# MASS TRAPPING OF COWPEA POD BORER *Maruca vitrata* (F.) (LEPIDOPTERA; PYRALIDAE) USING SEX PHEROMONES

*by*

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## DECLARATION

I, hereby declare that this thesis entitled **"MASS TRAPPING OF COWPEA POD BORER *Maruca vitrata* (F.) (LEPIDOPTERA; PYRALIDAE) USING SEX PHEROMONES"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

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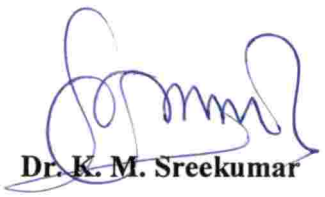
## CERTIFICATE

Certified that this thesis entitled **"MASS TRAPPING OF COWPEA POD BORER *Maruca vitrata* (F.) (LEPIDOPTERA; PYRALIDAE) USING SEX PHEROMONES"** is a record of research work done independently by Ms. EUREKHA SAVADATTI under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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# 1. INTRODUCTION

India is a major pulse growing country in the world with the production of 22.40 million tonnes from 29.28 million ha (GOI, 2017).The commonly grown major pulse crops in India are pigeon pea, mung bean, urd bean, chickpea, horse gram, cowpea and some of the minor pulse crops are dry bean, moth bean, lathyrus, lentil and peas (Mahalakshmi *et al.*, 2015).

The Legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) is an important insect pest of grain legumes in the tropics and subtropics. The Indo-Malaysian region is considered as the most probable origin of *Maruca vitrata* (CABI, 2002). It is widely distributed in Africa, Asia, and Central and South America (Singh and Taylor, 1978). Generally, larvae feed on 20 genera of plants, majority of which belong to family Papilionaceae (Akinfenwa, 1975).

The larval destructiveness at critical stages of crop growth *viz,* flowering and pod development stages, most of the economic plant parts such as flower buds, flowers and pods, it have become a major constraint to achieve the maximum productivity from grain legumes. Importance of *M. vitrata* as a pest on grain legumes results from its early establishment on the crop. The larvae web the leaves and inflorescence and feed inside flowers, flower buds and pods. Third instar larvae are capable of boring in to the pods, and occasionally into peduncles and stems (Taylor, 1967).

Chemical insecticides are widely used for the management of this pest often to the exclusion of other methods of control. However, it results in adverse effects (Sodavy *et al.*, 2000). Insecticides are ineffective and uneconomical against *M. vitrata* due to cryptic larval feeding habits (Sharma, 1998). Recently, *M. vitrata* has earned reduced susceptibility to insecticides that have been effective previously (Ekesi *et al.,* 1999). The limited success of these control methods suggest for the

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evolution of alternative methods like semiochemical based pest management that is precise, efficient, effective, inexpensive and environmentally safe.

A great deal of research has been undertaken concerning use of pheromones in pest management. While (*E,E*)-10,12-hexadecadienal (EE10,12-16:Ald) was identified as the major sex pheromone component of *M. vitrata* (Adati and Tatsuki,1999), (*E,E*)-10,12-hexadecadienol, (*E*)-10 hexadecenal (Downham *et al.*, 2003) and (*Z,Z,Z*)-3,6,9-tricosatriene (ZZZ3,6,9-23:H) (Hassan, 2007) were described as minor pheromone components.

A blend of these components attracted males in field trapping experiments in Benin, West Africa (Downham *et al.*, 2004) but not in Taiwan (Schlager*et al.*, 2012), India (Hassan, 2007), Thailand and Vietnam (Srinivasan *et al.*, 2013) in Southeast Asia. This may be due to the possible existence of polymorphism in blend composition of *M. vitrata* sex pheromone among populations from different geographical regions (Schlager *et al.*, 2012). Pheromone blends for each geographical regions are to be developed based on the variations in pheromone production and reception in *M. vitrata.* In India, M/s Pest Control India Private Limited, Bangalore develops commercial pheromone lure for *M.vitrata*. The data on field efficacy of the lure is scanty. Lack of consistency in performance of commercial synthetic lures become hurdle in pheromone trap based management of *M.vitrata* which may lead to loss of confidence on this technology among farmers. Hence, an investigation into the field efficacy of commercial sex pheromone lure available for *M. vitrata* was attempted. As the preliminary studies revealed the efficacy of pheromone low at field, level it demands further investigations to assess the mating behavior of the moths and ascertain the physiological and behavioural response of compounds to *M.vitrata* adults.

