered. Therefore, this report has significant public health importance for the district programme officer (Thiruvallur district, Tamil Nadu). Further, in Kerala, Karnataka, Andhra Pradesh, Utter Pradesh and West Bengal, various secondary

vectors have been identified, which include, *Ma. indiana, Cx. gelidus, Cx. whitmore, Cx. pseudovishnui, Cx. epidesmus, An. peditaeniatus, An. subpictus* and *Ma. uniformis* (Kelly Hope *et al.,* 2004; Pani *et al.,* 2004; Khan *et al.,* 1996; Arunachalam *et al.,* 2009). In Tamil Nadu also (Cuddalore, Villupuram and Tanjore), *Cx. gelidus* was reported as the secondary vector (Ramesh *et al.,* 2015; Samuel et al., 2015, 2016a,b, 2018). In the present study, *An. subpictus* was reported for

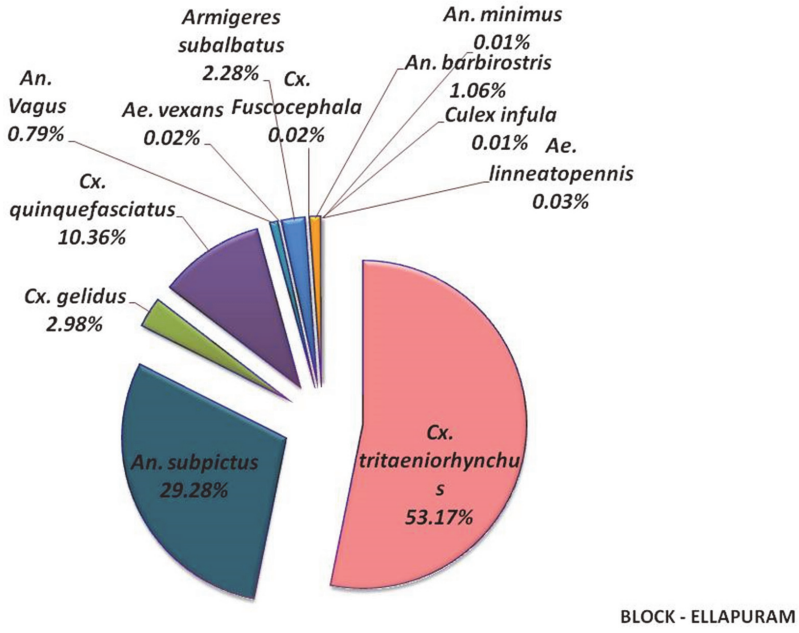

Fig. 3 JE vector abundance in Ellapuram - Thiruvallur district, Tamil Nadu, India

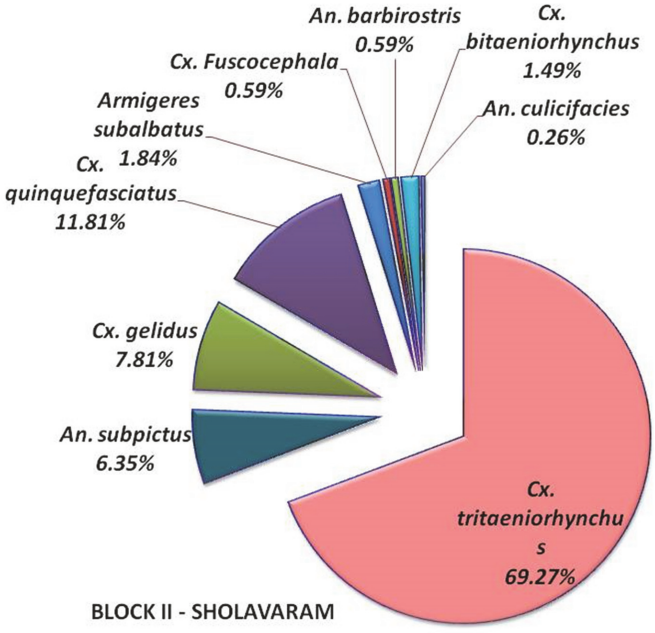

Fig. 4 JE vector abundance in Sholavaram - Thiruvallur district, Tamil Nadu, India

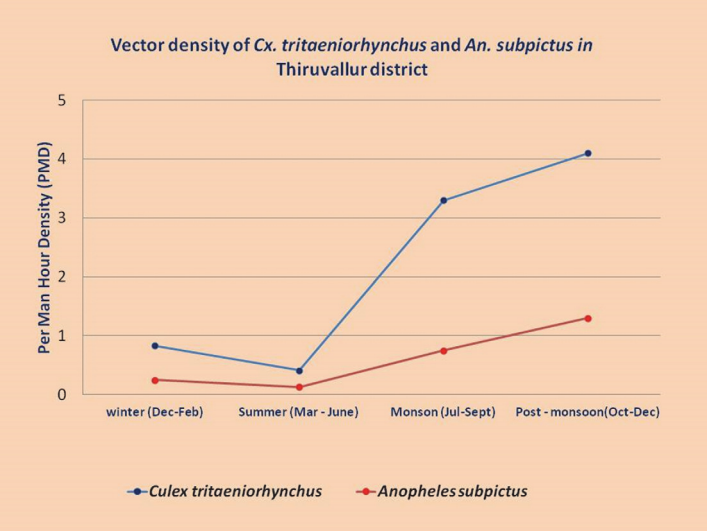

Fig. 5 JE vector abundance in Tiruvelangadu – Thiruvallur district, Tamil Nadu, India

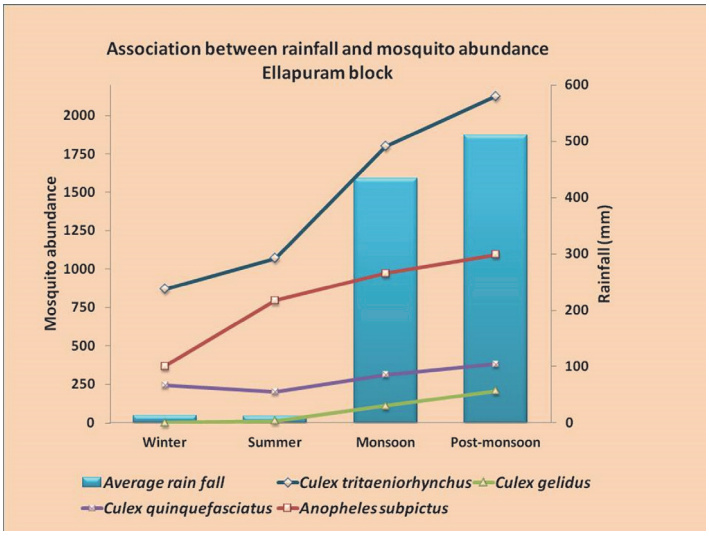

Fig. 6 Vector density of JE vector in Thiruvallur district, Tamil Nadu, India

the first time in the JE endemic district (Thiruvallur) as secondary species. Our findings corroborate with previous workers who have reported the *An. subpictus* as secondary vectors in JE prone areas such as Kuddapah (AP), Tirunelveli and Virudhu Nagar (TN) (Murthy *et al.,* 2002; Thenmozhi *et al.,* 2015; Anandh and Sevarkodiyon, 2017).

The population dynamics of JE vectors largely depend upon rice cultivation, water bodies, temperature and humidity in the rural areas. In these study sites, agricultural farming is the primary source of income for inhabitants, therefore agricultural farming favours prolific breeding of JE vectors especially *Cx. tritaeniorhynchus* followed by *An. subpictus*. Seasonal abundance of these mosquito species showed that *Cx. tritaeniorhynchus* and *An. subpictus* were very high in cool - dry (December to February) and cool - wet (July to September) seasons. This observation is in agreement with earlier findings (Ramesh *et al.,* 2015).

The per man hour density (PMD) ranged from 0.8 – 4.1 for *Cx. tritaeniorhynchus* and 0.25-1.3 for *An. subpictus* in the study period of January to December 2017 and similar trend was also observed in the subsequent year of field studies (2018). This entomological observation revealed a significant increase in vector abundance during monsoon season as reported inother areas like Andhra Pradesh and Tamil Nadu (Murthy *et al.,* 2002; Ramesh *et al.,* 2015; Malar *et al.,* 2015; Samuel *et al.,* 2016). JE vector abundance was high in rainy season (June to December). However, when vector density was plotted site-wise, there were two peaks, first during the summer and then during monsoon in Sholavaram blocks due to increase in density of the *Cx. tritaeniorhynchus*. A similar observation was reportedwith peak density of *Cx. quinquefasciatus* followed by *Cx. tritaeniorhynchus* in Bareilly district of Uttar Pradesh (Pantawane *et al.,* 2017). In the present study, the wild-caught mosquitoes of 902 pools collected from Thiruvallur district showed no positivity for JE virus. Similar results were reported by Changbunjong *et al.* (2013) in Thailand. During our study period, only ten sero-positive JE cases were reported by Department of Public Health and Preventive Medicine, Chennai. Therefore it is understood that JE positive cases recurrently occurring in these study areas (Ellapuram, Sholavaram and Tiruvelangadu blocks) but the titre values of JE virus transmission may be low and it is presumed that this may be due to the non-appearance of JE-virus positivity in mosquito pools during our study period.

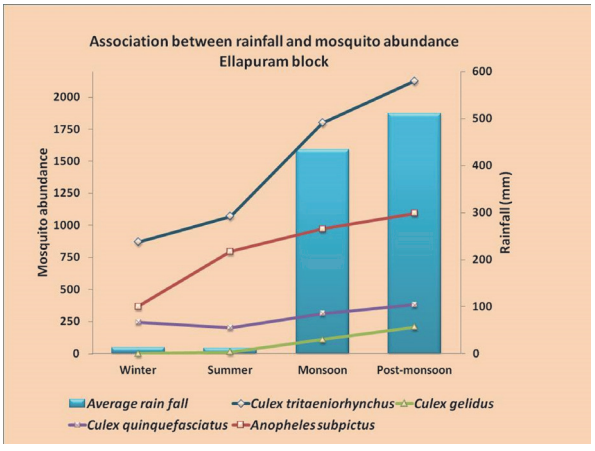

Fig. 7a Association between rainfall and mosquito abundance – Ellapuram block.

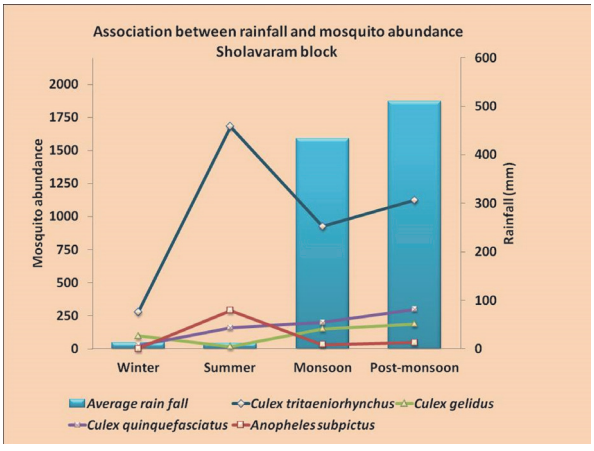

Fig. 7b Association between rainfall and mosquito abundance - Sholavaram block

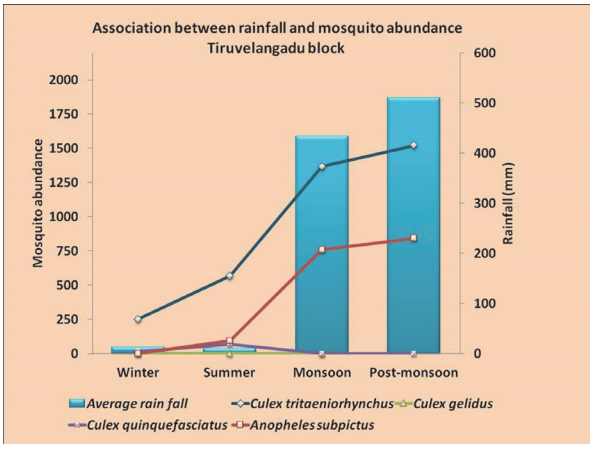

Fig. 7c Association between rainfall and mosquito abundance - Thiruvelangadu block

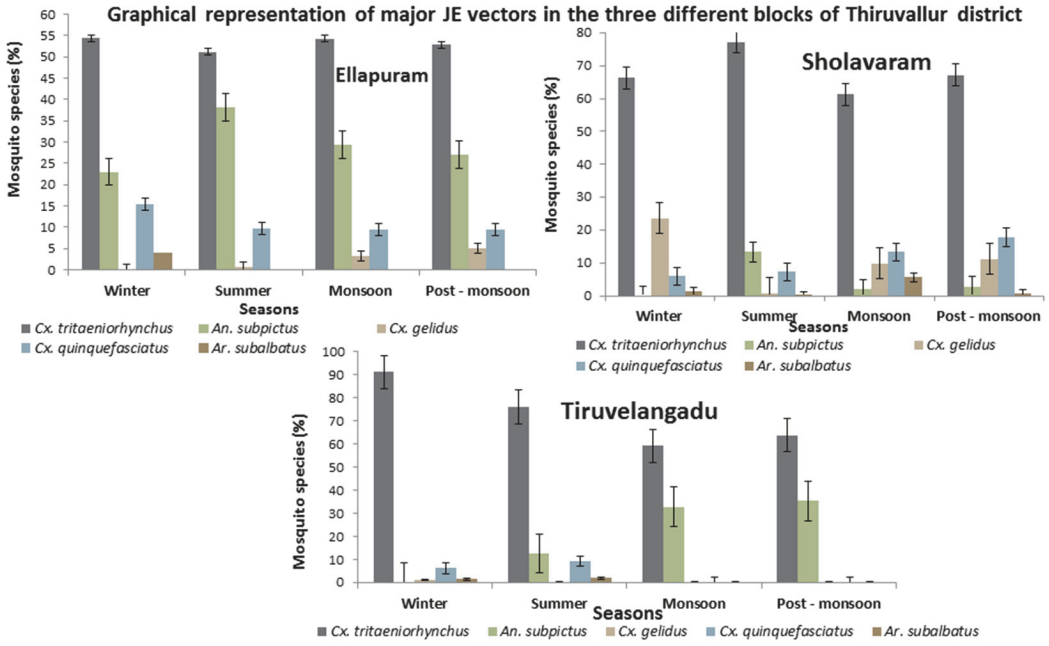

Fig. 8 Graphical representation of major JE vectors in the three different blocks of Thiruvallur district

In the present study it has been established that all three study sites continue to harbour mosquitoes responsible for JE transmission and *Cx. tritaeniorhynchus* continues to be the predominant JE vector in this region. Paddy cultivation and the close proximity of Pulicat bird sanctuary are the potential risk factors for JE transmission. Reported cases from the DDPH indicated that there has been a shift in JE incidence to higher age groups. Studies from different parts of India have established age-shift in the occurrence of JEV infection (Borathur *et al.,* 2013; Gunasekaran *et al.,* 2012). Vector surveillance along with serological studies in amplifier animals such as pigs and reservoir animals such as egret is a preferable approach which can be used as an early warning system (Baruah and Hazarika 2018).Pulicat bird sanctuary may lead the JEV transmission through migratory birds from

Table 1. Preferential resting places for Japanese encephalitis vectors

Mosquito resting places	Mosquito species Density			
	Cx. tritaeniorhynchus	*An. subpictus*	*Cx. gelidus*	*Cx. quinquefasciatus*
Thatched roof	+	++	+	+++
Sleeping mat	+	+	+	+++
Wooden shelf	+	+	+	+++
Clothes in rope	++	+	+	+++
Cattle shed	+++	++++	+	+
Pig shed	++++	++	++	++
Bushes	++	++	++++	+

+ Low density ++ Moderate density +++ High density ++++ Very high density

Table 2. Seasonal abundance of JE vector in Thiruvallur district, Tamil Nadu, India

Study site	Season	Mosquito species													
		Cx. tritaenior hynchus	*An. subpictus*	*Cx. gelidus*	*Cx. quinque fasciatus*	*An. Vagus*	*Ae. vexans*	*Armigeres subalbatus*	*Cx. Fuscoce phala*	*An. barbiro stris*	*Culex infula*	*Cx. bitaenior hynchus*	*Ae. linneato pennis*	*An. culicifa cies*	*An. minimus*
Ellapuram	winter	54.4	23.0	0.0	15.3	5.4	0.1	1.3	0.1	0.0	0.1	0.0	0.2	0.0	0.0
	summer	51.2	38.1	0.6	9.8	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	54.3	29.3	3.3	9.4	0.0	0.0	2.1	0.0	1.6	0.0	0.0	0.0	0.0	0.0
	post - monsoon	52.8	27.1	5.1	9.5	0.0	0.0	3.9	0.0	1.6	0.0	0.0	0.0	0.0	0.0
Sholavaram	winter	66.3	0.0	23.6	6.0	0.0	0.0	1.4	0.7	0.0	0.0	0.7	0.0	1.2	0.0
	summer	77.1	13.3	0.7	7.3	0.0	0.0	0.1	1.4	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	61.3	2.0	9.9	13.3	0.0	0.0	5.6	0.0	2.3	0.0	5.0	0.0	0.7	0.0
	post - monsoon	67.1	2.8	11.2	17.8	0.0	0.0	0.7	0.0	0.0	0.0	0.4	0.0	0.0	0.0
Thiruvelangadu	winter	91.2	0.0	1.1	6.2	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	summer	76.0	12.7	0.0	9.3	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	monsoon	59.1	32.8	0.0	0.0	0.0	0.0	0.0	3.1	5.0	0.0	0.0	0.0	0.0	0.0
	post - monsoon	63.8	35.3	0.0	0.0	0.0	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0

Table 3. Japanese encephalitis virus infection in field caught mosquitoes in Thiruvallur District, Tamil Nadu, India

Species	BLOCK I - ELLAPURAM			BLOCK II - SHOLAVARAM			BLOCK III – THIRUVELANGADU			TOTAL		
	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive	Number of Mosquitoes	Number of Pools	Number of pools Positive
Culex tritaeniorhynchus	5867	235	0	4001	160	0	3710	148	0	13578	543	0
Anopheles subpictus	3231	129	0	367	15	0	1698	68	0	5296	212	0
Culex gelidus	329	13	0	451	18	0	3	0	0	783	31	0
Culex quinquefasciatus	1143	46	0	682	27	0	87	3	0	1912	76	0
Anopheles Vagus	87	3	0	0	0	0	0	0	0	87	3	0
Aedes vexans	2	0	0	0	0	0	0	0	0	2	0	0
Armigeres subalbatus	252	10	0	106	4	0	19	1	0	377	15	0
Culex fuscocephala	2	0	0	34	1	0	76	3	0	112	4	0
Anopheles barbirostris	117	5	0	34	1	0	134	5	0	285	11	0
Culex infula	1	0	0	0	0	0	0	0	0	1	0	0
Culex bitaeniorhynchus	0	0	0	86	3	0	0	0	0	86	3	0
Aedes linneatopennis	3	0	0	0	0	0	0	0	0	3	0	0
Anopheles culicifacies	0	0	0	15	1	0	0	0	0	15	1	0
Anopheles minimus	1	0	0	0	0	0	0	0	0	1	0	0
Grand Total	11035	441	0	5776	231	0	5727	229	0	22538	902	0

another country. The study revealed that the potential primary vector and secondary vector in Thiruvallur district as *Cx. tritaeniorhynchus* and *An. subpictus* respectively and seasonal abundance of JE vectors were high during July- December which is a monsoon season in Tamil Nadu. However, in Sholavaram block there were two peaks during the year indicating that increase in mosquito density was due to high density of *Cx. tritaeniorhynchus*. In this block, first peak was observed during summer which indicates that the sporadic rain might be responsible for the vector abundance and also emphasizes fortnightly vector surveillance.