# Review of Literature

# 2. REVIEW OF LITERATURE

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## 2.1 COWPEA

Cowpea, *Vigna unguiculata* (L.) Walp. is an important annual legume crop in the tropics which is used for both vegetable as well as seed purpose. Cowpea is a rich source of protein and has high nutritional value. Due to its ability to fix atmospheric nitrogen, it has the potential to thrive well even in poor soils having less organic matter and low level of phosphorus. It is also a drought tolerant crop, which fetches a good price in Indian market. All these attributes make this an important vegetable as well as fodder crop.

Cowpea is attacked by wide array of insect pests that causes heavy yield loss up to 90% (Jackai and Daoust, 1986). Among the insect pests the important ones are aphids, *Aphis craccivora* Koch (Hemiptera: Aphididae); Serpentine leaf miner, *Liriomyza trifoli* (Diptera: Agromyzidae); Pod borers such as spotted pod borer, *Maruca vitrata* (F.) (Lepidoptera: Pyralidae); gram pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae); Blue butterflies, *Lampides boeticus* (L.) (Lepidoptera: Lycaenidae); Pod bugs such as *Riptortus pedestris* (F.) (Hemiptera: Coreidae); *Clavigrella gibbosa* Stal (Hemiptera: Coreidae); and *Nezara viridula* (L.) (Hemiptera: Pentatomidae).

Among the various pests, the legume pod borer, *Maruca vitrata* (F.) is an important one because of its extensive distribution, wide host range, and destructiveness (Taylor, 1967). It is a key pest of cowpea (Jackai, 1995) as well as other legume crops such as beans (Abate and Ampofo, 1996), and pigeon pea (Shanower *et al*., 1999).

The literature related to cowpea spotted pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) its taxonomy, distribution and host range, biology are collected and reviewed below:

## 2.2. TAXONOMY

*Maruca vitrata*. Fabricius. is the scientific name of Cowpea spotted pod borer synonymously called as *Maruca testulalis* Geyer and *Croshipora testulalis* Geyer. Classified under the family Crambidae of order Lepidoptera initially placed below Pyralidae, which is having single L seta on its ninth abdominal segment.

## 2.3 DISTRIBUTION AND HOST RANGE

The Indo-Malaysian region is considered as the most probable center of origin for the genus *Maruca*, including *M. vitrata,* which is found throughout the tropics (CABI, 2002).

It shows broad range of dispersal throughout Africa, South America, southern states of Australia and Asia (Singh, 1990). It is first reported as a pest of beans in Indonesia by Dietz (1914).It is also identified as serious pest of cowpea (Jackai, 1995), beans (Abate and Ampofo, 1996) ,black gram (Taylor, 1978), green gram (Visvanathan *et al.,* 1983), pigeon pea (Shanower *et al.*, 1999) and soybeans (Das and Islam, 1985) in Africa and Asia.

It is a serious pest of pigeon pea in Thailand (Buranapanichpan and Napompeth, 1982), Sri Lanka (Fellows *et al.*, 1977, Bangladesh (Das and Islam, 1985), Eastern Africa (Nyiira, 1971) and West Africa (Taylor, 1978). During off seasons in the absence of host plants *M. vitrata* survive on different hosts like wild leguminous trees and shrubs (Jackai and Singh, 1983).

Larvae of *M .vitrata* generally feed on nearly 39 African host species (Akinfenwa, 1975) of which most of them belong to family Leguminosae. It feeds on other 22 host plants belonging to Moraceae. Caesalpiniaceae, Mimosaceae, Annonaceae, Papilionaceae, Euphorbiaceae, Rubiaceae and Malvaceae (Atachi and Djihou, 1994).

## 2.4 LIFE CYCLE OF *Maruca vitrata*

The eggs of spotted pod borer are generally oval, milky white, translucent and dorsoventrally flattened when attached to plant surface. Female can lay 400 eggs singly or in batches of 2 -16 (Okeyo-Owuor and Ochieng, 1981; Jackai *et al.,* 1990). Eggs are usually faint with reticulate sculpture like markings possessing delicate chorion of triangular or rectangular forms. Eggs are generally laid on flower buds and flowers but oviposition found to happen on fresh leaves, leaf axils, shoots and fresh pods also (Taylor, 1967).

The egg-laying period lasts up to 3 days in a temperature range of 24-27°C (Ramasubramanian and SundaraBabu, 1988). The incubation period lasts for 2 to 4 days with an average of 3.31 days (Vishakantaiah and Jagadeesh Babu, 1980). It is also mentioned that the incubation period varies according to the host such as cowpea, pigeon pea, field bean, urdbean and mung bean.

There are five larval instars, which generally last for 8-16 days depending upon the climate and host plants. Earlier instars are dull white ,whereas the later instars poses black head with prominent dark black or brown spots running from head to thorax on dorsal and ventral surfaces of each body segment. So generally known by the name spotted pod borer. Larval spots tend to fade before pupation, mature larvae measures 17-20 mm in length. Larvae are generally active if falls down, web the leaves, and construct silken thread cocoon around them. Larval feed component also affects the biology of the insect and it preferentially feed on the reproductive organs of the host plant for about two week and then migrates to the pods before pupation. Larvae reared under artificial diet showed short duration (Chaitanya *et al.*, 2012).

Pupa are generally elongate measuring about 13mm in length. Early pupal stage will be greenish in colour later changes to dark brown when fully developed.

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They tends to construct silken cocoon found on dry leaves, flowers and other dead matters of plant. Pupal period lasts for 7-8 days (Chaitanya *et al.*, 2012).

Adult moths are medium sized and both sexes are morphologically identical. Forewings are dark brown with white spots and black edge whereas as hind wings are semi hyaline. Abdominal characters can differentiate adults of male and female. Generally, female possess brownish abdomen with hairy and bifid ovipositor and male with grey abdomen on last abdominal segments with sharp posterior end. (Okeyo-Owuor and Ochieng, 1981).

Adults when in normal resting condition stretch their wings in horizontal position where in other periods tend to fold their wings. Generally the lower surface of the leaves provide good microclimate and protect them from natural enemies. No sign of diapause is noticed. Life span of adults last from 6-10 days. Total biological period of insect completes within 18 to 35 days (Taylor, 1967; Akinfenwa, 1975).

## 2.5 NATURE OF DAMAGE OF *Maruca vitrata*

The larvae are voracious feeders of flower buds, flowers, and young pods (Singh and Jackai, 1990). Thus, infestation can occur at all stages of crop from seedling to pod forming stage. They cause damage to the crop by feeding on the young flower buds and boring into pods. The infested pod with bored holes plugged with excreta render the pods unmarketable and leads to considerable yield loss up to 20-80%.

## 2.6 BEHAVIORAL STUDIES ON *Maruca vitrata* AND OTHER PESTS

Several studies had been undertaken to know the basic biology of *Maruca vitrata* (Taylor, 1967; Singh, 1990), but no experiments exactly determined the adult emergence mating and circadian rhythm of insects except from few basic trials (Jackai *et al.*, 1990; Huang & Peng, 2001). This knowledge provides valuable

information for evolving tractable unified pest management approaches such as monitoring and timely application of insecticides. (Downham *et al.*, 2004).

### 2.6.1 Adult emergence

The study on adult emergence of *M. vitrata* carried out at 29±1°C with a relative humidity of 75-85 % and photoperiod 14L: 10D revealed emergence all over the day with 73% females and 86% males emerged during the dark period (Lu *et al.*, 2007a).

Haung and Peng (2001) reported that adult emergence takes place throughout the day; however, 31% of females and 55% of males emerged at night. Peak emergence was noticed at 03:00-05:00 for females and 13:00-15:00 h for males and sex ratio was found to be 0.48

A study on adult emergence of *Neoleucinodes elegantalis* Guenee (Lepidoptera: Crambidae) was conducted under laboratory condition at temperature of 23 ± 1°C and RH of 70%,12h photophase and the results revealed that adult emergence was restricted to scotophase. Female emergence started from 1$^{st}$ to 8$^{th}$ h with peak incidence during 4$^{th}$ hour of dark phase whereas, male showed emergence from second to 11$^{th}$ hour with peak incidence at 4$^{th}$ hour of scotophase as in case of female (Eiras, 2000).

### 2.6.2 Female calling behaviour

Studies on female calling activity of pod borer have not reached harmony regarding the mating behaviour and recurrence of courtship (Lu *et al.*, 2007a; Lu *et al.*, 2007b and Wen *et al.*, 2009). Moreover, the consequence of regular daily cycle and age on sex pheromone concentration and male responsiveness remains mysterious. Hence, better understanding about chemical communication can support to develop sex pheromone strategies to monitor these populations in the field.