Public Health Department of Tamil Nadu reported five, six and four JE sero-positive cases from the entire district of about 370 thousand population from 12 revenue blocks during the year 2016, 2017 and 2018 respectively. Only one and 2 sero-positive cases were reported from the study areas in 2016 and 2017 respectively. These areas are also covered under JE vaccination programme. These factors indicate that JE do not occur in epidemic proportion like the State of Uttar Pradesh, India. For such a low level of sporadic transmission, the viral load in vector mosquitoes is likely to be low and therefore, we were not able to detect JE positivity in RT-PCR assays. However, reporting of the JE sero-positivity in sporadic locations cannot be ignored. A close entomological and epidemiological investigation is suggested to monitor at regular intervals. The areas are covered under this study has the Pulicat bird sanctuary of about 480 sq. km shared with the adjacent State Andhra Pradesh. The Pulicat bird sanctuary harbour thousands of greater flamingos and it is the major feeding and breeding ground many migratory birds like storks and pelicans. Locations from the study areas are in close proximity to the bird sanctuary as well as the areas of Chennai Metropolitan city. If the transmission is allowed to set in the urban metropolitan areas in Chennai, it will lead to a major public health emergency. Therefore, further extensive vector surveillance activities to provide the lead for epidemiological intelligence and thereby the prevention of urban JE epidemics. The public health authorities need to create awareness among the communities on mosquito abundance, seasonal variations, vaccination and vector control strategies to prevent JE transmission. More sensitive PCR assays to pick up low level JE infection must be developed for a robust surveillance system in India.

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Insecticidal activity of cashew nut shell liquid against sucking pests of cowpea, *Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.

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ABSTRACT: Cashew nut shell liquid (CNSL), an important agro waste from cashew nut processing factories, was emulsified in water and assayed for insecticidal activity at concentrations ranging from 0.05 to 0.2 % against aphid, *Aphis craccivora* and pod bug, *Riptortus pedestris* infesting cowpea, *Vigna unguiculata sesquipedalis* under laboratory conditions by topical application. CNSL at various concentrations was found to have insecticidal properties against *A. craccivora* and *R. pedestris* wherein the speed of kill and efficacy varied with concentration and test insect. CNSL @ 0.1 % with mortality ranging from 95.83 to 100 per cent at 48 hours after treatment (HAT) was found effective against *A. craccivora* whereas a concentration of 0.2% was required against *R. pedestris* to achieve similar mortality (96.67 to 100 %) at 72 HAT. CNSL derived from two cashew nut processing methods (drum roasting and steam boiling) did not differ significantly in their insecticidal action. At concentrations of 0.1 and 0.2 %, mortality produced by CNSL was comparable to that of chemical insecticide, thiamethoxam 0.03% and significantly superior to the widely used botanical neem oil @ 2% against *A. craccivora* and *R. pedestris* respectively. *R. pedestris* that survived exposure to CNSL treatments exhibited developmental abnormalities and formation of nymphal adult intermediary indicating its possible insect growth regulatory effect.

KEYWORDS: CNSL, plant derived insecticide, *Riptortus pedestris, Aphis craccivora*

INTRODUCTION

Cow pea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc), commonly known as yard long bean is one of the most widely cultivated commercial vegetable crops of Kerala. Green pods of the crop harvested at short intervals are a cherished vegetable, fetching high returns to the cultivators. But the quality of the produce is at risk because of the insecticide residue left subsequent to over use of insecticides to tackle the incidence of insect pests. Sucking pests *viz*., cowpea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae) and pod bug, *Riptortus pedestris* (Fabricius) (Coreidae: Heteroptera) cause serious loss, affecting both quantity and quality of the produce forcing farmers to use synthetic insecticides at frequent intervals. Indiscriminate use of insecticides leads to ecological and health hazards necessitating exploration of alternatives. Plant derived insecticides being quickly biodegradable and safe to non target organisms are potential alternative to

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chemical pesticides. Plants synthesize and store many secondary metabolites capable of affecting insect development, reproduction and behavior. Characterization and use of such phytochemicals for pest control have yielded several botanical pesticides (Isman, 1994). Cashew plants, *Anacardium occidentale* produce and store phenolic secondary metabolites in the honey comb structure of its nut shell to ward off pests. This cashew nut shell liquid (CNSL) exude from the shells during cashew nut processing is a cheap by-product of cashew agro processing industry, available in plenty. CNSL possess insecticidal activity against termites (Asogwa *et al*., 2007), microorganisms (Lomonaco *et al*., 2009; Parasa *et al.,* 2011) and *Aedes aegypti* G. (Oliveira *et al*., 2011).

Anacardic acid, cardol and cardanol, the phenolic constituents of cashew nut shell was proved to have toxicity against sucking pests which was earlier indicated in the study of Oparaeke *et al*. (2005) wherein cashew nut shell extract found effective in reducing the pod sucking bugs. The toxicity of CNSL was earlier documented against chewing pests *viz*., coconut root grub (John *et al*., 2008), *Helicoverpa armigera* and *Spilarctia obliqua* (Mahapatro, 2011).The pesticidal efficacy of CNSL against chilli aphid, *Aphis gossypii* was reported earlier by Sundaran and Faizal (2018) where CNSL @ 0.2 % caused cent per cent mortality at 72 HAT. Andayanie *et al*. (2019) evaluated the efficacy of hexane extract of cashew nut shells against white fly, *Bemesia tabaci* wherein CNSL was reported to have antifeedant and anti oviposition activity besides causing mortality. In the present investigation the potential of CNSL as a natural insecticide against aphids and pod bugs of cowpea was evaluated with an aim to find a green alternative to synthetic insecticides.

MATERIALS AND METHODS

Laboratory bioassay studies were carried out at Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram during 2016-2019 to evaluate the insecticidal property of CNSL against major sucking pests of cowpea, *A. craccivora* and *R. pedestris*. *A. craccivora* was reared on sprouted green gram seeds maintained on wet cotton bed, kept in plastic containers. Gravid females were collected from the field and released to sprouted green gram seeds and the population thus maintained served as source culture. Second instar nymphs were collected from this source culture carefully, using a camel brush and used for experiments. Twenty numbers of aphids were used per treatment. The males and females of *R. pedestris* were collected from cowpea field and released on to the red gram pods maintained in the laboratory for egg laying. The nymphs that emerged from the eggs were transferred to fresh red gram pods. The second instar nymphs (10 numbers) from this source culture were used for each treatment.

Cashew nut shell liquid derived out of drum roasting method (CNSL-DR) of processing was purchased from Mahatma cashew exports, Kollam, Kerala and that derived from steam boiling technique (CNSL-SB) were collected from A.A nuts, Kollam. Seven different concentrations *viz*., 0.05, 0.06, 0. 07, 0.08, 0.09, 0.1 and 0.2 per cent of both CNSL- DR and SB were prepared using vegetable soap (0.6 %) as emulsifier and tested in the laboratory against *A. craccivora* and *R. pedestris* in completely randomized block design with three replications. Neem oil emulsion 2 % and thiamethoxam 25 WG 0.03% served as botanical and chemical check respectively.

The treatments were applied using potters precision spray tower @ 2 ml/replication ensuring uniform coverage of test insects and were subsequently transferred to treated pods kept in poly vinyl containers covered with muslin cloth. The insects sprayed with distilled water served as untreated check. The mortality of the test insects were observed at 24, 48 and 72 hours after treatment (HAT) for *A. craccivora;* 48, 72 and 96 HAT for *R. pedestris*. The treatment mortality was corrected with the mortality in untreated check (Abbot, 1925). The cumulative corrected percentage mortality was statistically analyzed after arcsine transformation. LC_{50} and LC_{90} was calculated using probit analysis in the statistical program, SPSS (Statistical Product and Service Solutions).

RESULTS AND DISCUSSION

Among the different treatments evaluated on the mortality of *A. craccivora*, thiamethoxam 25 WG 0.03% recorded cent per cent mortality and was superior to all other treatments at 24 HAT. This was followed by 0.2 % CNSL- DR, 0.2 % CNSL-SB and 0.1 % CNSL- DR with 76.67, 73.33 and 66.67 respectively which were on par with each other. The percentage mortality obtained in all other treatments including CNSL concentrations ranging from 0.05 to 0.09 were found to be inferior and on par with each other. At 48 hours after treatment also, the higher concentrations of CNSL *viz*, 0.2 % CNSL-SB, 0.1 % CNSL-SB and 0.2 % CNSL-DR were significantly superior (100 percent mortality) and their effect was equal to that of chemical check thiamethoxam 0.03%. Cent per cent mortality was observed at 72 HAT in all the treatments except neem oil emulsion 2 %, 0.6 % vegetable soap solution and untreated check (Table 1). CNSL emulsions caused mortality of *A. craccivora* at 24 HAT itself ranging from 30.00 per cent at 0.05 % to 76.67 per cent at 0.2 % and so LC_{50} and LC_{90} values were calculated by the probit analysis of dose-mortality responses at this level. The LC_{50} values obtained for the *A. craccivora* were 0.079 and 0.084 respectively for CNSL-DR and CNSL-SB with corresponding LC_{90} values of 0.250 and 0.275 (Table 2).

CNSL was comparatively slower in its action against heteropteran *R. pedestris* wherein a consistent mortality was observed only at 48 HAT, at which the chemical check thiamethoxam 0.03 % proved significantly superior (Table 1). 0.2 % CNSL-DR, 0.1% CNSL-SB and 0.2% CNSL-SB with per cent mortality of 73.33, 66.67, and 66.67 which were on par with each other and were significantly different from rest of the treatments. All other treatments containing CNSL emulsions were found to be inferior with values ranging from 26.67 to 50.00. At 72 HAT, cent per cent mortality was recorded in chemical check, 0.2% CNSL-DR and 0.1% CNSL-SB. These treatments along with CNSL-SB 0.2% with a mortality of 96.67 were significantly superior than rest of the treatments. The same trend continued at 96 HAT also. Apart from mortality developmental irregularities like nymphal adult intermediary formation was noticed in *R. pedestris* that survived exposure to CNSL.

At 48 HAT, 0.2 % concentration of CNSL emulsion caused only 73.33 per cent mortality, hence the LC_{50} and LC_{90} values were calculated by probit analysis of dose mortality responses studied at CNSL concentrations ranging from 0.05 to 0.5 %, the results of which are presented in Table 2. Mortality of *R. pedestris* increased with increase in concentration of CNSL. The LC_{50} values obtained for *R. pedestris* at 48 HAT were 0.095 and 0.102 per cent respectively for CNSL-DR and CNSL-SB. The corresponding LC_{90} values were 0.275 and 0.334 per cent respectively for CNSL-DR and CNSL-SB at 48 HAT.

CNSL at various concentrations was found to have insecticidal properties against *A. craccivora* and *R. pedestris* wherein the speed of kill and efficacy varied with concentration and test insect. *A. craccivora* succumbed to the treatments as early as 24 HAT where as *R. pedestris* took slightly more time to get killed. The mortality of the pest was found to increase with increase in concentration of CNSL. 0.2 % CNSL-DR and SB and 0.1 % CNSL-DR which produced 76.67, 73.33 and 66.67 per cent mortality respectively of *A. craccivora* at 24 HAT and was found to be superior over other concentrations of CNSL though inferior to chemical check (100 per cent). But at 48 HAT, higher concentrations of CNSL *ie*., 0.1 and 0.2 % recorded cent per cent mortality and was on par with chemical check thiamethoxam 0.03 %. Thiamethoxam was reported to be an effective chemical insecticide against the sucking pests in cotton (Nagger and Zidan, 2013), okra (Ghosh *et al.,* 2016) and green gram (Sujatha and Bharpoda, 2017). In the present study CNSL was found to be as effective as chemical pesticide thiamethoxam against *A. craccivora,* though it took slightly more time to kill the insects. The lower concentrations of CNSL also yielded cent percent mortality at 72 HAT confirming its efficacy comparable to thiamethoxam. No significant difference was observed in the insecticidal action of CNSL @ 0.1 and 0.2 % against *A. craccivora*, which indicates

Table 1. Effect of different concentrations of cashew nut shell liquid emulsion on *Aphis craccivora* and *Riptortus pedestris*

Treatments		Corrected mortality per cent*							
		24 HAT		48 HAT		72 HAT		96 HAT	
		Aphis craccivora	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*
T1	0.05 % CNSL – DR	30.00 (32.99)^{h}	0.00 (0.00)^{c}	54.17 (47.39)^{c}	26.67 (30.98)^{fg}	100.00 (90.00)^{a}	65.56 (54.08)^{cd}	100.00 (90.00)^{a}	74.07 (59.47)^{de}
T2	0.06 % CNSL - DR	43.33 (41.05)^{fg}	0.00 (0.00)^{c}	70.83 (57.90)^{bc}	30.00 (33.20)^{efg}	100.00 (90.00)^{a}	68.89 (56.08)^{bcd}	100.00 (90.00)^{a}	77.78 (61.85)^{cde}
T3	0.07 % CNSL - DR	46.67 (43.06)^{fg}	0.00 (0.00)^{c}	70.83 (58.08)^{bc}	33.33 (35.20)^{def}	100.00 (90.00)^{a}	72.22 (58.30)^{bcd}	100.00 (90.00)^{a}	77.78 (61.85)^{cde}
T4	0.08 % CNSL - DR	43.33 (41.14)^{fg}	0.00 (0.00)^{c}	66.67 (55.49)^{bc}	40.00 (39.22)^{cde}	100.00 (90.00)^{a}	75.93 (60.68)^{bc}	100.00 (90.00)^{a}	81.48 (64.73)^{cd}
T5	0.09 % CNSL - DR	53.33 (46.90)^{def}	0.00 (0.00)^{c}	66.67 (54.80)^{bc}	50.00 (44.98)^{c}	100.00 (90.00)^{a}	75.93 (60.68)^{bc}	100.00 (90.00)^{a}	85.19 (67.62)^{c}
T6	0.1 % CNSL - DR	66.67 (54.76)^{bcd}	0.00 (0.00)^{c}	95.83 (83.09)^{a}	50.00 (44.98)^{c}	100.00 (90.00)^{a}	79.26 (62.89)^{b}	100.00 (90.00)^{a}	92.59 (77.00)^{b}
T7	0.2 % CNSL - DR	76.67 (61.20)^{b}	0.00 (0.00)^{c}	100.00 (90.00)^{a}	73.33 (58.98)^{b}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
T8	0.05 % CNSL - SB	30.00 (32.99)^{h}	0.00 (0.00)^{c}	54.17 (47.39)^{c}	20.00 (26.55)^{g}	100.00 (90.00)^{a}	61.85 (51.90)^{d}	100.00 (90.00)^{a}	70.37 (57.09)^{e}
T9	0.06 % CNSL - SB	33.33 (35.20)^{gh}	0.00 (0.00)^{c}	58.33 (49.81)^{c}	26.67 (30.98)^{fg}	100.00 (90.00)^{a}	68.89 (56.08)^{bcd}	100.00 (90.00)^{a}	77.78 (61.85)^{cde}
T10	0.07 % CNSL - SB	46.67 (43.06)^{fg}	0.00 (0.00)^{c}	79.17 (63.07)^{b}	33.33 (35.20)^{def}	100.00 (90.00)^{a}	70.00 (56.98)^{bcd}	100.00 (90.00)^{a}	77.78 (61.85)^{cde}
T11	0.08 % CNSL - SB	46.67 (43.06)^{fg}	0.00 (0.00)^{c}	70.83 (57.39)^{bc}	36.67 (37.21)^{def}	100.00 (90.00)^{a}	75.93 (60.68)^{bc}	100.00 (90.00)^{a}	85.19 (67.62)^{c}
T12	0.09 % CNSL - SB	50.00 (44.98)^{ef}	0.00 (0.00)^{c}	83.33 (66.17)^{b}	43.33 (41.14)^{cd}	100.00 (90.00)^{a}	75.93 (60.68)^{bc}	100.00 (90.00)^{a}	85.19 (67.62)^{c}
T13	0.1 % CNSL – SB	63.33 (52.75)^{cde}	0.00 (0.00)^{c}	100.00 (90.00)^{a}	66.67 (54.97)^{b}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
T14	0.2 % CNSL - SB	73.33 (58.98)^{bc}	13.33 (21.14)^{b}	100.00 (90.00)^{a}	66.67 (54.76)^{b}	100.00 (90.00)^{a}	96.67 (83.85)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
T15	0.6% soap solution (emulsifier)	3.33 (6.14)^{j}	0.00 (0.00)^{c}	29.17 (32.57)^{d}	0.00 (0.00)^{h}	70.83 (57.39)^{b}	0.00 (0.00)^{f}	100.00 (90.00)^{b}	11.11 (19.46)^{g}
T16	2 % Neem oil emulsion	10.00 (18.43)^{i}	0.00 (0.00)^{c}	29.17 (32.57)^{d}	3.33 (6.14)^{h}	75.00 (60.49)^{b}	7.04 (12.63)^{e}	100.00 (90.00)^{b}	22.22 (27.61)^{f}
T17	Thiamethoxam 25 WG 0.03 %	100.00 (90.00)^{a}	63.33 (52.75)^{a}	100.00 (90.00)^{a}	86.67 (68.83)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
	C.D.(0.05)	**(8.023)**	**(2.360)**	**(12.865)**	**(7.654)**	**(3.896)**	**(7.827)**	**NS**	**(7.264)**
	SE(m)	2.779	0.818	4.457	2.652	1.350	2.712	-	2.503

*Corrected with Abbot's formula over control, Mean of 3 replication comprising 20 aphids and 10 *Riptortus pedestris* nymphs each (Values in the parentheses are arcsine transformed values).
DR- Drum roasting SB- Steam boiling HAT-Hours after treatment.

Table 2. Median Lethal concentration of cashew nut shell liquid emulsions against *Aphis craccivora* and *Riptortus pedestris*

CNSL	Lethal Dose	Estimate (Per cent)		Fiducial limits (per cent)				Chi square value	
				Lower bound		Upper bound			
		Aphis craccivora	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*	*Aphis craccivora*	*Riptortus pedestris*
CNSL-DR	LC_{50}	0.079	0.095	0.066	0.082	0.091	0.109	5.325	2.849
	LC_{90}	0.250	0.275	0.198	0.221	0.356	0.381		
CNSL-SB	LC_{50}	0.084	0.102	0.071	0.088	0.098	0.119	5.898	7.530
	LC_{90}	0.275	0.334	0.216	0.261	0.394	0.479		

*CNSL- cashew nut shell liquid, DR- Drum roasting, SB- Steam boiling

the suitability of the lower dose (0.1 per cent) for management of *A. craccivora*. The pesticidal efficacy of CNSL against chilli aphid, *Aphis gossypii* was reported earlier by Sundaran and Faizal (2018) where CNSL @ 0.2 % caused cent per cent mortality at 72 HAT.