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The study on calling behaviour of *Nephopterix* sp. (Lepidoptera: Pyralidae) was found to be age-dependent. The insects were most likely to call when they are at 2 to 5 days old. The effects of age on the outset of calling and the time consumed for calling were irregular. The inception of mating also changed irregularly with age but time used up for mating reduced directly with age. Results also conveyed that change in calling and mating sequence with age was match up with enlarged reproductive pubescence of females (Witethom, 1992).

The study conducted by Mazomenos *et al.* (2002) on pheromone biology of *Palpita unionalis* (Rossi) (Lepidoptera: Pyralidae) was carried out in 14L: 10D. The results revealed that insects emerged 2 to 3 hours prior to lights on during scotophase. Female started calling during second day after emergence with maximum calling at $4^{th}$ hour of scotophase with 68.5 %.Calling action was commenced during the 5 to 6 h after lights out and ceased during 7 to 8 h at night. The mean inception of calling proceeded from 6.4 h on $2^{nd}$ day of night to 5.1 h on $7^{th}$ day of night. The mean time consumed for calling was almost identical from 2 to 5 days of dark period and reduced for the succeeding two scotophases ($6^{th}$ and $7^{th}$ day).

The study on calling activity of virgin females *Condylorrhiza vestigialis* Guenee (Lepidoptera: Crambidae) carried under lab conditions. Most of them initiated calling after emergence during first hour of scotophase. Maximum calling commenced between $7^{th}$ and $10^{th}$ hour of night. The span of calling proliferated with age until the $4^{th}$ hour of scotophase and number of calling session increased notably with age (Ambrogi *et al.*, 2009).

### 2.6.3 Mating behaviour

Mating behaviour in moths show some general pattern described below:

On initiation of the calling by females, the males become active and they initiate flight and searching with their antennal movements. The courtship behaviour

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of male mainly consisted of perception of female calling signals, searching wing fanning and approaching the receptive female and copulation. Once male moths reach the calling female goes around and then head towards the front of the female. On their orientation, facing head to head, the antennae is exposed to one another and then exhibit wing expansion. This act followed by the male going over the female and then turns back. Both male and female expand their abdomen, they contact through extruding abdominal tips, and mating takes place.

Published literature on mating behaviour of *Maruca vitrata* is scanty except from few preliminary experiments.

Mating behaviour of *M. vitrata* was observed at 29±1 ºC with 75-85 % RH and 14L: 10D(Lu *et al.*, 2007a) noticed Mating was to found to happen from 19:00 to 05:00 h and duration ranged from 20 to 90 min.

Mating behaviour of *M.vitrata* moth depends on age with maximum frequency noticed during 5$^{th}$ hour of night in case of 3 day old moth. It is also noticed that single mating peak happened in case of one, 6 and 7 days old moth whereas in 2-5 days old moths dual mating peaks are noticed (Lu *et al.*, 2008).

Haung and Peng (2001) revealed that adults of *M.vitrata* started to mate at 21:00h, mating time lasted for 44.4±34.3 min and highest mating frequency occurred in 3-day-old moths.

The mating of *Maruca vitrata* occurred between 4$^{th}$ and 12$^{th}$ hour of scotophase at temperature 20-25 ºC with RH of 80%. Peak activity happened between 8$^{th}$ and 9$^{th}$ hour after scotophase initiated. While in conveying mating readiness, the male proceed towards the female sideways and then hangs around for few minutes. It is also noticed that highest mating and oviposition found in 4 or 5 nights of pairing. Among females, most of them mated only once but in males, there was multiple

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mating. For oviposition and mating, male, female ratio of 1:1 gave best results (Jackai *et al.*, 1990).

A study conducted by Lu *et al.* (2007a) on mating of *M. vitrata* in China revealed that mating behaviour depends on age and occurs throughout the dark phase. Mating frequency peaked between 23:00 and 24:00 h for 3-day-old moths. Advanced outset of time and peak mating was observed in older moths, which is not seen in case of younger moths. As the temperature increased, highest mating peaks observed in young moths but outset time and mating duration found to be high in old aged moths at cool temperature. Shortened photoperiod results in delayed mating.

The study on mating behaviour of *Nephopterix* sp. (Lepidoptera: Pyralidae) was found to be age-dependent. The insects were most likely to mate when they are at 2 to 5 days old. The occurrence of mating also changed non-linearly with age but time used up for mating reduced directly with age. Results also conveyed that change in mating sequence with age was match up with enlarged reproductive pubescence of females (Witethom, 1992).

Eiras (2000) reported that mating happens only during the scotophase period during the $4^{th}$ and $10^{th}$ h of darkness, with the maximum occurrence at the $7^{th}$ h. Recently emerged *N. elegantalis* moths are occasionally mated with 2.8% whereas 2 day old moths mated with 26.3% and 4 day old moth mated with 27.5%.

Study conducted by Xu *et al.* (2008) on *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) showed emergence throughout the day with peak incidence at scotophase. Most of the time mating is observed during scotophase that peaked during emerged day. Most of the mating peaked at second part of scotophase.

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### 2.6.4 Studies on female sex pheromone

In moth's female, that produce sex pheromone for which male elicit specific reaction that is mainly responsible for procreation of species initiates sexual communication (Wyatt, 2009). Sex pheromone is main key of sexual cue employed by moths. (Carde and Minks, 1995; Linn and Roelofs, 1994). Generally, female moth's produces chemical cues in very small amount to attract mate from a vast distance.

Sex pheromone production and release typically depends on age of moths, its mating level and pheromone gland development (Shorey *et al.,* 1968; Miller and Roelofs, 1978; Schal *et al.*, 1987; Raina *et al.,* 1986; Babilis and Mazomenos, 1992; Tang *et al.*, 1992 and Del Mazo-Cancino *et al.*, 2004).

In moths sexual communication system consist of 2-7 compounds with chain of 10-12 carbon atoms, which includes aldehydes, acetates and alcohol produced in specific portion. Pheromones traps can be used efficiently in crop protection for species-specific integrated pest management technique. The new techniques have been for monitoring adults of *Maruca vitrata* by using pheromone traps (Bottenberg *et al.*, 1997; Downham*et al.,* 2002).

Gut *et al.* (2004) reported that monitoring of adult emergence using sex pheromones helps to decide when to spray insecticides. El-Sayed *et al.* (2006) reported that pheromone traps are sensitive to check low population density and are effective for tracing the invasive species in the development stage.

### 2.6.5 Pheromonal components of *Maruca vitrata* and other insects

Experiments show that purified synthetic extract (*E*, *E*)-10, 12-hexadecadienal (*E*10, *E*12–16: Ald) obtained from the abdominal tip of *M. vitrata* with 99% isomeric purity attracted male moths (Adati and Tatsuki, 1999).

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They observed that (*E, E*)-10, 12-hexadecadienal (E, E)-10, 12-16: Ald) as an electroantennogram (EAG) active component in the extract from the abdominal tip of female *Maruca* which is effective component to trap males.

According to Downham *et al.* 2003, traps baited with a three-component blend of (*E, E*)-10, 12-hexadecadienal and (*E, E*)-10, 12-hexadecadienol and (*E*)-10-hexadecenal in the ratio 100:5:5 caught significant number of male moths.

Unmole (2009) reported that synthetic pheromone having (E, E -10, 12-16: Ald) alone or in combination with one or more minor components (E, E 10, 12-16: OH and E 10-16: Ald) are effective to catch males of *M.vitrata* but there will be geographical variation among populations.

According to Schlager *et al.* (2015), the pheromone containing a mixture of (E, E) -10, 12 –hexadecadienal (major compound) and (E, E)-10, 12 hexadicadienol and (E)-10-hexadecenal (minor compound) are most attractive to trap males of *M. vitrata.*

Males of *Maruca vitrata* where trapped in water traps baited along with virgin female moths thus gave a cue for pheromone extraction from female moths in Kenya.(Okeyo-Owuor and Agwaro, 1982).

Cork *et al.* ( 2001) conducted experiment and done analysis for female extracts from brinjal fruit and shoot borer, *Leucinodes orbonalis* Guenee (Lepidoptera: Crambidae) of Taiwanese and Indian origin established (E)-11-hexadecenyl acetate (E11-16:Ac) as the vital pheromone component with 0.8-2.8 per cent of the related (E)-11-hexadecen-1-ol (E11-16:OH).