The bioefficacy of CNSL against *R. pedestris* indicated that at 48 HAT, the higher concentrations of CNSL tried *viz*., 0.2 % of both CNSL-DR and SB and 0.1 % CNSL-SB produced significantly higher mortality of 73.33, 66.67 and 66.67 respectively, though inferior to chemical check (86.67 per cent). Lower concentrations registered a mortality ranging from 20 to 50 per cent. But at 72 HAT, the mortality obtained in 0.1 and 0.2 % CNSL-DR and CNSL-SB (96.67 to 100 per cent respectively) was comparable with that of chemical check thiamethoxam. Similar trend was noticed at 96 HAT also, wherein cent per cent mortality was noticed in 0.1 and 0.2 % emulsions of both CNSL-DR and CNSL-SB as against a much low mortality of 22.22 per cent observed in conventional botanical pesticide neem oil. The results indicate the suitability of CNSL as plant derived insecticide against heteropteran sucking pests.

The main constituents of nut shell liquid of cashew are anacardic acid (60-65 %), cardol (15-20%), cardanol (10%) and traces of methyl cardol (Tyman *et al*., 1987). Since high temperature during processing is likely to decarboxylate thermally unstable anacardic acid, the composition of technical CNSL vary depending up on the processing conditions (Trevisan *et al*., 2005). Hence CNSL obtained from two widely employed processing methods *viz*., drum roasting and steam boiling (CNSL-DR and CNSL-SB), which vary considerably in the processing temperature was tested in this study to document difference if any in their insecticidal property. No significant difference was observed between the same concentrations of CNSL-DR and SB against the test insects indicating that insecticidal property of CNSL was unaffected by the difference in processing of cashew nuts. Cashew industry in Kerala mainly employs drum roasting method of processing and CNSL- DR is available in plenty at a cheaper rate.

Several malformations, possibly related to defective molting like nymphal adult intermediary formation was observed in *R. pedestris* that survived CNSL treatments. This point to the possible insect growth regulatory action of CNSL. Similar insect growth regulatory action was earlier reported by Zanuncio *et al*. (2016) for botanicals like neem oil at higher doses against predatory stink bug, *Podisus nigrispinus*. Dorn *et al*. (1986) reported insect growth regulatory action of azadirachtin on *Oncopeltus fasciatus* Dallas (Hemiptera: Lygaeidae) due to its effect on JH biosynthesis and catabolism.

The present study indicates the suitability of CNSL as a plant derived insecticide for the management of heteropteran, *R. pedestris* also, wherein a

comparatively higher dose was required and took slightly more time to achieve significant mortality than the homopteran, *A. craccivora.* CNSL @ 0.2 % was required to get an effect comparable to chemical pesticide against *R. pedestris* as against a dose requirement of 0.1 % against *A. craccivora*. The LC_{90} values of CNSL- DR against *A. craccivora* and *R. pedestris* was computed as 0.25 and 0.275 respectively in the dose mortality response studies. Since the field dose is normally fixed above LC_{90} value, CNSL @ 0.275 % or above need to be fixed for the field management of sucking pests complex. The toxicity of cashew nut shell extract against sucking pests was earlier indicated in the study of Oparaeke *et al*. (2005), where extracts of cashew nut shell + garlic bulb and cashew nutshell + African pepper mixed in the ratio of 10:10 % w/w were found effective in reducing the pod sucking bugs. Andayanie *et al*., 2019 evaluated the efficacy of hexane extract of cashew nut shells against white fly, *Bemesia tabaci* wherein a concentration of 0.75 % was reported to have antifeedant and anti oviposition activity besides causing mortality. CNSL up to 3.00 % did not cause any phytotoxic effect. Though the highest mortality of *B. tabaci* was obtained with 6.00 % CNSL, it caused phytotoxic symptoms on soybean leaves.

CNSL irrespective of concentration tested produced significantly superior mortality against both homopteran and heteropteran pests than the widely exploited botanical pesticide neem oil 2% indicating the potential of CNSL as an alternative plant derived insecticide for the management of sucking pests. Olotuah and Ofuya (2010) based on a screen house standardisation fixed a dose of 1% ethanolic extract of CNSL which when evaluated in the field conditions were found to yield more pod protection of cowpea than cypermethrin treatment from the attack of *A. craccivora* and *Maruca testulalis*. Present study reveals the possibility of utilising the technical CNSL obtained from cashew processing industry at a much lower dose of 0.2 % for the management of sucking pests of cowpea. Hence further field investigations on the effectiveness of CNSL need to be explored considering the phytotoxicity aspects. The toxicity of CNSL was earlier documented against chewing pests *viz*., coconut root grub (John *et al*., 2008), *Helicoverpa armigera* and *Spilarctia obliqua* (Mahapatro, 2011). The pesticidal property of CNSL is attributed to the presence of the phenolic compounds; cardanol and cardol (Venmalar and Nagaveni, 2005).

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Temporal variation of mayfly community (Ephemeroptera) in response to ecological attributes in Gadana river, Tamilnadu, India

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ABSTRACT: In the study of diversity and distribution of Ephemeroptera in Gadana River and relationship between Ephemeropteran community and ecological factors, revealed a total of 2056 specimens belonging to 25 genera and 7 families. The diversity and distribution of Ephemeroptera were higher during the rainy season, contrasted with non-rainy period. The high scores of Shannon index and Simpson's index indicate that Gadana River is hale and healthy and it bolsters more diverse taxa. The pH values accomplish greatest range in the months of January and August; it legitimately impacts on diversity of mayflies. Leptophlebiidae and Baetidae were the most ubiquitous families present in the Gadana River. Canonical Correspondence Analysis (CCA) shows that rainfall, pH, DO and water temperature were to be the significant stressor in altering the community structure of mayflies.

Keywords: Ephemeropteran community, rainfall, pH, Canonical Correspondence Analysis, Simpsons Index, Shannon Index

INTRODUCTION

Ephemeropterans also called as mayflies, serves as a bioindicators of water quality. The aquatic larval stages, namely the naiads undergo a series of moults as they grow, the precise number being variable within a species, depending on external factors, such as temperature, food availability and current velocity. They are important components of aquatic assemblages in freshwater environments due to their high abundance and richness and therefore have an important role in nutrient cycling, since they process large amounts of organic matter from the riparian vegetation and periphyton in the aquatic environment (Moulton *et al*., 2004). So they serve as very good indicators of conservation importance and of centres of endemicity and they can be used to identify significant localities at much smaller scale than those identified by studies on vertebrates. Mayflies are very touchy to contamination and can accordingly just be discovered near water that is of a high calibre. The diversity and distribution of aquatic invertebrates in fresh water ecosystem are determined by environmental variables such as

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rainfall (Kim *et al*., 2013), current velocity, water temperature, dissolved oxygen (Bueno *et al*., 2003), rainfall (Bispo *et al*., 2004), conductivity (Scheibler and Debandi, 2008), depth (Mollozzi *et al*., 2011) and the type of substrate (Buss *et al*., 2004). These variables show spatial and temporal variability and are consequently expected to drive auxiliary changes in lotic invertebrate communities.

Gadana river which resides in southern Western Ghats has already been explored to biomonitoring studies. It is based on the diversity and distribution of family Baetidae (Kubendran *et al.,* 2017 a). Aim of the study was on the diversity and distribution of all families of Ephemeroptera present in Gadana river and to assess the relationship between Ephemeropteran community and environmental variables.

MATERIALS AND METHODS

Study area: Gadana River originates from Agasthyamalai Biosphere Reserve in southern Tamil Nadu part of Western Ghats of India. Three tributaries such as Pambar, Kallar and Iluppaiyar join to form the Gadana River. It is a 33 km long, drains about 7112 acres which gets together witha Major River called Tamiraparani near Thiruppudai Marudur village in Ambasamudram taluk, Tirunelveli district, south Tamil Nadu. It has its origin in the Sivasailam peak of Western Ghats at an elevation of 1564 m above M.S.L. at a Lat. 81°48' N and Long. 77° 19' E and flows down the eastern slopes of Western Ghats. The stream has well developed riffles, pools, cascades and runs. Channel substrates are bedrock, boulder, gravel, cobble and sand covered with leaf litter. Banks of this stream are exceptionally unstabilised by coconut farms and agricultural lands. Random sampling was carried out in three study sites of Gadana river. Site I is upstream of river, site II is near temple path way and site III is near Dam outlet which are closely associated with cultivated land and polluted human inhabiting area. Samplings were done from August 2018 to January 2019.

Measuring water quality parameters: The physicochemical parameters of stream water such as pH, water temperature, air temperature, dissolved oxygen and water flow were analysed for every month by using the guidelines of APHA (2005). The mean rainfall data of different months were collected from meteorological data.

Ephemeroptera collection: The Mayflies were quantitatively sampled by using 1m wide Kick-net (Burton and Sivaramakrishnan, 1993) and surber sampling. The organisms were then carefully picked from the net surface and were preserved immediately in 70% ethyl alcohol. These samples were transported to the laboratory for further processing and identification was done using stereomicroscope (Magnus MSZ-TR).

Laboratory sorting, identification and enumeration: All samples from the river were identified with the help of field guide by Sivaramakrishnan *et al*. (1998) and by other taxonomic literatures (Balachandran and Ramachandra, 2011; Sivaramakrishnan *et al.,* 2009).

Shannon and Simpson indices and Canonical Correspondence Analysis (CCA) were calculated with the help of PAST software (Hammer *et al.,* 2001).

RESULTS AND DISCUSSION

Sampling of larva of Ephemeroptera in Gadana river from August 2018 to January 2019 resulted in a total of 2056 specimens belonging to 25 genera and 7 families (Table 1). The genera are listed in table 1 which includes maximum of 25 genera in the months of September, October, November and December, whereas in the month of January only 20 genera were noted.

Species richness and Abundance were higher in the month of November followed by October and December it coincides with North East Monsoon period and it yields about 641.4mm rainfall. This shows that during the rainy seasons, the diversity and distribution of Ephemeroptera were higher compared to non-rainy period (January which has low abundance). It is likewise clear that South West Monsoon yields only less diversity and distribution compared to North East Monsoon (October to December), since Western Ghats regularly receives higher rainfall during the North East Monsoon

period as opposed to to South West Monsoon. So this study revealed that Ephemeropteran community is directly in relationship with the high amount of rainfall, as their diversity and distribution continues expanding during the rainy period.

The Shannon index value normally lies between 0.0 – 5.0 and it exceeds 4.5 very rarely. Indices values above 3.0 indicate that structure of habitat is stable or balanced. The values under 1.5 indicate that ecosystem is broken or degradation in structure of habitat. Shannon index (Fig. 2) was highest in October 2018 (3.095) and lowest in January 2018 (2.614). Simpson's index (Fig. 1) was also supports the results of Shannon index and the index was highest in October 2018 (0.951) and lowest in August (0.905). Both the index values which show that Gadana river which have more stable ecosystem in the months of September to December and it have less diversity in the months

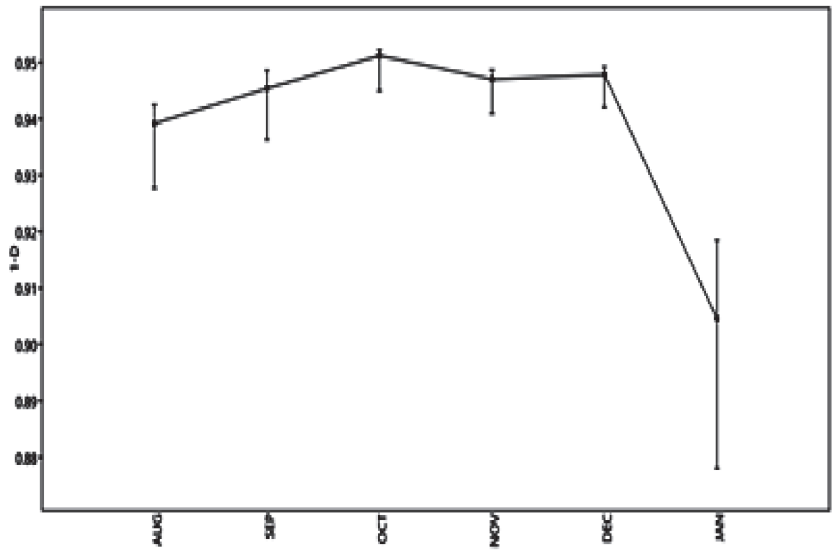

Fig. 1: Simpson index of mayflies in different months

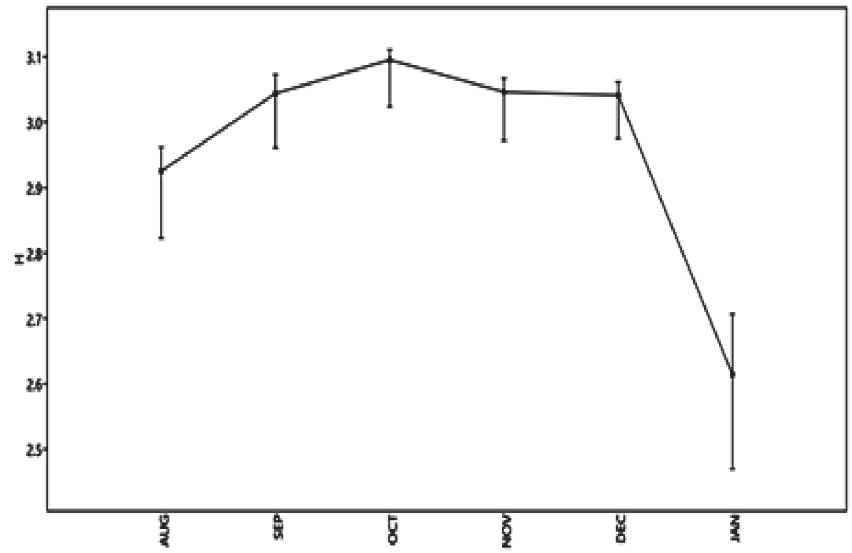

Fig. 2: Shannon index of mayflies in different months

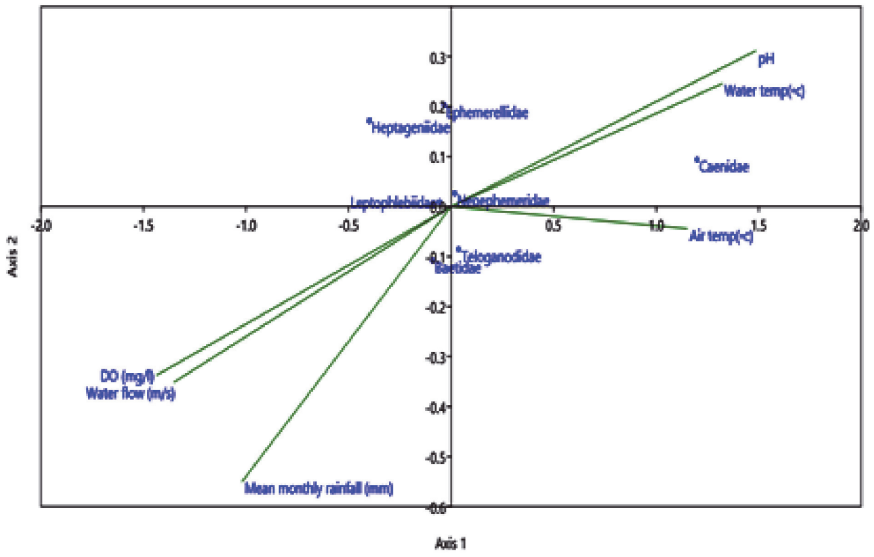

Fig. 3 Canonical Correspondence Analysis (CCA) of mayfly community and ecological attributes in Gadana river

of low rainfall months like August and January but that it also of less concern. The high scores of Shannon index and Simpson's index, indicate that Gadana river is healthy, clean or unpolluted river and it supports more diverse taxa

Water temperature and air temperature varied moderately among months and it was higher in the month of January (22æ%c and 24æ%c). The water flow in the month of January was found to be 0.21 m/s which were very much less compared to other months. During November, the water flow was high which supported more diverse taxa. Normal dissolved oxygen (DO) level in the running water was 4.6 – 8.6 mg/l (Srinivasan *et al.,* 2019) and in all the months it falls in the normal range. The pH values attain maximum range in the months of January (7.9) and August (7.4) and found to be alkaline in these months and this is due to anthropogenic activity during that months (Table 2). Due to this the Ephemeropteran community gets affected and which supports only more tolerant species like Caenis sp.

Leptophlebiidae and Baetidae were the most numerous and ubiquitous families, comprising seven and eight species respectively(Table 1).

Canonical Correspondence Analysis (CCA) predicts relationships between Ephemeropteran communities and environmental variables and it allows integrated analysis of taxa and environmental attributes (Ter Braak and Smilauer, 2002). From the CCA results (Fig. 3), it is clear that family Caenidae which shows positive relationship with high levels of pH and water temperature and it is adversely associated with high levels Dissolved Oxygen, rainfall and water flow though other families' shows negative correlation with elevated levels of pH and water temperature. High rainfall in the months of October, November and December which underpins families like Heptageniidae, Ephemerellidae and Baetidae whereas it have negative correlation with family Caenidae. From the previous investigations in southern India (Selvakumar *et al.,* 2014), it is apparent that family Caenidae is a poor indicator of water quality. Our outcomes also substantiates with that. So CCA results showed that mayflies were significantly associated with ecological attributes in Gadana River. Results additionally anticipated that rainfall, pH, DO, water flow and water temperature turns into the significant components in administering the community structure of mayflies. The prior investigations in Western Ghats uncover that pH

Table 1. List of Ephemeroptera collected in Gadana river

FAMILY	GENUS/ SPECIES	Number of individuals collected in different months					
		AUG	SEP	OCT	NOV	DEC	JAN
Baetidae	*Baetis acceptus*	12	14	22	27	26	05
	Centroptella similis	11	11	14	13	18	03
	Cloen bimaculatum	13	10	16	09	15	04
	Indobaetis michaelohubbardi	08	11	16	14	17	02
	Labiobaetis pulchellus	01	01	07	04	03	00
	Acentrella (Liebebiella) vera	01	01	01	03	03	00
	Tenuibaetis frequentus	05	08	14	15	16	00
Caenidae	*Caenis sp*	24	15	07	04	02	29
	Clypeocaenis bisetosa	13	11	09	08	02	18
Ephemerellidae	*Torleya nepalica*	08	09	04	09	11	01
Heptageniidae	*Afronurus kumbakkaraiensis*	04	22	17	23	34	01
	Epeorus petersi	00	12	10	15	29	00
	Thalerosphyrus flowersi	00	24	18	29	24	00
Leptophlebiidae	*Choroterpes (Euthraulus) alagarensis*	21	34	28	37	34	12
	Choroterpes (Euthraulus) nambiyarensis	12	39	25	31	33	11
	Edmundsula lotica	03	12	11	11	07	01
	Indialis badia	12	11	20	23	18	02
	Isca purpurea	11	17	23	33	19	05
	Nathanella saraswathiae	07	12	15	19	19	02
	Notophlebia jobi	03	10	16	17	21	04
	Thraulus gopalani	08	08	18	24	23	07
Neoephemeridae	*Potamanthellus caenoides*	02	09	10	08	09	04
Teloganodidae	*Teloganodes sartorii*	19	23	31	37	21	10
	Teloganodes indica	11	15	28	39	37	09
	Dudgeodes palnius	10	14	21	26	21	13
	Total number of individuals	219	353	401	478	462	143

and DO turn into the crucial component in controlling the population dynamics of mayflies (Selvakumar *et al.,* 2012; Kubendran *et al.,* 2017 b). Our outcomes additionally validates with that as well. Beyene *et al.* (2008) found that rainfall turns into the significant part in mayfly diversity and they recorded richer diversity of macroinvertebrates in the wet season in an Ethiopian highland river and this work additionally records a similar outcome. This work provides stable information on the present status of water quality and temporal variations in reference to community structure of mayflies in Gadana river and serves as a model ecosystem for the biomonitoring studies.