Kuenen *et al.* (2010) identified four vital components from extracts of pheromone glands in *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) in the ratio of 100:100:5:5. They also identified other minor components such as (11E, 13Z)

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- hexadecadienal; (11Z, 13E)-hexadecadienal, (Z)-11-hexadecenal; hexadecanal, (11Z, 13Z)-hexadecadien-1-yl acetate; (3Z, 6Z, 9Z, 12Z, 15Z)-pentacosapentaene (C25pentaene), hexadecan-1-ol; and a second pentaene these components did not show any attraction for male insects.

Baker *et al.*, 1991 extracted three important sex pheromone components from pheromone gland discharge of the carob moth, *Ectomyelois ceratoniae* (Zeller)(Lepidoptera: Pyralidae) and performed EAG and GC-MS analysis by various techniques. They identified (Z, E)-9, 11, 13-tetradecatrienal as a major component.

E1–10–hexadecenal (E10–16: Ald) as a major component and two hexadecanal (16: Ald) and (Z)-10-hexadecenal (Z10-l6: Ald) were identified as minor components of the sex pheromone extracts of *Conogethes punctiferalis* Guenee. Investigation of single sex pheromone discharge done by capillary gas chromatography specified that the comparative ratio of 16: Ald, Z10–16: Ald and E10–16: Ald was equal to 13: 6.6:80.4 respectively (Liu *et al.*, 1994).

Sex pheromone extracted from virgin female moths sweet potato vine borer *Omphisa anastomosalis* (Guenee) (Lepidoptera: Crambidae) found single EAG Active component (10E, 14E)-10, 14-hexadecadienal (E10E14-16: Ald and identified other minor components (10E) - 10- hexadecenal (E10-16: Ald), (14E)-14-hexadecenal (E14-16: Ald and hexadecanal (16: Ald) at 0.7:3.0:3.1% respectively. It is also found that there was no increase in attraction when major compounds are combined with minor components (Wakamura *et al.*, 2010).

### 2.6.6 Bioassay pheromone constituents of *Maruca vitrata* and other pests

Mondhe (2001) tested the 3-component *M. vitrata* pheromone and natural female ovipositor extracts. He reported that for synthetic blend, most of the male moths were unresponsive (> 80 percentage), but a few flew upwind and hovered in

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front of the pheromone source (< 10 percentage each). Statistically there were no significant differences in response levels between the 3-component *M. vitrata* lure and the hexane solvent control. On the contrary, he found that female ovipositor washings resulted in 57 per cent of tested male moths making source contact or landing on the source.

Study conducted by Hassan (2007) in wind tunnel experiment revealed that most of the male attracted to the major component, EE10, 12-16: Ald, which was found to be equivalent with female ovipositor washings extract. The attraction significantly improved by addition of 10 per cent E10-16: OH to the major component. The poly unsaturated hydrocarbons ZZZ3,6,9-23:H and ZZZZZ3,6,9,12,15-23:H (two new components of *M. vitrata* pheromone) elicited behavioral responses from male moths in association with the major component.

Experiment conducted by Wang *et al.* (2015) showed that males of *Maruca vitrata* elicited electroantennogram response to primary sex pheromone component EE 10, 12-16 Ald . There were significant differences among the tested males with different day-ages. Third day male moth after emergence showed higher antennal response. Similarly, in wind tunnel experiment male after emergence elicited major response during third day old and found to decline in later age.

The extract from the pheromone gland of the female moth jasmine bud borer, *Trichophysetis cretacea* (Butler) (Lepidoptera: Crambidae) which is an important pest of jasmine in China was analyzed by (GC-MS) gas chromatography coupled to mass spectroscopy. (Z)-11-hexadecenol (Z11-16: OH), (Z)-11-hexadecenal (Z11-16: Ald) and (Z)-11-hexadecenyl acetate (Z11-16: Ac) were recognized as sex pheromone components (Peng *et al.*, 2012). From the review, it is clear that there are differences in the field efficacy of pheromone for the test insect *M.vitrata*. So, testing the performance of the commercial lures available in our country is imperative.

# Materials and Methods

# 3. MATERIALS AND METHODS

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This study was conducted to assess the practical utility of synthetic sex pheromone of *Maruca vitrata* developed by Pest control India Ltd. Bangalore to mass trap spotted pod borer *Maruca vitrata* and to study the response of male moths to the synthetic lure components and investigate female calling of *Maruca vitrata*.

## 3.1 FIELD STUDIES

The experiment was conducted at instructional farm at college of Agriculture Padannakkad during September –December in 2017. Cowpea was raised in five-cent area at two isolated plots separated by 500m in which one was kept as control plot. Two *M.vitrata* commercial pheromone traps procured from M/s Pest Control India Limited Bangalore were installed in each plot. Number of adult moths trapped at weekly intervals were recorded.

Survey was conducted during September-December 2017 in cowpea growing areas of the district such as Periye, Bedakam and at the instructional farm of College of Agriculture Padannakkad. Also, at Pattambi in Palakkad district. Different types of traps such as funnel, bucket, delta and water traps were installed based on the visual observation of damage caused by *M.vitrata* feeding (plate 1). Observations on the number of moths caught were recorded at weekly intervals.

## 3.2 BEHAVIORAL STUDIES ON *Maruca vitrata*

These studies were done at ICAR-NBAIR, Hebbal, Bangalore

Generally, the female moths releases pheromone during night which cause specific reaction in male moths. There will be constant antennal movements in males as the sign of receptive Pheromonal molecules. Then the male will move towards female which finally results in courtship and mating.

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A) Delta trap

B) Bucket trap

C) Funnel trap

D) Water trap

**Plate 1. Types of traps installed during survey of *M.vitrata* infested area**

### 3.2.1 Rearing of *Maruca vitrata*

Larvae of *Maruca vitrata* were collected from the field during July – September from infested flowers and young pods of cowpea (Plate 2). Larvae were maintained in rearing room at the temperature of 27±2°C and relative humidity of 65-70%, photoperiod of 12L: 12D. Larvae were reared individually to prevent cannibalism by keeping them in transparent plastic containers of 7 cm diameter and 12 cm height size with absorptive paper. First and second instar larvae were provided with flowers and flower buds of cowpea as food. Later instars were fed with pods, flower and flower buds. Waste, excreta and other extraneous material were timely removed. After pupation, pupae were separated based on genital characters and kept in separate boxes. Emerged adults were differentiated based on genital characters in which male poses a sharp forked abdominal tip whereas female shows bifid and hairy ovipositor (Hassan, 2007).

### 3.2.2 Study on adult emergence

Well-developed silken-cocooned late pupae (plate 3) (N=160) were selected and kept in separate containers individually on tissue paper (Ke *et al.*, 1985).The pattern of emergence was recorded at hourly from 0-24 hours. The number of emerged moths were recorded per unit time for each sex (12 L: 12D). Observations were taken during scotophase with help of LED lamp of 2 to 3 watts, which was covered with red cellophane. Emerged moths were separated to males and females based on abdominal features as mentioned earlier (Plate 4). Mean percentage of moths emerged per hour was recorded.

### 3.2.3 Female calling behaviour

Calling behaviour of female moths were observed in laboratory during scotophase to understand the calling pattern. Twenty female moths of one-day-old (0-24h) were confined to a transparent cylindrical plastic container of 12.5 cm dia; 24

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cm height size individually and provided with ventilation. Moths were provided with 10% honey soaked cotton ball as food. The calling of the female moths of 1-8 day old were observed throughout scotophase (lights off at 18:00 and on at 06:00 h) within every 15 minutes interval. Light was provided using LED 3w lamp with red cellophane during scotophase. ANOVA was used to analyze the results.

### 3.2.4 Study on mating behaviour

Soon after the emergence, 15-30 active pairs of one-day-old moths were selected and placed individually in clear ventilated cylindrical plastic containers of 12.5 cm diameter 24 cm height size and left to mate. Observations were initiated instantly after pairing at every 15 min interval up to 8 days. Moist filter paper was provided to maintain humidity and honey soaked cotton balls in the containers. Number of pairs initiated to mate were observed for every 15 min and calculated for each hour. In addition, onset time of mating and duration of mating for each pair recorded. The courtship behavior such as male advancement towards female, exposing its hair pencils and mating position were observed. Virgin male with mated female and mated male with virgin female were observed separately to know the possibility of multiple mating.