Table 2. Physico-chemical parameters of the Gadana river

PARAMETERS (Mean Values)	AUG	SEP	OCT	NOV	DEC	JAN
Water flow (m/s)	0.34±0.09	0.53±0.11	0.68±0.17	0.76±0.16	0.57±0.07	0.21±0.04
pH	7.4±0.1	7.2±0.2	7.1±0.1	7.0±0.0	7.0±0.3	7.9±0.2
Air temp(æ%c)	24±1.2	23±0.8	23±0.7	21±0.8	22±1	24±1.2
Water temp(æ%c)	21±1	20±0.6	19±0.8	17±0.8	18±0.9	22±0.9
DO (mg/l)	8.2± 0.1	8.6± 0.2	8.9± 0.1	8.7± 0.1	8.7± 0.2	7.8± 0.2
Mean monthly rainfall (mm)	17.32	92.1	254.3	283.7	103.4	12.6

Table 3. Correlations of environmental gradients with the axes of canonical correspondence analysis (CCA) in Gadana river

VARIABLES	AXIS 1	AXIS 2
Eigenvalue	0.0779	0.0178
Water flow	**0.0329**	**0.1012**
pH	2.2732	1.9390
Air temp	2.2797	1.8036
Water temp	2.5323	2.1199
DO	1.8165	1.3216
Rainfall	**-1.5381**	1.1417

(Bold values indicates significant differences)

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Parasitism potential of *Diadegma argenteopilosa* (Cameron) (Hymenoptera : Ichneumonidae), an internal larval parasitoid of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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ABSTRACT: *Diadegma argenteopilosa* (Cameron) (Ichneumonidae: Hymenoptera) is an internal larval parasitoid of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera), a notorious and polyphagus pest of pulses and vegetables in India. Attempt has been made to initiate their mass multiplication for successful biocontrol programme. Behavioral studies, food stuffs, host selection aspects plays a crucial role in mass multiplication of biocontrol agents. Therefore, present work was conducted to study the optimum host age, specificity and host density for maximum progeny production of the parasitoid under laboratory conditions and later their release in the field for the management of pest species. The parasitoid caused highest mortality in the pest larvae of second instars, 4 day old larvae were attacked most with high percent parasitism, 39.00%. Optimum density for maximum progeny production of *D. argenteopilosa* was 20, which generate maximum parasitism (43.00%). Host specificity by exposing the parasitoids towards different host species and analyse parasitoid preference by *S. litura* > *Helicoverpa armigera* (Hubner) > *Mythimna separata* Walker > *Achaea janata* (Linnaeus). Nutritional requirement of parasitoid was tested with different foodstuffs and found 50% honey best suited for maximum longevity 8.2 and 11.4 days for males and females respectively. The longevity ratio also female biased, 1: 1.39 (Male: Female). From the results it concludes that *D. argenteopilosa* fed with 50% honey solution, exposed to 3-5 day old caterpillars of *S. litura* at density of 20 gave maximum progeny production and effectively utilized in the biocontrol programme.

KEY WORDS: Parasitoid, potential, biocontrol, pest management.

INTRODUCTION

Biological pest management is a complementary approach in devising a robust pest management strategy. Development in pest management strategies improves the status of farmer community. Farmers are facing problem of pests and diseases of crop plants. Long ago the pests and diseases could be controlled with environmental factors but then it shifted to era of chemical control. Due to pesticidal residue, pest resistance, pest resurgence, cost of sprays, lack of labor, secondary pest outbreak and phytotoxicity of pesticides the farmers now deliberately moved towards biological control of agricultural pests. Biological control of different pests with biocontrol agents enhance the crop yield and also improve the quality of produce. The above fact clearly indicates that there is extreme need of

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minimizing pesticidal use. Therefore, attempts have been made with the use of biocontrol agents for effective control of agricultural pests as ecofriendly control measure.

Armyworm *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera) is a polyphagous pest of agricultural crops in India. Several management practices were implemented to achieve the control of the pest, *viz.* biological, chemical, mechanical, cultural etc. Among biocontrol agents *Diadegma argenteopilosa* (Cameron) (Ichneumonidae: Hymenoptera) is most common and potent internal larval parasitoid. Host searching and selection of host density by parasitoid counts the success of biocontrol programme for any pest species (Bhosale, 2018). The high percent of parasitism is desirable character of an ideal parasitoid. In mass production and colonization of parasitoids in biocontrol strategies *viz.* shape, size, nutritional suitability and host age plays very important role (Vinson, 1976; Vinson and Iwantsch, 1980).

Leong and Oatman (1968), Lewis and Vinson (1971), Lingren and Nobel (1972), Romeis and Shanower (1996), King (1998), Wackers (2001), Eliopoulos (2007), Dhillon and Sharma (2007), Sathe and Bhosale (2011), Khatri *et al.* (2012), Sathe *et al.* (2012), Han *et al.* (2013), Bhosale and Bhosale (2019) made investigations on optimum age, density and specificity of hosts and nutritional requirement of ichneumon parasitoids. The present study was carried with *D. argenteopilosa*, an internal larval parasitoid of *S. litura* to find out the optimum age of host for obtaining maximum progeny of parasitoids, which will help in mass rearing and field release for effective biocontrol program.

MATERIALS AND METHODS

Rearing of host species

S. litura larvae were reared in small perforated plastic container (7x8 cm, Diameter x Height). After adult emergence they may transferred in oviposition cage 25x25x25 cm (LxWxH). First instar caterpillars usually hatch after 2 days from oviposition. These larvae were collected and further used for experiment. During the course of study, the host caterpillars were fed with castor (*Ricinus communis* L.) leaves. Similarly, the other host species used to conduct the host specificity experiment were reared their natural food like, *Helicoverpa armigera* (Hubner) on gram and *Achaea janata* (Linn.) on castor leaves and *Mythimna separata* Walker on leaves of maize *Zea mays* L.

Rearing of parasitoid

Adults of *D. argenteopilosa* were reared in ventilated wooden cage (30x30x30 cm, LxWxH). *D. argenteopilosa* are very minute and are negatively geotropic, hence cages must be made with glass walls on three sides and top of the cage while one wall was made up of very fine mesh cloth for proper handling of parasitoids. The adults of *D. argenteopilosa* were fed with 50% honey solution. Adults of parasitoids released for oviposition in the rearing cages for 24 h with different age and densities of *S. litura* caterpillars. After 24 h, adults were removed and hosts reared for further analysis. The cocoons of parasitoids then transfer into separate container and adults of *D. argenteopilosa* emerges out that can be used for experimental purpose.

Nutritional requirements and adult longevity of parasitoid on different foodstuffs

Emerged adults of *D. argenteopilosa* are fed with different foodstuffs to analyze the ideal feed for getting highest longevity. Honey acts as natural food for any parasitoid, hence three concentrations of 100, 50 and 10 percent were made to analyze the longevity of parasitoid. However, 50 percent glucose and sucrose; and juice of citrus and apple can also provide as a food for study.

Effect of host age on parasitism

To determine the effect of host age on parasitism, 20 larvae of *S. litura* of known age (ranging from less than 1 day to 13 days old) were exposed to single mated female of *D. argenteopilosa* in a glass cage for 24 hrs. The larvae were removed and placed in separate containers for further observations. Daily records of cocoon construction and parasitoid emergence from each container were observed.

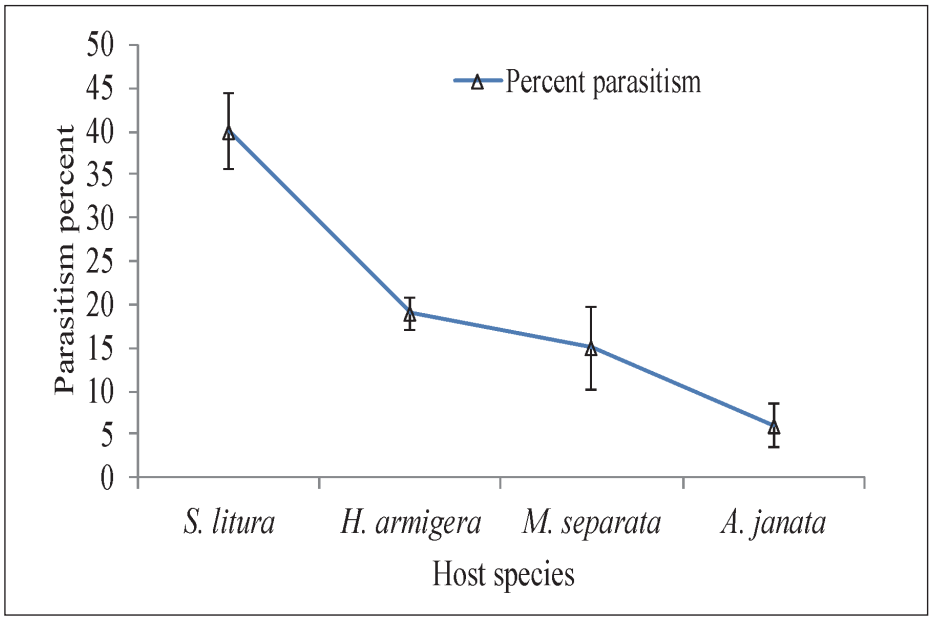

Fig. 1 Host specificity of *D. argenteopilosa*
*Each value is the mean of five replicates with error bars indicating standard error of mean (SEM).

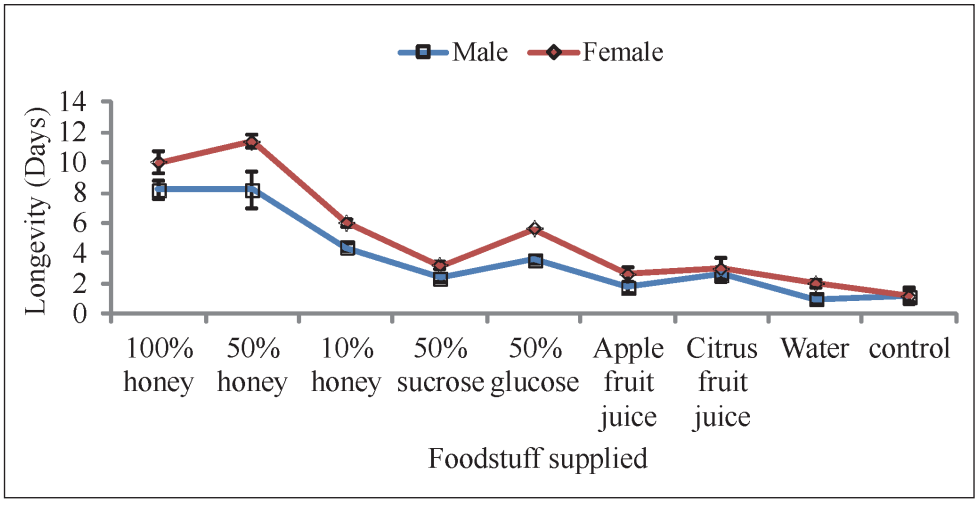

Fig. 2 Adult longevity of *D. argenteopilosa* with different foodstuffs
*Each value is the mean of five replicates with error bars indicating standard error of mean (SEM).

Host density for optimum parasitization

S. litura caterpillars (4-5 day old) were exposed in densities of 10, 20, 30, and 40 towards mated females of *D. argenteopilosa* for 24 hrs in oviposition cage 25x25x25 cm (LxWxH). The host larvae were reared into plastic containers to record further development or parasitoid emergence.

Host specificity for optimum parasitization

Host specificity was conducted by exposing the mated females of parasitoid towards caterpillars of different host species like *S. litura*, *H. armigera*, *M. separata* and *A. janata*. The hosts were placed in the oviposition cage for 24 h. Hosts were released in 20 densities to record optimum

parasitism. Afterwards the hosts were reared on the natural diet and observe the emergence of parasitoid or further lifecycle of host species.

The experiments were carried out at 25±2°C, 60±5% RH and 12hr photoperiod. During the course, castor leaves were provided as a food to the caterpillars of *S. litura* and other appropriate food for other experimented host species, while the parasitoids were fed with 50% honey solution. Each experiment was repeated 5 times for confirming the result. The statistical analysis was made by one way ANOVA using the statistical software package SAS 9.3(32) English. The percent values were transformed to arcsine values before analysis.

RESULTS AND DISCUSSION

D. argenteopilosa was most effective in controlling the caterpillars of *S. litura* than other biocontrol agents associated with particular pest. The results of host specificity experiment (Fig. 1) revealed that the parasitoid prefer *S. litura* as the primary host with 42.00% parasitism. Among tested hosts, parasitoid showed 19.00 percent parasitism for *H. armigera*, 15.00 percent parasitism for *M. separata* and 6.00 percent parasitism for *A. janata*. The order of preference for parasitism shown by the parasitoid was *S. litura*> *H. armigera* > *M. separata* > *A. janata*. Adult longevity of *D. argenteopilosa* with different foodstuff were analyzed and plotted (Fig. 2.) Parasitoid survived longer with 50% honey solution with maximum male: female longevity ratio (1: 1.39). The maximum longevity of male and female when fed with 50% honey solution was 8.2 and 11.4 days respectively. Hence, it could be best suited for mass rearing of parasitoid in the laboratory. The parasitoid caused highest mortality in the second instar caterpillars (Table 1). The caterpillars of 3-6 days old are preferred for parasitism whereas, beyond 12 days old were not attacked by the parasitoid. Four day old caterpillars were attacked most with high percent parasitism (39.00%).

The results of optimum host density for maximum progeny production of parasitoid showed that the number of parasitoids obtained from host density 20 was highest with 43.00 percent parasitism,

Table 1. Host age related parasitism by *D. argenteopilosa*

Host age (days)	% Parasitism	% Mortality	% Moth emergence
1	4.00 (±2.20)^{ef}	7.00 (±3.70)^{a}	89.00 (±2.50)^{ab}
2	10.00 (±3.70)^{de}	8.00 (±1.20)^{a}	82.00 (±3.70)^{abcd}
3	23.00 (±3.70)^{abc}	8.00 (±1.00)^{a}	69.00 (±3.00)^{de}
4	39.00 (±2.50)^{a}	9.00 (±2.90)^{a}	52.00 (±1.90)^{e}
5	28.00 (±3.70)^{ab}	5.00 (±2.50)^{a}	67.00 (±4.60)^{de}
6	23.00 (±3.40)^{abcd}	9.00 (±1.20)^{a}	68.00 (±2.70)^{cde}
7	13.00 (±0.00)^{bcd}	11.00 (±5.10)^{a}	76.00 (±5.10)^{bcd}
8	11.00 (±1.90)^{cde}	8.00 (±3.40)^{a}	81.00 (±1.90)^{abcd}
9	10.00 (±1.90)^{cde}	9.00 (±3.30)^{a}	81.00 (±4.60)^{abcd}
10	3.00 (±1.20)^{ef}	10.00 (±3.30)^{a}	87.00 (±2.40)^{a}
11	3.00 (±2.00)^{ef}	7.00 (±2.70)^{a}	90.00 (±2.50)^{abc}
12	0.00 (±0.00)^{f}	11.00 (±4.00)^{a}	89.00 (±4.00)^{a}
13	0.00 (±0.00)^{f}	6.00 (±3.40)^{a}	94.00 (±3.40)^{ab}
CD (P=0.05)	12.88	18.12	12.75

*The data presented are the mean of five replicates. Different letters indicate the significant difference (One way ANOVA) P<0.05 Tukey's standardized range (HSD) test. Figures in parentheses are standard error of mean (SEM).

Table 2. Host density dependent parasitism by *D. argenteopilosa*

Host density	% parasitism	% Mortality	% Moth emergence
10	29.00 (±1.61)[b]	31.00 (±0.24)[a]	40.00 (±1.61)[a]
20	43.00 (±1.12)[a]	18.00 (±1.47)[a]	39.00 (±2.38)[b]
30	35.67 (±0.84)[a]	15.33 (±0.61)[a]	49.00 (±1.14)[ab]
40	27.80 (±0.87)[ab]	14.60 (±0.30)[a]	57.60 (±1.16)[ab]
50	22.00 (±0.43)[ab]	15.50 (±0.37)[a]	62.50 (±0.64)[ab]
CD (P=0.05)	12.58	8.08	11.99

*The data presented are the mean of five replicates. Different letters indicate the significant difference (One way ANOVA) P<0.05 Tukey's standardized range (HSD) test. Figures in parentheses are standard error of mean (SEM).

compared to those produced from other host densities 10, 30 and 40 with 29.00, 35.67, 27.80 and 22.00 mean percentage of parasitism (Table 2).

Bhosale and Bhosale (2019) reported the host density 20 of *Plutella xylostella* (L.) (Plutellidae: Lepidoptera) for obtaining maximum progeny production (41.00%) of the parasitoid *Diadegma insulare* (Cameron). Likely, Sathe and Bhosale (2011) reported the host density 100 for obtaining maximum progeny production (38.50%) of the parasitoid *D. insulare*. Similarly, Cardona and Oatman (1971), reported 90 host density of *Keiferia lycopersicella* (Walsinghum) as optimum number for maximum parasitism by *Pseudapanteles* (=*Apanteles*) *dignus* Muesebeck. In *P. diguns*, they reported the percentage of parasitization increased with the increase in number of hosts (30, 60 and 90) up to host density 90 per replicate. A decrease in parasitization observed in all replicates when 120 larvae were offered. In present study percent parasitism was found decreasing beyond 20 host density that suggesting the suitability of the larval number. In present findings the parasitoid preferred *S. litura* later *H. armigera*, *M. separata* and then *A. janata*. Similarly, Pawar *et al.* (1989), studied the parasitism of *C. chlorideae* on *H. armigera*, they found average percentage parasitism of first to third instar larvae, which are only parasitised by parasitoid, parasitism found on associated crop was 44·2 on sorghum, 33·1 on chickpea, 32·6 on pearl millet, 7·1 on groundnut and 4·2 on pigeon pea.

Lingren *et al.* (1970) stated the host age preference of *C. chlorideae* towards four lepidopterous host species *Prodenia ridinia* (Craner), *Prodenia praefica* Grote, *Trichopulsia ni* (Hubner) and *Pseudoletia unipuncta* (Hawarth). They reported that caterpillars 1-8 day old of all hosts were susceptible for parasitism, 2-6 day old being most acceptable. In present findings 2-9 day old caterpillars of *H. armigera* were susceptible, 3-7 day old caterpillars readily accepted and 4-5 day old being most suitable for parasitism. Nikam and Basarkar (1981) studied the reproductive potential of *C. chlorideae* and reported maximum parasitization at host density 40. In present findings 20 host density shows maximum parasitism (43.00%). Eliopoulos (2007), studied the importance of food supplements for *Venturia canescens* ichneumon parasitoid of stored product pests and found honey is the best supplement for *in-vitro* parasitoid rearing.

D. argenteopilosa has been successfully initiating the biocontrol program for managing the *S. litura*. Parasitoid can be mass reared in laboratory scale on 50% honey solution. For getting maximum progeny of parasitoid exposed towards second instar *S. litura* caterpillars with 20 host density. The mass rearing of parasitoid *D. argenteopilosa* may initiate the biocontrol programme for *S. litura*.

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Intrinsic rate of natural increase of an isochnoceran louse *Goniocotes jirufti* (Ansari, 1947) (Insecta: Phthiraptera)

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ABSTRACT: The ischnoceran lice, *Goniocotes jirufti* (Ansari, 1947) infesting the black partridge, *Francolinus francolinus* were reared *in vitro* condition (35 ± 1°C, 75-82% RH, at feather diet), to record the incubation period, adult longevity and daily egg rate. The data obtained from *in vitro* experimentation were used to construct the life table and to determine the intrinsic rate of natural increase (rm). The value of rm of aforesaid species was computed as 0.042. At this rate the doubling time of its population appeared to be 16.50 days. In comparison to the other species studied so far, *G. jirufti* seems to breed moderately.

KEY WORDS: *In vitro,* biotic potential, ischnocera lice, black partridge

INTRODUCTION

The intrinsic rate of natural increase is referred as the rate of increase per head of a population under specific physical conditions. Different authors have given, different names to intrinsic rate of natural increase i.e. Chapman (1931) referred it is a biotic potential; Stanley (1946) called it as environmental index. The intrinsic rate of natural increase of twelve avian ischnocera e.g., *Brueelia amandava* Rekasi, 2005 parasitizing red munia, *Amandva amandva* L. (Gupta *et al.* 2007); *Brueellia cyclothorax* Burmeister 1838 from house sparrow, *Passer domesticus* L; *Sternoedoecus bannoo* Ansari 1955 from common myna, *Acridotheres tristis* L; *Neopsittaconirmus elbeli* Guimaraes 1974 parasitizing Indian parakeet, *Psittacula eupatria* L; *Columbicola columbae* Linnaeus, 1758 from rock pigeon, *Columba livia* G.; *Anaticola crassicornis* (Scopoli, 1763) from Mallard duck, *Anas platyrhynchos* L (Saxena *et al*., 2009); *Brueelia plocea* Lakhsminarayana 1968 from common baya, *Ploceus philippinus* L. (Arya *et al*., 2009); (*Goniocotes gallinae* De Geer 1778 parasitizing domestic fowl, *Gallus gallus domesticus* L. (Saxena *et al.,* 2007); *Upupicola upupae* Shrank from common hoopae, *Upupa epops* (Agarwal *et al.,* 2011); *Columbicola bacillus* Giebel 1866 parasitizing Eurasian collared dove, *Streptopelia decaocta* F. 1838 (Singh *et al.,* 2012), *Lipeurus caponis* Linneus 1758 parasitizing Domestic fowl, *Gallus gallus domesticus* (Kumar and Hasan, 2016) have been noted on the basis of data obtained through *in vitro* experimentation. The value of intrinsic rate of natural increase of three mammalian lice (sheep louse, *Bovicola ovis* Schrank 1781, rodent louse, *Geomydoecus oregonus* Price & Emerson 1971 Goat biting louse, *Bovicola caprae* Gunlt 1843) have also been indicated by the workers (Murray and Gordon,

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1969; Rust ,1974; Rashmi *et al.,* 2010). Since, the values of 'rm' of the species studies so far, varied considerably. Hence, it was found worthwhile to work out the life table statistics of one more ischnoceran louse. In the present paper, an attempt has been made to compute the intrinsic rate of natural increase of *Goniocotes jirufti* Ansari 1947 infesting black partridges, *Francolinus francolinus,* on the basis of data obtained through *in vitro* experimentations.

MATERIALS AND METHODS

Some feathers bearing fresh eggs were gently cut from black partridges, *Francolinus francolinus* the host body and incubated in culture vials (at 35±1°C, 75-82% RH), to record the incubation period. The humidity was maintained in culture vials by placing 50-100 m.l. of saturated solution of salts (Witson and Bates, 1960). Freshly emerged nymphal instars were reared on the host feather diet, to determine the duration of three nymphal instars. Likewise, the colonies of apparently freshly moulted healthier adult lice were reared *in vitro* condition (in batches) to determine the adult longevity. Culture vials were examined daily.

The data obtained from *in vitro* experimentation were used to construct the life table and compute the intrinsic rate of natural increase, rm ($e^{-rmx}lxmx=1$; where e=base of natural logarithms; x = age of individuals in days; lx = number of individuals alive at age x as a proportion of one; mx = number of female offspring produced/ female in the age interval x), net reproductive rate (Ro= lxmx), the innate capacity of increase (rc= logeRo/ Tc), the precise generation time (T= loge Ro/ rm), the finite rate of increase (=erm) and the doubling time of population (DT= log2/ log Σ) on the lines suggested by Evans and Smith (1952), Howe (1953) and also followed by Saxena *et al*. (2007, 2009), Gupta *et al.* (2007) and Arya *et al*. (2009).

RESULTS AND DISCUSSION

The mean incubation period of the eggs appeared to be 5.70 ± 0.95 days (range, 4-8 days, n=118). The average duration of first, second and third instar nymphs ranged from 5.61 ± 0.77 days (range, 4-days, n=106), 5.67 ± 0.88 days (range, 4-7 days, n=93), 5.41 ± 0.82 days (range, 4-7 days, n=46) respectively (Fig. 1). The average adult life span of males and females was found to be (15.52 ± 6.66 days (range, 2-26 days, n=150, 16.64 ± 7.66 days (range, 2-30 days, n=150) (Fig. 2, 3).

The life table was constructed on the basis of lines suggested by the aforesaid workers. Studies on population structure of *G. girufti* indicated that male, female ratio in natural population is 1:1.35. Thus, maternal frequency (mx = average number of female egg produced) was determined by multiplying the daily average egg rate by a factor of 0.57. While preparing the survivorship table, it was assumed that all the eggs laid were fertile and the nymphal mortality (larval mortality) would be negligible on the body of host (Table 1).

The gross reproductive rate of *G. jirufti* (mx - average number of daughter eggs expected to be produced by a female living through entire reproductive period) seems to be 13.892 (Table 2). Likewise, the net reproductive rate (Ro) appeared to be 4.606. The mean length of generation (∑xlxmx/Ro) was determined as 37.09 days. The value of intrinsic rate of natural increase was computed by using trial values of r to find the figure which satisfied the equation $\sum e^{-rmx}lxmx= 1$. In table 1, put the values rm = 0.042 for each age, the summation of $\sum e^{-rmx}lxmx$ proved to be 1.008. By this value of rm (0.042) the precise corrected generation time (T=logeRo/rm) appeared to be 36.33. Likewise, at this value of rm (0.042) the doubling time (DT = loge2/log∑) of *G. jirufti* appeared to be 16.5 days.

Evans and Smith (1952) constructed the life table of human head louse *Pediculus humanas* after making several assumptions as done in present case also. A review of literature indicates that the intrinsic rate of natural increase of twelve ischnoceran species have been recorded, so far (Gupta *et al.,* 2007; Saxena *et al.,* 2007, 2009; Arya *et al*., 2009, Agarwal *et al.,* 2011; Singh *et al.,* 2012; Kumar and Hasan, 2016). The value of gross reproductive rate of the species studies by aforesaid workers varied from 4.7-29.2 days. The net reproductive rate varies from 2.9-14.4. The values of rm of the

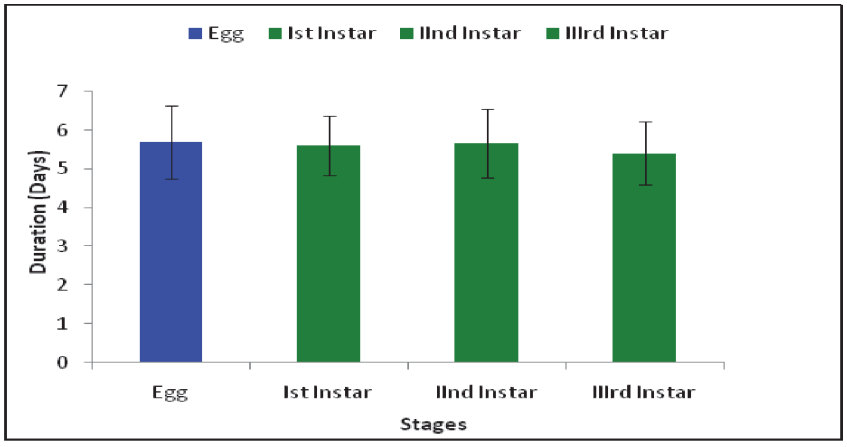

Fig. 1 Duration of different life stages of *G. jirufti* (Ansari, 1947).

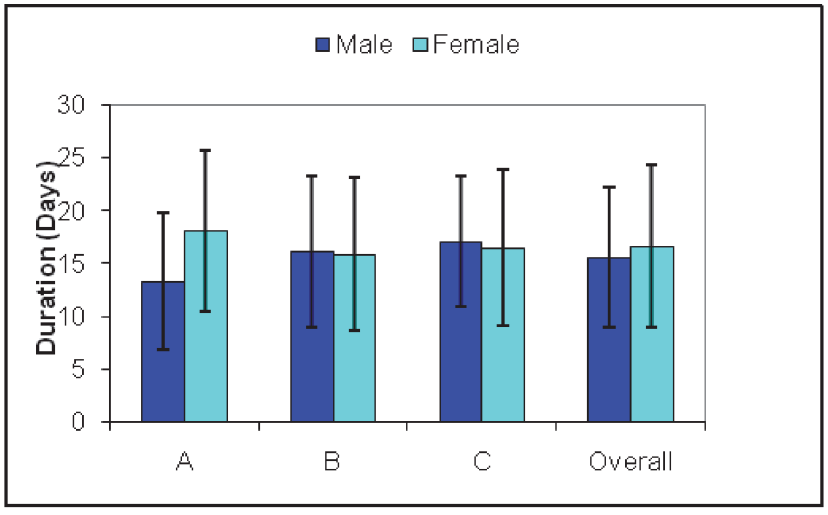

Fig. 2 Adult longevity of males and females of *G. jirufti* (Ansari, 1947).

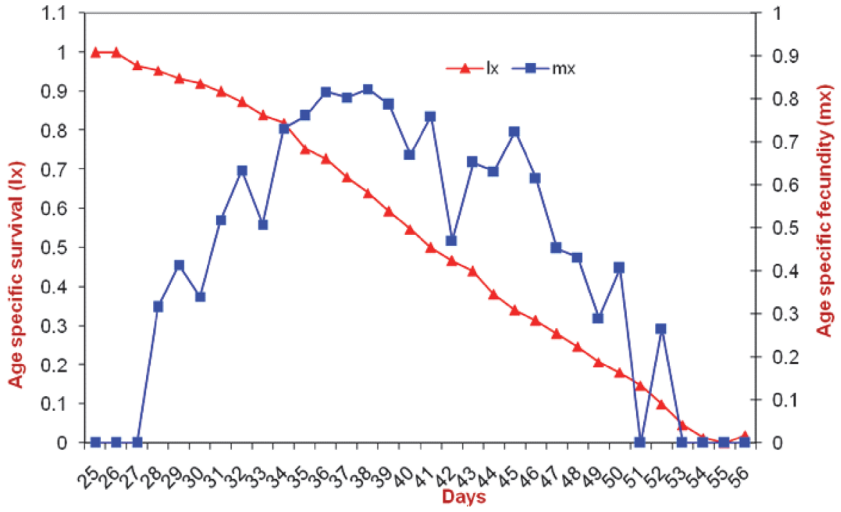

Fig. 3 Age specific survival and fecundity of *G. jirufti* (Ansari, 1947) in *in vitro* condition (35+1C, 75-82% RH, at feather diet).

Table 1. Life table and intrinsic rate of natural increase of *Goniocotes jirufti*

X	lx	mx	lxmx	X1xmx	rmx	e-rmx	e-rmx1xmx
0-23	Immature stage of *Goniocotes jirufti*						
24-25	Pre- oviposition period						
25	1.000	0.000	0.000	0.000	1.050	0.350	0.000
26	1.000	0.000	0.000	0.000	1.092	0.336	0.000
27	0.967	0.000	0.000	0.000	1.134	0.322	0.000
28	0.953	0.315	0.171	4.788	1.176	0.309	0.053
29	0.933	0.414	0.220	6.392	1.218	0.296	0.065
30	0.920	0.341	0.179	5.358	1.260	0.284	0.051
31	0.900	0.519	0.266	8.246	1.302	0.272	0.072
32	0.873	0.634	0.315	10.093	1.344	0.261	0.082
33	0.840	0.508	0.243	8.026	1.386	0.250	0.061
34	0.820	0.732	0.342	11.628	1.428	0.240	0.082
35	0.753	0.761	0.327	11.438	1.470	0.230	0.075
36	0.727	0.817	0.338	12.175	1.512	0.220	0.075
37	0.680	0.804	0.312	11.529	1.554	0.211	0.066
38	0.640	0.823	0.300	11.408	1.596	0.203	0.061
39	0.593	0.787	0.266	10.374	1.638	0.194	0.052
40	0.547	0.671	0.209	8.360	1.680	0.186	0.039
41	0.500	0.760	0.217	8.881	1.722	0.179	0.039
42	0.467	0.471	0.125	5.267	1.764	0.171	0.021
43	0.440	0.652	0.163	7.026	1.806	0.164	0.027
44	0.380	0.632	0.137	6.019	1.848	0.158	0.022
45	0.340	0.725	0.141	6.327	1.890	0.151	0.021
46	0.313	0.617	0.110	5.069	1.932	0.145	0.016
47	0.280	0.452	0.072	3.393	1.974	0.139	0.010
48	0.247	0.432	0.061	2.918	2.016	0.133	0.008
49	0.207	0.290	0.034	1.676	2.058	0.128	0.004
50	0.180	0.407	0.042	2.090	2.100	0.122	0.005
51	0.147	0.000	0.000	0.000	2.142	0.117	0.000
52	0.100	0.267	0.015	0.790	2.184	0.113	0.002
53	0.047	0.000	0.000	0.000	2.226	0.108	0.000
54	0.013	0.000	0.000	0.000	2.268	0.104	0.000
55	0.000	0.000	0.000	0.000	2.310	0.099	0.000
							1.008

Table 2. Intrinsic rate of natural increase of different ischnoceran lice.

Species	Gross reproductive rate	Net reproductive rate (females egg per female)	Mean length of generation	r	D	References
Brueelia amandava (*Amandava amandava*)	4.98	3.31	35.4	0.031	23.45	Gupta *et al*. 2007
Brueelia cyclothorax (*Passer domesticus*)	4.7	2.9	34.2	0.032	21.35	Saxena *et al*. 2009
Sturnidoecus bannoo (*Acridotheres tristis*)	9.3	5.0	33.1	0.049	14.21	Saxena *et al*. 2009
Neopsittaconirmus elbeli (*Psittacula eupatra*)	7.9	5.2	33.5	0.050	13.93	Saxena *et al*. 2009
Columbicola columbae (*Columba livia*)	9.9	8.0	39.4	0.053	14.2	Saxena *et al*. 2009
Anaticola crassicornis *(Anas platyrhynchos)*	29.2	14.4	36.6	0.074	9.01	Saxena *et al*. 2009
Brueelia plocea (*Ploceus phillipinus*)	7.74	3.74	28.19	0.045	15.41	Arya *et.al*. 2009
Goniocotes gallinae (*Gallus g. domesticus*)	12.49	8.3	36.9	0.059	11.73	Saxena *et al*. 2007
Upupicola upupae *(Upupa epops)*	6.08	3.67	37.15	0.035	19.1	Agarwal *et al*. 2011
Columbicola bacillus *(Streptopelia decaocta)*	12.37	6.20	35.93	0.054	12.95	Singh *et al* 2012
Bovicola caprae *(Copra hircus)*	11.62	6.73	35.27	0.055	12.6	Rashmi *et al* 2010
Lipeurus caponis *(Gallus gallus domesticus)*	12.53	3.9	29.64	0.046	16.1	Kumar and Hasan 2016
Goniocotes jirufti *(Francolinus francolinus)*	13.89	4.606	37.09	0.042	16.50	Present study

different species varied from 0.031-0.074. Finally, the value of doubling time of different species has been recorded as 9.0 -23.5 days (Table 2). In comparison to earlier studies species, the black partridge louse, *G. jirufti* appears to be moderate breeder as its rm equaled 0.042 and the doubling time remained 16.50 days.

As far as the mammalian lice are concerned, the value of rm for sheep louse, *B. bovis* has been estimated as 0.053 per day (thus, doubling in 13-14 days) (Murray and Gordon, 1969). The value of rm for rodent louse, *Geomydoecus oregonus* remained too low (0.006 per day indicating doubling after every 112 days) (Rust, 1974). The data clearly shows that the reproductive potentials of different phthirapterans exhibit considerable diversity.

Presumably, the fast breeding species may build their population at faster rate (than moderate and slow breeders) and consequently may cause extensive damage to feathers of the host. On the other hand, slow breeders may exhibit low prevalence and intensity of infestation and thus causing minimal effect on host plumage. The moderate breeders like *G. jirufti* presumably are

supposed to exhibit intermediate condition in this regard.

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Surveillance of *Aedes* (*Stegomyia*) mosquitoes in and around International Airport, Kerala - Assessment of vector control efforts

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ABSTRACT: Vector-borne Diseases (VBDs) such as malaria, dengue, chikungunya, zika virus and yellow fever are reported in over 100 countries and put up to 60% of the world's population at risk of infection; more than 500 million cases are reported each year. The International Health Regulations (IHR) emphasizes to look after international seaports/airports and surrounding areas up to 400 meters free of *Aedes aegypti* mosquito and other vectors of epidemiological significance. Vector surveillance and control at Port of Entry (PoE) is an essential activity for the implementation of IHR. Hence Entomological surveillance was done inside and the residential areas around Cochin International Airport during 2013 to 2019. *Aedes* larval indices in both inside and residential areas outside the airport were found to be below the critical level in all these years. However the study showed no *Aedes* positivity inside the airport during 2014, 2016, 2018 and 2019. Effectiveness of vector control measures implemented in and around the airport is deliberated.

KEY WORDS: Vector-borne diseases, Port of Entry, International Health Regulations

INTRODUCTION

Vector-borne diseases are among the major cause of human sufferings in terms of morbidity and mortality, on one hand, and the stunting the social and economic growth of the country on the other. International travel and transport network play a significant role in the rapid spread of VBDs all over the world. Arboviral diseases such as Dengue fever, Chikungunya, Yellow fever and Zika virus are growing global concern due to geographic expansion of vectors and pathogens. Globalization and industrialization have opened and expanded trade and commerce, which in turn have provided impetus to increased air traffic. The rapid global growth of connectivity has been responsible for the spread of vectors and the disease (WHO, 2008; Strickman and Kittayapong, 2003; WHO, 2012). Among the invasive mosquitoes recorded all over the world, *Aedes* species are particularly frequent and grave. As several of them are potential vectors of diseases, they present significant health concerns.

Aedes mosquitoes originally found in tropical and subtropical zones carry a variety of pathogens that

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can be transmitted to humans. *Ae. aegypti* mosquito is the main vector that transmits the viruses that cause dengue, chikungunya, yellow fever and zika virus. *Ae. albopictus* is also playing the role as vector for the transmission of dengue virus as well as competent vector of 22 arboviruses, including West Nile and Yellow fever (Gubler, 2003). *Aedes* mosquito is considered a highly domesticated mosquito, very adapted to living with man, preferring to rest indoors and to feed on humans during daytime hours. The *Aedes* mosquitoes generally breed in water holding containers found in and around the houses, such as those used for water storage, flower vases, mud containers, metal containers, used tires, plastic utensils and other receptacles that collect rain water (Sheela Devi *et al*., 2012).

The incidence of VBDs proliferating rapidly due to many factors including uncontrolled urbanization that promote breeding of vector mosquitoes. World Health Organization (WHO) in 2010 stratified the current situation of DF/DHF in India under category A, which means a major public health problem, leading cause of hospitalizations and death among children. To convey the global threat due to the entry and establishment of vectors and emergence of vector-borne diseases, through point-of- entry (PoE), WHO brought Member States under a common umbrella of the International Health Regulations (IHR) in 1969 to which all the Member States were signatory. In May 2005, the 58thWorld Health Assembly adopted the new International Health Regulations (IHR), which came into force in July 2007 (WHO, 2012). At present there are 22 International airports and 12 seaports in the country, which act as PoE. In accordance with IHR, all International airports and seaports should be remain free from all types of vector mosquitoes with a range of 400 meters around the ports to achieve the ultimate aim of public health security (WHO, 2016). Thus, vector surveillance and control become a vital component for the implementation of IHR. In order to assess the effectiveness of vector control measures adopted in and around the airport, eco-entomological survey was undertaken in Cochin International Airport Limited (CIAL).

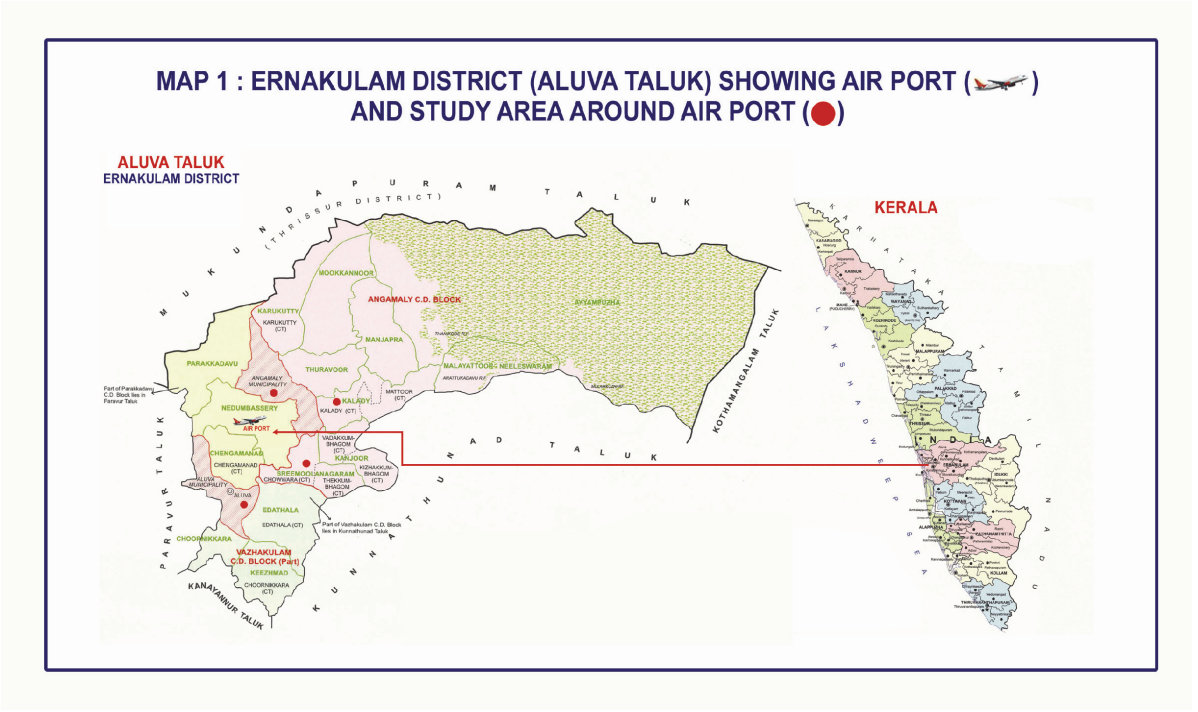
MATERIALS AND METHODS

Study area: Cochin International Airport Ltd. (CIAL) is located in Nedumbassery, about 25 Km Northeast of the city. Nedumbassery is a suburb of the city of Cochin and it lies between the two Municipalities of Aluva and Angamali in the Greater Cochin region. Nedumbassery is also an integral part of the Cochin Metropolitan area. The Entomological surveillance was undertaken in these urban and rural areas, situated around the Air port, from 2013 to 2019 (Map 1). CIAL is the largest and busiest airport in Kerala constructed under public-private partnership. As of 2019, CIAL caters to 61.8% of the total air passenger movement in Kerala. The coordinates of Cochin International airport are 10^0 091'19'' N and 76^0 23' 28'' E.

Entomological surveillance: *Aedes* survey was done in all the operational areas of Cochin International Airport and in randomly selected 100 residential houses around the airport from 2013 to 2019. In each year the survey was done in the months of November-December. Standard entomological techniques were used for survey. Larval survey was carried out in all types of water holding containers to detect the breeding of *Aedes* (*Stegomyia*) mosquitoes in and around the Airport. All accessible larval breeding habitats like discarded tires, earthen, plastic, metal containers, cement tanks, etc. were inspected. The collected larvae were identified microscopically/ after adult emergence as per guidelines (WHO, 1995).

The type of breeding habitats and their location were recorded on a predesigned proforma for classification. The data on larval survey were analyzed and calculated in terms of House index/ Premise index (HI/PI), Container index (CI), Breteau index (BI) and the preferred breeding habitats of *Aedes* mosquitoes also assessed. The dry containers seen scattered in the premises were also examined as these can act as potential breeding sources of *Aedes* mosquitoes during summer rains/ monsoon.

After the completion of the work, the report was sent to Air port health officer, Cochin International Airport (CIAL) for necessary action. The copy of

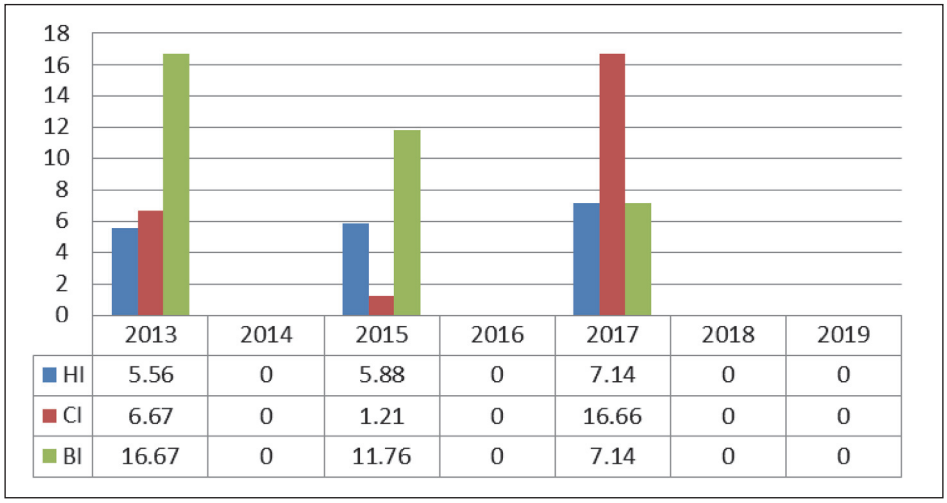


the report was sent to the Director, CIAL for follow up. The vector control activities done by the CIAL health authorities in each year on the basis of the report of NCDC, Kerala branch would be assessed by the surveillance team in the succeeding year. The observations were analyzed and assessed the progress of the activity in each year.

Residential area: Cochin Airport is located in Nedumbassery. It lies between Aluva and Angamaly Municipalities. There are nine panchayaths in Angamali C.D. Block, of which Kalady and Sreemoolanagaram panchayaths are situating adjacent to the Airport. To assess the *Aedes* mosquito prevalence around the Airport and also to assess the effectiveness of vector control measures done by the local bodies and local health system, NCDC, Kerala branch has undertaken regular vector surveillance in randomly selected wards of Angamaly Municipality (urban) and Kalady and Sreemoolanagaram Panchayaths (rural) from 2013 to 2019 (Map 1). During each Entomological surveillance, 100 houses were randomly selected from the target area and the data was analyzed statistically. After the completion of the work, the report was sent to DMO (H) of concerned district for necessary action. The copy of the report was sent to Director of Health Services, Kerala State for follow up. The vector control measures including the source reduction activities with community participation and awareness campaign done in each year based on the recommendations/suggestions of the study team would reflect in the subsequent surveillance activity. In order to assess the effectiveness of vector control measures implemented in the target area, both the qualitative (ecological conditions of the house premises, mosquitogenic and local hygiene conditions, etc.) and quantitative observations (*Aedes* larval indices and potential breeding sites of vector mosquitoes) were noted and compared with the previous observations.

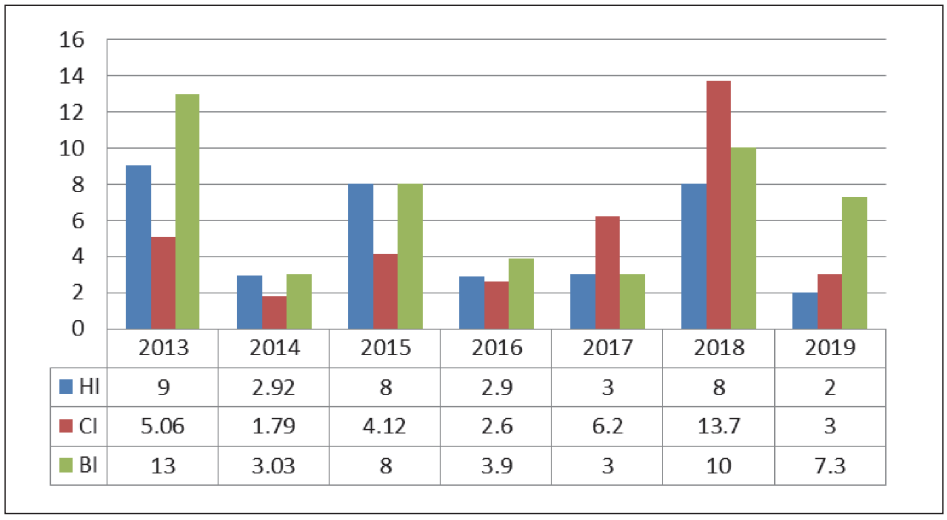
RESULTS AND DISCUSSION

Airport area: Entomological surveillance was done in CIAL during 16th and 17th of December 2013. Though a total of 45 water holding containers at 18 premises were examined, only 03 containers were found positive for *Aedes* larvae. The Premise index (PI), Container index (CI) and Breteau index (BI) were 5.56%, 6.67% and 16.67 respectively (Fig. 1). It is to be noted that the Premise index (PI) > 10% and Breteau index (BI) > 20 are considered



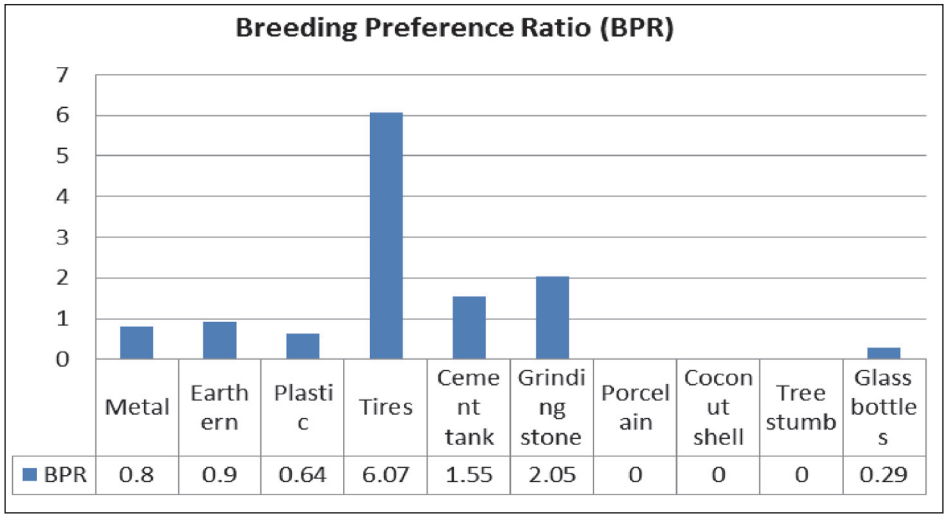
	2013	2014	2015	2016	2017	2018	2019
HI	5.56	0	5.88	0	7.14	0	0
CI	6.67	0	1.21	0	16.66	0	0
BI	16.67	0	11.76	0	7.14	0	0

Fig. 1 *Aedes* Larval Indices inside the Cochin Airport



	2013	2014	2015	2016	2017	2018	2019
HI	9	2.92	8	2.9	3	8	2
CI	5.06	1.79	4.12	2.6	6.2	13.7	3
BI	13	3.03	8	3.9	3	10	7.3

Fig. 2 *Aedes* Larval Indices around Cochin Airport



	Metal	Earthern	Plastic	Tires	Cement tank	Grinding stone	Porcelain	Coconut shell	Tree stumb	Glass bottles
BPR	0.8	0.9	0.64	6.07	1.55	2.05	0	0	0	0.29

Fig. 3 The preferred breeding habitats of *Aedes* mosquitoes around CIAL

as critical. In the present study all the *Aedes* larval indices are below the critical level. The number of *Aedes* positive containers is a decisive factor in determining the status of Breteau index (BI). Hence, if the positive containers are less, naturally the BI will also be low. Thirty nine different types of dry containers were seen scattered in the air port premise. Hence it has been suggested to intensify source reduction activities and removal or disposal of dry containers to make the premise clean and free from vector breeding sources. As part of routine Entomological surveillance in International Air ports, the survey was done in CIAL in 2014. However the study team could not found any vector breeding sources in the airport premise indicating the CIAL authority's commitment in fulfilling the responsibility. NCDC, Kerala branch did the Entomological surveillance continuously for a period of seven years i.e., from 2013 to 2019. On an average 22 premises were checked for *Aedes* breeding in the Airport in each vector surveillance. However no *Aedes* breeding could be detected in 2014, 2016, 2018 and 2019. In the other years, all the *Aedes* larval indices were below the critical level. In a study on the breeding prevalence of vectors of dengue/chikungunya and yellow fever, Sharma and Kumar (2015) could not find the breeding of *Aedes* mosquitoes inside Chennai sea port. While studying the breeding habitats of vector mosquitoes in Marmugao Port Trust (MPT), Goa, Patel *et al*. (2017) also reported a similar situation. Though 13 water holding containers at 33 premises were examined at New Mangalore Port Trust (NMPT) no containers were found positive for *Aedes* larvae (Rajendran *et al*., 2019). The CIAL authorities took much care in executing the recommendations of the study team in each year (Table 1). This is a classic example to illustrate the effectiveness of vector control measures in reducing mosquito breeding habitats in the Airport premise.

Residential area: As Entomological surveillance has done in CIAL from 2013 to 2019 and during these years, the survey has also been done in the residential areas around the Air port. In each survey 100 houses were randomly selected around the Air port to detect vector breeding sources. In 2013, the House index, Container index and Breteau index in the survey area (Angamaly Municipal area, Ward Nos. 15, 16, 17) were 9.0%, 5.06% and 13 respectively. All the *Aedes* larval indices were below the critical level. The report, in each year, was sent to DMO (H), Ernakulam and Secretary of the concerned Local Self Government (LSG) for necessary action. In every year the health department in association with LSG is implementing 'pre-monsoon drive' to clean the environment by destroying the mosquito breeding habitats. Though the larval indices are below the critical level in all the years, it never attained 'zero level' as has been witnessed inside CIAL (Fig. 2). This indicates the lack of community participation in vector control activity in the survey area. Air port premise being a closed environment and being under the control of a well secured system, effective vector control implementation is possible, if the authorities are committed. The same cannot be anticipated in an open environment where the owners are different and many. Hence it is only through regular awareness campaign the community participation could be made possible for vector control. Though number of dengue fever cases and deaths are increasing every year with the onset of monsoon, it is surprising to note that mosquito control is not yet become a felt need of the community. Though the households are creating mosquitogenic conditions in their own premises, many of the households of Kerala waiting the health workers to come and clean the environment. Dengue vector control is simple and can be achieved through regular practice of source reduction activity in our own premises. But unfortunately, the mindset of most of the inhabitants is disheartening the local health workers. Such an attitude of the community should change. People who are hailing from high literacy and health consciousness should think that it is our duty to get rid of breeding sites of mosquitoes at least from our own premises. Many investigators emphasized the importance of active involvement of community in controlling vector breeding habitats in a locality and thus to control vector-borne diseases (Sheela Devi, 2011; Rajendran *et al*., 2020).

It is observed from the present study that *Aedes albopictus* was the species seen in different habitats of the survey area. Prior to 2013, NCDC

Table 1. Details of entomological surveillance inside Cochin International Airport

Year	*Aedes* Larval Indices	Observation	Recommendation/ Suggestions	Activities done by Airport authorities	Interpretation
2013	PI-5.56% CI-6.67% BI-16.67	1. A total of 45 different water holding containers were checked, in which 03 containers were found positive for *Aedes albopictus* larvae. *Aedes aegypti* was absent. 2. 39 dry containers / utensils were seen scattered inside the airport.	1. As *Aedes* breeding was detected regular source reduction activities need to be carried out. 2. Many dry containers seen inside the airport is a potential risk factor for *Aedes* breeding during rains. Hence these containers need to be removed or disposed of safely.	Base line data	Premise index >10% and BI>20 as considered critical. All the *Aedes* larval indices are below the critical level in the present study.
2014	All the three larval indices are zero	1. Of the 20 premises searched for the presence of *Aedes* breeding, , no water holding containers were seen. However, dry containers/ utensils were seen scattered which can act as potential source for Aedes breeding during rains.	Dry containers seen scattered inside the airport need to be removed and disposed of safely.	The suggestions of NCDC, Kerala branch has been taken care by the CIAL (Cochin International Airport Limited) authorities.	Source reduction activities are perfectly done. No *Aedes* breeding sources found inside the airport
2015	PI-5.88 CI-1.21 BI-11.76	1. Out of 165 containers searched in 17 premises inside the airport area, only 02 containers found positive for *Aedes albopictus.* 2. Many dry containers/utensils were also seen scattered inside the airport area.	Timely source reduction activities should be continued to sustain the indices low.	Care has been given for source reduction activities.However efforts should be extended to locate and remove the mosquito breeding sources.	All the *Aedes* larval indices are below the critical level in the present study.
2016	All the three larval indices are zero	Out of 15 containers searched in 25 premises inside the airport, no water holding containers found for breeding of *Aedes* larvae.	Regular weekly vector surveillance and source reduction activities are to be done inside the airport.	The source reduction activities have been done as per the recommendation of NCDC, Kerala branch.	Vector control measures are perfectly done. No *Aedes* breeding sources found inside the airport

Year	*Aedes* Larval Indices	Observation	Recommendation/ Suggestions	Activities done by Airport authorities	Interpretation
2017	PI-7.14 CI-16.66 BI-7.14	1. Out of 18 water holding containers searched, 03 of them found positive for *Aedes albopictus.* 2. *A* few dry containers were found scattered inside the airport.	Timely source reduction activities should be continued to sustain the larval indices low.	Vector control measures have been done including source reduction activities.	All the *Aedes* larval indices are below the critical level in the present study.
2018	All the three larval indices are zero	A total of 22 premises have been searched for *Aedes* breeding. However none of the water holding containers was found breeding of *Aedes* mosquito.	The unwanted dry containers are to be removed and disposed of safely.	Vector control measures have been done as per the suggestion of NCDC, Kerala branch.	All the premises are comparatively clean and no mosquito breeding sources located in the area.
2019	All the three larval indices are zero	None of the water holding containers was found breeding of *Aedes* mosquito.	As dry containers are seen inside the airport premise, source reduction activities should be intensified.	Vector control measures have been done as per the suggestion of NCDC, Kerala branch.	Vector control measures are perfectly done. No *Aedes* breeding sources found inside the airport

PI-Premise Index, CI-Container Index, BI- Breteau Index

team could collect *Aedes aegypti* mosquitoes few times in and around the Cochin airport. The availability of the source/containers seen scattered in the peri-domestic environment may influence the site selection of *Aedes* mosquitoes for oviposition. The details of Entomological surveillance from 2013 to 2019 around the Air port were taken for analysis. The Breeding Preference Ratio (BPR) was calculated in order to find out the most preferred habitat selection of *Aedes* mosquitoes (Fig. 3). It has been found that around the Cochin air port, the BPR with respect to *Aedes* mosquitoes was more in Tires (6.07) followed by Grinding stone (2.05) and Cement tank (1.55). Many researchers identified the used automobile tires holding rain water as key breeding sites of *Aedes* mosquitoes (Gill et al., 2000; Sheela Devi, 2011; Sharma *et al.,* 2015; Rajendran *et al*., 2020).

Of the total dry containers/sources seen in the residential areas around Cochin Air port, 46.19% were plastic containers. During summer rain/ monsoon, the dry containers may get filled with rain water and pave for the breeding of *Aedes* mosquitoes. In order to avoid mosquito breeding, either these containers are to be removed or kept properly covered. *Aedes* breeding could be noted in the residential areas around the Air port area. The closeness of the residential area to the Air port enhances the chances of spill over of breeding of *Aedes* mosquitoes in the air port area.

Vector-borne disease control across international borders is one of the important public health issues. India is having international ground crossings and bordering districts with Nepal, Bhutan, Myanmar, and Bangladesh. The country is connected with air and water with other part of the world with entry points at airports and seaports. Transmission dynamics across borders are generally similar to Indian climatic conditions.

The risk due to the introduction of vectors, pathogens and diseases from one country to another

would be reduced if the airports and seaports were kept free of mosquito breeding, as required by International Health Regulations. A careful supervision of the airports and seaports by trained vector control personal is needed to prevent the breeding of vector mosquitoes. In most of the airports, the vector control is being done through outsourcing services. It will be appropriate if the airport health authorities can monitor the vector control activities from time to time. Regular entomological surveillance is required to identify the factors favoring the breeding of vector mosquitoes and the potential vector breeding sites. This basic knowledge is essential in formulating appropriate vector control strategy in the target area.

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Tolerance of *Metarhizium anisopliae* Sorokin isolates to selected insecticides and fungicides

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ABSTRACT: An experiment was conducted to assess the compatibility of the popular insecticides like spinosad, cypermethrin, imidacloprid and chlorantraniliprole as well as fungicides copper oxychloride, carbendazim and hexaconazole with native isolates of *M. anisopliae* (MC 2, MC 4, MC 7). Among the isolates, MC 2, MC 7 and MC 4 were found compatible with insecticides spinosad, imidacloprid and chlorantraniliprole as well as fungicide copper oxychloride. Isolates MC 2 and MC 7 exhibited highest growth with only 3.70 and 5.18 per cent inhibition in the PDA medium amended with highest dose of copper oxychloride (0.30 g/ l) when compared to MC 4 (7.03 % inhibition). Among the three isolates tested, the isolate MC 7 was more compatible with highest growth at all higher doses of chlorantraniliprole (0.35 ml/L), spinosad (0.38ml/ l) and imidacloprid (0.15g/ l) by recording least per cent growth inhibition (11.00, 11.41 and 14.44 per cent inhibition respectively). The insecticide cypermethrin was slightly toxic to all the isolates of *M. anisopliae* and fungicides, carbendazim and hexaconazole were not compatible with the *M. anisopliae* isolates.

KEY WORDS: Entomopathogenic fungi *Metarhizium anisopliae*, compatibility, insecticides and fungicides

INTRODUCTION

Entomopathogenic fungi (EPF) are fungal microorganisms that are pathogenic to pests. *Metarhizium anisopliae* is one among them and is effective against several species of insects including beetles, termites, leafhoppers, mosquitoes and lepidopterans. It has been recovered from a variety of crop ecosystem, rendering it an ideal candidate for exploration on stress tolerant isolates. Incompatibility of insecticides and fungicides with fungi is one among the abiotic stresses. Combined use of mycoinsecticides and chemical insecticides is a promising pest control option for minimizing adverse chemical effects and also reduces frequency of application of mycoinsecticide. Several studies have reported that *M. anisopliae* is a dominant species in intensively cultivated arable lands and it was thought to be due to the ability of *M. anisopliae* to tolerate agricutltural chemicals and mechanical disturbances (Vanninen and Hokkanen, 1988; Vanninen, 1995). More than that, many researches have been implemented for exploring the compatibility of entomopathogens with insecticides (Silva *et al*., 2013; Kassab *et al*., 2014; Sain *et al*., 2019).

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Combined use of incompatible insecticides and fungicides might inhibit the entire functioning of entomopathogenic fungi which adversely affect the system of integrated pest management. Therefore, identification of the fungal isolates which are compatible to pesticides will retain biocontrol potential and will be effective in managing pests.

MATERIALS AND METHODS

Experiments on compatibility of different insecticides and fungicides with *M. anisopliae* native isolates were conducted at College of Horticulture, Vellanikkara, Thrissur, Kerala during 2018 under laboratory conditions. Native isolates of *Metarhizium anisopliae* namely MC 2, MC 4, MC 7 isolated from Moncompu in Alappuzha district were tested as per the protocol of Grover and Moore (1962), following the method of poisoned food technique. Four insecticides *viz*., spinosad, cypermethrin, imidacloprid and chlorantraniliprole as well as three fungicides namely copper oxychloride, carbendazim and hexaconazole were used in this experiment (Table 1). Compatibility of the isolates was evaluated by exposing them to different doses *i.e*., lower dose, recommended dose and higher dose (recommended dose as per package of practices, KAU). Desired quantity of chemical was measured out and mixed thoroughly in the sterilized molten potato dextrose agar medium and poured onto the Petri plates. Control plates without the addition of fungicides and insecticides were also maintained. A five millimeter mycelial disc of each isolate was placed at center of the medium and kept for incubation at 26 ± 2°C for 14 days. Radial growth was measured and the percent reduction of growth (Vincent, 1927) compared to control was calculated using the formula,

Percent inhibition = (C-T) × 100/C where,

C = radial growth of isolate in PDA plate (cm)

T = radial growth of the salt amended PDA plate (cm)

Sporulation of the isolates was also observed and visually analysed. Depending upon the growth inhibition values, the pesticides are again classified on a 1-4 index where one denotes harmless (< 50 % reduction in growth), two is slightly harmful (50-79 %), three designates moderately harmful (80-90 %) and four implies harmful (> 90%) according to Hassan's classification scheme (Hassan, 1989). Data was accorded to Analysis of Variance (ANOVA) employing Web Agri Stat Package (WASP 2.0). Multiple comparisons between the treatment means were done with Duncan's Multiple Range Test (DMRT) and appropriate transformations were considered according to the method elucidated by Gomez and Gomez (1984).

Table 1. Details of insecticides and fungicides used in the study

Chemical name	Trade name and formulation	Doses used (ml or g/ l)		
		Lower	Recommended	Higher
Spinosad	Taffin, 45 SC	0.28	0.33	0.38
Cypermethrin	Cyperguard, 25 EC	0.35	0.40	0.45
Imidacloprid	Admire, 70 WG	0.05	0.10	0.15
Chlorantraniliprole	Coragen, 18.5 SC	0.25	0.30	0.35
Copper oxychloride	Fytolan, 50 WP	0.20	0.25	0.30
Carbendazim	Bavistin, 50 WP	0.50	1.00	1.50
Hexaconazole	Contaf, 5 EC	1.50	2.00	2.50

RESULTS AND DISCUSSIONS

In general, growth of all isolates reduced at higher doses of insecticides and fungicides. A considerable decrease in the growth and sporulation was noticed in the PDA amended with fungicides when compared to insecticides (Table 2). In the case of PDA amended with spinosad at different doses, all the isolates showed a growth inhibition of less than 19.50 per cent. Isolate MC 7 was superior among the isolates with least inhibition from 8.11 to 11.41 per cent at higher doses of spinosad. The extent of inhibition increased as the dose of spinosad increased in the medium. Sporulation was adversely affected only at higher doses of insecticide (Table 3). Isolate MC 7 produced medium sporulation even

Table 2. Effect of insecticides and fungicides on the growth of Metarhizium anisopliae isolates

Sl. No.	Insecticides and fungicides	Growth inhibition over control (%) *			Standard error	Grade
		MC 2	MC 4	MC 7		
1.	Spinosad					
	@ 0.28 ml/l	4.61(2.97)^{a}	4.42(2.54)^{ab}	3.62(2.12)^{b}	0.332	1
	@ 0.33 ml/l	16.64(9.59)^{a}	10.71(6.16)^{b}	8.11(4.67)^{c}	0.313	1
	@ 0.38 ml/l	17.40(10.02)^{b}	19.23(11.10)^{a}	11.41(6.59)^{c}	0.359	1
2.	Cypermethrin					
	@ 0.35 ml/l	53.00(32.22)^{a}	51.51(30.75)^{b}	53.74(33.00)^{a}	0.336	2
	@ 0.40 ml/l	56.23(33.72)^{c}	61.90(38.48)^{a}	59.20(36.03)^{b}	0.335	2
	@ 0.45 ml/l	60.71(36.90)^{b}	64.04(40.11)^{a}	62.92(38.40)^{a}	0.370	2
3.	Imidacloprid					
	@ 0.05 g/l	13.64(7.87)^{a}	10.30(5.95)^{b}	7.10(4.03)^{c}	0.363	1
	@ 0.10 g/l	19.20(11.10)^{a}	16.70(9.59)^{b}	11.93(6.80)^{c}	0.385	1
	@ 0.15 g/l	21.50(12.41)^{a}	17.43(10.02)^{b}	14.44(8.30)^{c}	0.339	1
4.	Chlorantraniliprole					
	@ 0.25 ml/l	0.00(0.00)^{b}	1.85(1.06)^{a}	0.00(0.00)^{b}	0.210	1
	@ 0.30 ml/l	2.96(1.69)^{b}	4.81(2.75)^{a}	0.00(0.00)^{c}	0.287	1
	@ 0.35 ml/l	8.88(4.88)^{a}	7.77(4.45)^{a}	1.11(0.84)^{b}	1.117	1
5.	Copper oxychloride					
	@ 0.20 g/l	1.11 (0.63)^{c}	4.81(1.69)^{b}	2.96(2.75)^{a}	0.257	1
	@ 0.25 g/l	2.59(1.48)^{c}	5.92(3.39)^{a}	4.07(2.33)^{b}	0.314	1
	@ 0.30 g/l	3.70(2.12)^{c}	7.03(4.03)^{a}	5.18(2.97)^{b}	0.314	1
6.	Carbendazim					
	@ 0.50 g/l	86.33 (59.61)^{b}	93.40 (68.38)^{a}	58.51 (35.77)^{c}	0.336	4
	@ 1.00 g/l	100	100	100	-	4
	@ 1.50 g/l	100	100	100	-	4
7.	Hexaconazole					
	@ 1.50 ml/l	100	100	100	-	4
	@ 2.00 ml/l	100	100	100	-	4
	@ 2.50 ml/l	100	100	100	-	4

Values given in the parentheses are arcsine transformed values

Table 3. Effect of insecticides and fungicides on the sporulation of *Metarhizium anisopliae* isolates

Sl. No.	Insecticides and fungicides	Sporulation of the isolates		
		MC 2	MC 4	MC 7
1.	Spinosad			
	@ 0.28 ml/l	+++	+++	+++
	@ 0.33 ml/l	++	+	++
	@ 0.38 ml/l	-	-	++
2.	Cypermethrin			
	@ 0.35 ml/l	+	++	++
	@ 0.40 ml/l	-	+	+
	@ 0.45 ml/l	-	-	++
3.	Imidacloprid			
	@ 0.05 g/l	+	+	++
	@ 0.10 g/l	-	+	+++
	@ 0.15 g/l	-	-	+++
4.	Chlorantraniliprole			
	@ 0.25 ml/l	+++	+++	+++
	@ 0.30 ml/l	++	+++	+++
	@ 0.35 ml/l	+	+	++
5.	Copper oxychloride			
	@ 0.20 g/l	-	+++	+++
	@ 0.25 g/l	++	++	+++
	@ 0.30 g/l	+	+	++
6.	Carbendazim			
	@ 0.50 g/l	++	-	+++
	@ 1.00 g/l	-	-	-
	@ 1.50 g/l	-	-	-
7.	Hexaconazole			
	@ 1.50 ml/l	-	-	-
	@ 2.00 ml/l	-	-	-
	@ 2.50 ml/l	-	-	-

+++ : high sporulation, ++ : medium sporulation, + : sparse sporulation, - : no sporulation (visual observation)

at highest dose of spinosad. Cypermethrin on the other hand, caused inhibition of more than 50 per cent in all isolates even at the lowest dose (Table 2).

All the isolates were equally incompatible with cypermethrin, suggesting that combined application of cypermethrin and *M. anisopliae* should not be advisable. The isolates had less than 22 per cent growth inhibition at all doses of imidacloprid, with growth inhibition of isolates ranging between 7.10 and 21.50 per cent. The isolate MC 7 was consistently superior to other isolates in terms of growth and sporulation. The inhibition was dose

dependent for all isolates, with highest degree of inhibition being at the highest dose of the insecticide (0.15 g/ l). The three isolates were also found to be compatible with chlorantraniliprole at all doses used in this study. The growth inhibition caused was less than nine per cent for all isolates at all doses. Significantly superior radial growth along with high sporulation at higher doses was exhibited by the isolate MC 7 when compared to other two isolates (Table 2).

Screening of isolates for fungicide compatibility was also carried out. Less than eight per cent growth inhibition was observed for all isolates at different doses of the copper oxychloride (COC), proving its compatibility with *M. anisopliae*. Among the three isolates tested, MC 2 recorded least growth inhibition of 1.11 to 3.70 per cent at different doses of copper oxychloride (Table 2). However, high sporulation was exhibited by the isolate MC 7 with medium sporulation even at the highest dose of COC (0.30 g/ l) [Table 3]. Growth of all isolates was considerably inhibited even at the lowest dose of carbendazim (0.50 g/ l). Total growth inhibition was observed in all isolates at recommended dose of 1 g/ l and above (Table 2). At 0.50 g/ l, the inhibition in the growth of all isolates was ranged between 58.51 and 86.33 per cent. MC 2 and MC 7 registered medium and high sporulation respectively at the lowest dose of fungicide. No sporulation was observed at higher doses [Table 3]. All isolates of *M. anisopliae* resulted in 100 per cent growth inhibition at all doses of hexaconazole (1.50, 2, 2.50 ml/ l) depicting that the isolates obtained in the present study were incompatible with fungicide hexaconazole. Based on Hassan's classification scheme, insecticides spinosad, imidacloprid, chlorantraniliprole and fungicides copper oxychloride were categorized to index 1, insecticide cypermethrin to 2 and fungicides carbendazim and hexaconazole to index 4.

Combined application of pesticide and entomopathogenic fungi provides satisfactory control against many agricultural pest. But use of incompatible pesticides in soil could hamper the growth and development of beneficial fungi. In this context, experiment was conducted in order to screen the native isolates of *M. anisopliae* for tolerance to different insecticides and fungicides. Compatibility of four isolates of *M. anisopliae* from Punjab and Pakistan with a number of pesticides had been reported by Akbar *et al*. (2012). The isolate M70 recorded highest radial growth of 6.81 cm and a spore yield of 1.26×10^8/ml in PDA amended with recommended dose of spinosad whereas imidacloprid, indoxacarb, cypermethrin, acetamiprid supported only moderate conidial germination. The study concluded that insecticides like spinosad, imidacloprid and acetamiprid were more compatible with *M. anisopliae* than other insecticides tested. The insecticide spinosad and imidacloprid were more compatible than cypermethrin with *M. anisopliae* isolates in the present study and was in tune with the findings of Akbar *et al*. (2012).

Mochi *et al*. (2005) reported that imidacloprid had no effect on the survival and growth of *M. anisopliae*. Imidacloprid had been found as compatible with *M. anisopliae* by several authors. Quintela and McCoy (1998) reported that combined application of *M. anisopliae* and imidacloprid resulted in higher mortality of root weevil grub, *Diaprepes* sp. in soil. Moreover in the study of Neves *et al*. (2001) also confirmed compatibility of imidacloprid with *M. anisopliae*. But imidacloprid was found moderately toxic at maximum dose and incompatible at minimum dose with entomopathogens as stated by Filho *et al*. (2001). Study of Joshi *et al*. (2018) found complete inhibitory action of fungicides carbendazim and hexaconazole on the growth of *M. anisopliae*. According to Mochi *et al*. (2005), CO_2 production by *M. anisopliae* was suppressed in soil for 4-6 days when co- applied with fungicides (COC, tebuconazole *etc*), but after that there is no significant difference between the respiratory activity of *M. anisopliae* in fungicide treated and untreated soil. The tested acaricides, herbicides and insecticides had only less impact on respiratory activity of fungi and hence suggested for the combined application with fungi. The insecticides used in the present study were more compatible with *M. anisopliae* isolates than the fungicides used and were in line with the reports of Mochi *et al*. (2006) who studied the effects of insecticides and

fungicides in the growth of *M. anisopliae*. Most of the fungicides were incompatible with the entomopathogens while there was a greater compatibility between insecticides and *M. anisopliae*. Laboratory bioassays alone doesn't determine the effective compatibility of entomopathogens with pesticides hence additional field or greenhouse studies are required to confirm the compatibility or incompatibility of pesticides with biocontrol agents before they recommend in crop management strategies..

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Biology of anthocorid predator, *Blaptostethus pallescens* Poppius (Heteroptera: Anthocoridae)

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ABSTRACT: Biology of anthocorid predator *Blaptostethus pallescens* Poppius was studied on eggs of alternate host *Corcyra cephalonica* (Stainton). Eggs of *B. pallescens*, thrust within the plant tissue, hatched after a mean incubation period of 5.78 days. Nymphs, when reared on UV sterilized eggs of *C. cephalonica* under ambient conditions, developed normally with five instars, each having a mean duration of 2.63, 1.92, 2.01, 2.50 and 5.10 days, respectively. Females laid eggs after a pre-oviposition period of 4.2 days. Average fecundity of bugs was 134.04 eggs. Mean longevity of females was found to be higher (52.03 days) than that of males (40.18 days).

KEYWORDS: Bug, *Blaptostethus pallescens*, *Corcyra cephalonica, life history*

Minute pirate bugs belonging to the family Anthocoridae are found in all zoogeographical regions of the world and are perceived as potential biocontrol agents of arthropod pests. They are predacious on small lepidopteran larvae, mites, aphids, thrips, psocids, and storage pests. Natural populations of anthocorid bugs have been successful in maintaining the pest infestations to a low level and hence, remain the most sought-after natural enemies for pest management across several countries like France, the United Kingdom, the Netherlands and Germany (Ballal and Yamada, 2016).

The anthocorid bug, *Blaptostethus pallescens* Poppius (Hemiptera: Anthocoridae) has been reported as a promising biocontrol agent of spider mites (Ballal *et al.,* 2009) and other arthropods of significance, especially under protected cultivation. This makes them an attractive proposition for pest management in polyhouses of Kerala, with over 600 polyhouses growing high value crops like salad cucumber and capsicum. However, information regarding the biology of the bug under Kerala conditions is non-existent. Hence a study was conducted to investigate the biology of *B. pallescens* on eggs of factitious host, *Corcyra cephalonica* as a preliminary step for assessing its potential against soft bodied insects.

The biology of the anthocorid bug was studied at 28 ± 2 ^{0}C and 70% RH in the AICRP on BCCP, College of Horticulture, Vellanikkara during September - December, 2018. The culture of *B. pallescens* was obtained from National Bureau of Agricultural Insect Resources, Bengaluru and were multiplied on eggs of rice meal moth (*C. cephalonica*) as per the procedure described by Ballal *et al*. (2003).

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Adults of uniform age were provided with pieces of bean pods as ovipositional substrate. The bean pods with eggs laid on them were collected after 24 h and were transferred to a separate plastic box with tissue paper lining and observed daily for hatching. Freshly emerged nymphs (0-24 h old) were transferred singly into individual glass vials of 5 ml capacity using a fine camel hair brush. Nymphs were provided with an adequate supply of UV sterilized *C. cephalonica* eggs. A thin piece of paper strip was provided inside each glass vial to facilitate movement of the nymph. A total of 115 nymphs were maintained in this fashion. The vials were observed daily under stereo microscope (30X) till adulthood to record total developmental period. Newly emerged 0-24h old male and female bugs were formed into 50 pairs. Each pair was then transferred to 35 ml test tubes (10 cm height x 9 cm diameter) and were given UV sterilized rice meal moth eggs as feed and sections of bean pods for oviposition. The bean pods were replaced daily. The number of eggs laid each day were counted under stereo microscope (30X). Longevity of males and females were also recorded.

Duration of life stages of *B. pallescens*

Life cycle of *B. pallescens* constituted of three different developmental stages namely, egg, nymph and adult. Duration of different developmental stages of *B. pallescens* recorded during the study is presented in table 1.

Eggs were bottle shaped and inserted singly into the tissue of bean pods with only the operculum visible outside. In a few instances, eggs were also laid among the cotton strands. Newly laid eggs were creamy white but later turned pink. Mean incubation period was found to be 5.782 ± 0.131 days. The nymphs emerged through the operculum, which opened like a lid.

Nymphal stage consisted of five instars.

The first instar nymphs, upon hatching, were pale to slightly pink in colour with dark red eyes. The duration of first instar ranged from 2 to 5 days, with an average of 2.636 ± 0.057 days.

The second instar nymphs were uniformly pink in colour. The duration of second instar ranged from 1 to 3 days, with an average of 1.926 ± 0.041 days. Second instar nymphs had the shortest stadium.

The third instar nymphs were uniform reddish and were darker in colour than the second instar. Wing pads were visible. Duration of third instar ranged from 1 to 3 days with an average of 2.018 ± 0.022 days.

The dark reddish brown fourth instar nymphs had well developed wing pads. Duration of fourth instar ranged from 2 to 5 days with an average of 2.500 ± 0.060 days.

The fifth instar nymphs were reddish black in colour with well-developed wing pads. They had the longest duration that ranged from 4 to 7 days with a mean value of 5.102 ± 0.051 days.

Fifth instar nymphs moulted to adults. Adults were black in colour with functional wings. Sexual dimorphism was evident in *B. pallescens*. Females were larger in size than males and had broader abdomen with ventral copulatory tubes. The abdomen was narrow with a slight kink towards the left side in case of males. Mean longevity of females at 52.03 ± 1.336 days, was greater than that of males with a corresponding value 40.18 ± 1.163 days.

The findings of the present investigations on duration of developmental stages are in agreement with those of similar studies previously reported. Sobhy *et al*. (2014), for instance, had reported a mean incubation period of 5.53 days at 25 ^{0}C. However, Ballal *et al*. (2003) had observed the mean incubation period of *B. pallescens* to be 4.5 when reared on *C. cephalonica* eggs. The higher mean incubation period observed in the present study could have been due to differences in ambient conditions under which the study was conducted. Observations by Sobhy *et al*. (2014), who reported that the developmental time of *B. pallescens* was significantly shorter at higher temperatures supports the above conclusion.

Shorter incubation period for *B. pallescens* eggs has also been reported on other hosts such as *Sitotroga cerealella* (4.6 days) by Gupta *et al*. (2018) and on *Oligonychus coffeae* (4.4 days) by Srikumar *et al*. (2017).

Table 1. Duration of life stages of *Blaptostethus pallescens* on *Corcyra cephalonica* eggs

Life stage	Mean days ± SE	Range
Egg*	5.782 ± 0.131	4-15
Nymph**		
First instar	2.636 ± 0.057	2-5
Second instar	1.926 ± 0.041	1-3
Third instar	2.018 ± 0.022	1-3
Fourth instar	2.500 ± 0.060	2-5
Fifth instar	5.102 ± 0.051	4-7
Total nymphal period	13.46 ± 0.104	13-23
Adult***		
Male	40.18 ± 1.163	25-63
Female	52.03 ± 1.336	34-70

* Mean of 234 observations **Mean of 115 observations ***Mean of 50 observations

The mean larval duration of 13.46 observed in the present study broadly agreed with those of previous reports. Tawfik and El- Husseini (1971), who reared *B. pallescens* on different prey like lepidopterous larvae, aphids and mites, reported that the bug had five nymphal instars with duration of 2-6, 2-3, 2-3, 2-4 and 4-6 days. However there are reports on longer nymphal period. Devi (2012) also recorded mean nymphal duration of *B. pallescens* to be 18.3 days, while, Ballal *et al.* (2003) had reported a shorter duration of 16.3 days on eggs of *Corcyra cephalonica*.

The mean adult longevity of 40.18 and 52.03days for males and females respectively, are identical to the average longevity of 42.4 and 58.2 days for males and females respectively, reported by Ballal *et al.* (2003), who also reared the bugs on eggs of *C. cephalonica*. Several studies have also reported adult longevity values that vary from the above findings, albeit on different hosts. Thus, Gupta *et al.* (2018) reported a mean longevity of 47.4 and 31.25 days respectively for females and males of *B. pallescens* on *Sitotroga cerealella*. Srikumar *et al.* (2017), however, reported a much lower longevity of 33.57 and 28.01 days for females and males of the bug respectively when reared on tea mite, *O. coffeae*. It is apparent that the above variations could be due to the differences in the hosts on which the bugs were reared.

Reproductive biology of *Blaptostethus pallescens*

Post mating, females laid eggs after a mean pre-oviposition period of 4.2 ± 0.164 days. Egg laying continued for an average of 39.42 ± 1.029 days and was followed by a mean post oviposition period of 8.64 ± 0.807 days. Number of eggs laid per day ranged from 0 to 15.

The adults readily mated when paired. Female bugs laid eggs after a mean pre-oviposition period of 4.2 days which was identical to the 4.1 days was reported by Devi (2012) as well as the 4.05 days at 25 ^{0}C by Sobhy *et al.* (2014).

After the pre oviposition period, egg laying continued for an average of 39.42 ± 1.029 days. Oviposition period was followed by a mean post oviposition period of 8.64 ± 0.807 days.

The observations on oviposition and post oviposition periods of *B. pallescens* females showed wide variation with the previous reports. Both values were greater than the oviposition period of 20.92

(at 25 ^{0}C) days and post oviposition period of 4.45 reported by Sobhy *et al.* (2014) as well as the 12.0 and 1.7 days respectively, reported by Devi (2012).

Fecundity

Adult females of *B. pallescens*, on an average, laid 134.04 eggs within a range of 99-211 in its lifetime. This is comparable with the mean production of 143 nymphs reported by Ballal *et al*. (2003) as well as 136 nymphs reported by Srikumar *et al*. (2017). However, a number of studies have reported substantially lower fecundity when the bug was reared on different hosts. For instance, Tawfik and El- Husseini (1971) recorded considerable variation in fecundity, with values of 78, 13.2 and 5.7 eggs when the bugs were fed with lepidopteran larvae, aphids and mites respectively. Devi (2012) recorded an average fecundity of 53.0 eggs on *C. cephalonica* while Gupta *et al.* (2018) noted that a female bug on an average laid 91.25 eggs when reared on another lepidopteran, *S. cerealella*. El- Basha (2016) observed that the mean fecundity of *B. pallescens* varied significantly based on the crop on which the host (*T. urticae*) was reared. Total lifetime fecundities observed on bean, brinjal, pepper and cucumber were 70.0, 54.3, 48.0 and 22.9 eggs respectively.

Sex ratio

B. pallescens exhibited a female biased sex ratio of 1.276: 1. Several authors like Tawfik and El-Husseini (1971), Ballal *et al.* (2003), and Srikumar *et al.* (2017) have reported identical female: male ratio of 1.2: 1. Devi (2012) recorded a sex ratio of 1.1:1 while Gupta *et al.* (2018) documented a ratio of 1.44:1 and 1.5:1 when reared on *Sitotroga cerealella* and *Corcyra cephalonica* eggs respectively. The findings of present study broadly agree with the previous reports.

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Butterflies (Lepidoptera) of Thusharagiri, Kerala, India

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ABSTRACT: Survey conducted on butterflies of Thusharagiri, Kozhikode, Kerala State identified 59 species under 6 families; 29 species under Nymphalidae, nine species belongs to Papilionidae, seven each in Pieridae and Hesperiidae, six species belong to Lycaenidae, and one species in Riodinidae. The information regarding the diversity of butterflies forms a baseline data for future studies. © 2020 Association for Advancement of Entomology

KEYWORDS: Butterflies, Thusharagiri, Lepidoptera, Nymphalidae

The earliest known butterfly fossils are from mid Eocene epoch, in between 40-50 million years ago. Their development is closely linked to the evolution of flowering plants and which are probably evolved from moths. Butterflies are sensitive biota, which get severely affected by environmental variations and changes in forest structure (Pollard, 1991). They are the food chain of birds, reptiles, amphibians, spiders and predatory insects. They also respond to disturbances and changes in the quality of habitat, and are thus a good indicator species to evaluate changes in habitat and landscape structure variations (Kremen 1992; Kocher and Williams 2000). Butterflies and their caterpillars are dependent on specific host plants for food, thus the diversity of butterflies indirectly reflects overall plant diversity especially that of shrubs and herbs in the given area (Padhye *et al*., 2006). Most of them are strictly seasonal and prefer only particular set of habitats (Kunte 1997).

Butterflies are found throughout the world and are seen in large number (about 45,000 species) throughout tropical belt, which are categorized into 6 different families (Lamas, 2008), however they are not found in Antarctica. India has around 1,501 species of butterflies, out of which 334 species are reported from the Western Ghats and 37 species are endemic to the Western Ghats (Evans 1932; Kunte 2000). Of the 334 species of butterflies of Western Ghats, 316 species have been reported from Kerala (Palot *et al*., 2012).Very little documentation has been done on butterfly fauna in Kerala. Some of the earlier documentation on butterfly fauna from Kerala and adjacent areas include Mathew and Rahamathulla (1993), who had reported 100 species of butterflies from Silent Valley National Park, Sudheendrakumar *et al*. (2000), who reported 124 species of butterflies from Parambikulam Wildlife Sanctuary. Arun (2003), reported 75 species from Siruvani Reserved

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Forests; Ambrose and Raj (2005) reported 24 species from Kalakkad-Mundanthurai Tiger reserve; Eswaran and Pramod (2005) reported 75 species from Anaikatty near Coimbatore; Prasad *et al. (*2010) recorded 52 species from Kerala University campus, Thiruvananthapuram, while Toms *et al*. (2010) reported 109 species from Mahatma Gandhi University campus, Kottayam. Susanth and Rajasree (2012) studied butterflies in different habitats of Vazhachal- Athirapilly reserve forest. In 2014 Aneesh *et al.* reported 139 species of butterflies under six families from Kerala agricultural university campus, Trissur, Kerala, India. An attempt has been made to document diversity of butterflies in Thusharagiri, Kozhikode, Kerala and the findings are presented in this paper.

Study area Thusharagiri is located at 11.28 North, 76.3 East at an elevation of 15 m. It is located about 51 km away from Kozhikode town."Thusharagiri" means "snow-capped mountains". The major economy of the region comprises tourism and agriculture. It is a picturesque location, famous for its waterfalls. It comprises Erattumukku, MazhavilChattom and Thumbithullumpara. Of the three waterfalls, the highest one falls from an altitude ranges from 21.4°C to 33.5°C, and the average humidity of the region is about 52%. The butterfly fauna of Thusharagiri was surveyed from January 2020 to March 2020. The survey was conducted weekly from morning 10 AM to12.30 PM. The butterfly species were also photographed from different angles to enable positive identification of the species. Photographs were taken in Nikon D3500. Butterflies were primarily Species iidentified directly in the field with the help of field guides. Species identity was confirmed with the help of the field guides by Kunte (2000) and Kehimkar (2008), taxonomy and nomenclature have been updated after Kunte *et al*. (2011). The observed butterflies were categorized into 6 families. Butterflies observed were categorized into three groups based on their abundance during the period of study. Accordingly, those species observed 60–100 % of the survey days were categorized as common, 40–60 % as uncommon, 40–60 %, and below 40% as rare.

A total of 59 species belonging to six families were identified from Thusharagiri. Of these four species are endemic to Western Ghats and six species protected under various schedules of the Indian Wildlife (Protection) Act, 1972. Family Nymphalidae commonly called brush footed butterflies, 29 species belongs to this family, which is the largest family. Family Pieridae is commonly called whites and yellows, 7 species belongs to this family. 6 species belongs to the family Lycaenidae. They are known as blues. 7 species belongs to the family Hesperiidae, which is called skippers because of skipping and bounding flight exhibited by its members. Family Riodinidae is represented by one species, commonly called as judies and punches. 9 species belongs to the family papilionidae, usually called as swallow tails. The study showed that the family Nymphalidae is the most diverse butterfly family in Thusharagiri. The above observations are quite significant and it emphasizes the importance of Thusharagiri water fall area in the conservation of biological diversity.

Table 1. Number and percentage distribution of species under different Families

Sl.No	Family	Species Number	Percentage
1	Papilionidae	9	15.25
2	Pieridae	7	11.86
3	Nymphalidae	29	49.15
4	Lycaenidae	6	10.16
5	Hersperidae	7	11.86
6	Rionidae	1	1.69

CHECKLIST OF BUTTERFLIES OF THUSHARAGIRI

Habitat: Semi-evergreen and Riparian forest

Sl.No	Common Name	Scientific Name	Remarks
Family: Hesperiidae (Skippers)			
01.	Brown Awl	*Badamia exclamationis*	Common
02.	Suffused Snow Flat	*Tagiades gana*	Uncommon
03.	Bicolour Ace	*Sovia hyrtacus*	Rare, WG Endemic
04.	Chestnut Bob	*Iambrix salsala*	Common
05.	Coon	*Psolos fuligo*	Common
06.	Blank Swift	*Caltoris kumara*	Uncommon
07.	Indian Dartlet	*Oriens goloides*	Common
Family: Papilionidae (Swallowtails)			
08.	Southern Blue bottle	*Gaphium sarpedon*	Common
09.	Tailed Jay	*Graphium agamemnon*	Common
10.	Common Rose	*Pachliopta aristolochiae*	Common
11.	Malabar Rose	*Pachliopta pandiyana*	UncommonWG Endemic
12.	Crimson Rose	*Pachliopta hectar*	Common, **Schedule 1**
13.	Southern Birdwing	*Troides minos*	UncommonLargest butterfly in India
14.	Common Mormon	*Papilio polytes*	Common
15.	Red Helen	*Papilio helenus*	Common
16.	Blue Mormon	*Papilio polymnester*	Uncommon
Family: Pieridae (Whites and Yellows)			
17.	Three-spot Grass Yellow	*Eurema blanda*	Common
18.	Common Emigrant	*Catopsilia pomona*	Common
19.	Chocolate Albatross	*Appias lyncida*	Common, **Schedule 2**
20.	Common Albatross	*Appias albino*	Common.Migration observed (100 individuals per 1 minute
21.	Plain Puffin	*Appias indra*	Common
22.	Painted Saw tooth	*Prioneris sita*	Uncommon **,Schedule 4**
23.	Psyche	*Leptosia nina*	Common
Family: Riodinidae (Judies and Punches)			
24.	Plum Judy	*Abisara bifasciata*	Common
Family: Lycaenidae (Blues)			
25.	Pale Four-Line blue	*Nacaduba hermus*	Uncommon
26.	Common Cerulean	*Jamides celenobhairana*	Common

Sl.No	Common Name	Scientific Name	Remarks
27.	Metallic Cerulean	*Jamides alecto*	Common
28.	Common Pierrot	*Castalius rosimon*	Common
29.	Tiny Grass Blue	*Zisula hylax*	Common
30.	Common Hedge Blue	*Acytolepis puspa*	Common
Family: Nymphalidae (Brush-footed butterflies)			
31.	Blue Tiger	*Tirumala limniace*	Common
32.	Dark Blue Tiger	*Tirumala septentrionis*	Common
33.	Double-branded Crow	*Euploea sylvester*	Common
34.	Common Crow	*Euploea core*	Common
35.	Malabar Tree Nymph	*Idea malabarica*	UncommonWG Endemic
36.	Common Nawab	*Polyura athamas*	Common
37.	Common Evening Brown	*Melanitis leda*	Common
38.	Great Evening Brown	*Melanitis zitenius*	Uncommon
39.	Tamil Treebrown	*Lethe drypetis*	Common
40.	Common Five-ring	*Ypthima baldus*	Common
41.	Common Four-ring	*Ypthima huebneri*	Common
42.	Tawny Coster	*Acraea violae*	Common
43.	Small Leopard	*Phalanta alcippe*	Uncommon
44.	Cruiser	*Vindula erota*	Common
45.	Tamil Yeomen	*Cirrochro athais*	UncommonWG Endemic
46.	Rustic	*Cupha erymanthis*	Common
47.	Commander	*Moduza procris*	Common
48.	Common Sergeant	*Athyma perius*	Uncommon
49.	Colour Sergeant	*Athyma nefteinarva*	Uncommon
50.	Common Lascar	*Pantoporia hordonia*	Common
51.	Common Sailer	*Neptis hylas*	Common
52.	Sullied Sailer	*Neptis natahampsoni*	Rare
53.	Chestnut-streaked Sailer	*Neptis jumbah*	Common
54.	Clipper	*Parthenos sylviaviens*	Uncommon, **Schedule 2**
55.	Grey Count	*Cynitia lepidea*	Common, **Schedule 1**
56.	Common Map	*Cyrestis thyodamas*	Common
57.	Chocolate Pansy	*Junonia iphita*	Common
58.	Grey Pansy	*Junonia atlites*	Common
59.	Great Egg fly	*Hypolimnas bolina*	Common, **Schedule 1**

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