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**Plate 2.Larva of *M.vitrata* from infested cowpea**

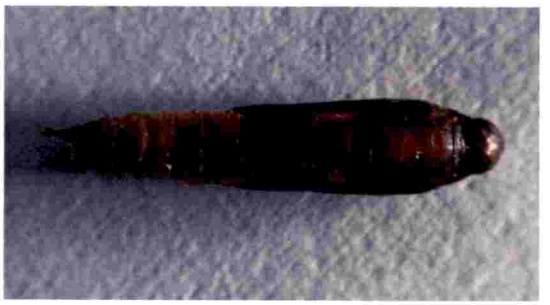

**Plate 3. Late pupa in silken cocoon and pupa of *M.vitrata***

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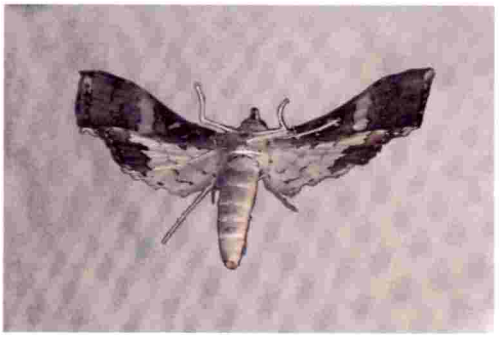
A .Male moth with forked abdomen tip



B. Female with tapered abdomen

**Plate 4. Male and Female moths of *M.vitrata***

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## 3.3 ELECTROANTENNOGRAPHY (EAG) ASSAY FOR TESTING COMMERCIAL LURE

Electroantennography (EAG) bio- assay is achieved for rapid confirmation and screening of different combinations or a blend of pheromone compounds since it is a flexible method for finding electrophysiological activity of chemicals using alive insects. Electroantennography is the sum of olfactory receptor recorded simultaneously by an electrode in insect antenna where chemosensory organ is located (Barsi, 1998). When the insect antenna is exposed to chemicals its receptors generates depolarization, which is recorded by microelectrode located on either sides of insect antenna. This method gives essential hint for further diagnosis of the bioactive chemical compounds.

Electroantennogram studies were conducted by using GC (Agilent technologies model) coupled to electroantennogram detector (syntech model CS55) (plate.5). Antenna of unmated 2-3 day old male of *M.vitrata* was dissected and kept in an electronic holder and continuous signal was established. Care was taken to reduce the noise by using freshly prepared antenna and appropriate electrolyte. The septa from BCRL was dissolved in 100 micro lit of hexane following standard protocols. From the extract 20 micro lit was taken in a bit of Whatman filter paper, which was kept inside air delivery unit. A puff of stimulus was given for a duration of 500ms and the amplified signals were recorded using GC-EAD software supplied by syntech. A stimulus of hexane was used as control stimulus. These studies were done at ICAR-NBAIR, Hebbal, Bangalore.

### 3.3.1 Gas Chromatography -Mass spectrometry (GC-MS) studies for identification of Pheromonal active compounds.

The gas chromatographic technique is useful in separating the several chemical compounds and checking the purity of isolated compound. Failure to ensure the

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isolated material in pure form will result in thwarted identification of bioactive material. Though 100 per cent separation of several chemical constituents is not ensured in GC, criteria of analysis of a compound were established to detect the purity of the synthesized compound. The effective use of GC for the analysis of pheromones and the determination of their purity needs a clear understanding of the chromatographic process. Capillary Gas Chromatography is the most useful method now available for pheromone analysis for which it provides maximum resolution needed for the separation of complex mixtures and the sensitivity to detect minute amounts of impurities. These studies were done at ICAR-NBAIR, Hebbal, Bangalore.

For identification of the Pheromonal active compound in the commercial lure of the PCI, it was dissolved in hexane using standard protocol. One mic lit of sample injected in to GC-MS .GC-MS analysis was done using Agilent model 6890 N GC interfaced to a 5790C mass selective detector (MSD) (plate.6). In GC-MS, the column used was HP-5MS Phenyl Methyl Siloxane capillary non-polar column. The column oven was maintained in temperature programme 70-260°C for 26min. The mass spectra where matched with whiley mass spectra library reading a compound with more than 95% purity where identified and matched.

### 3.3.2 Coupled gas chromatography - Electroantennography (GCEAD)

These studies were done at ICAR-NBAIR, Hebbal, Bangalore, Synthetic pheromone compound was injected to Agilent GC was fitted with the Flame ionization detector (FID). The split injector with the split ratio of 50:1 was used and HP 5MS phenyl methyl siloxane capillary non polar column (30 m: 0.25 mm: 0.25 µm) was used for the separation of compounds and a flame ionization detector (FID; 250°C) which was ignited using high purity Hydrogen gas and reference gas zero air (99.999%) (White and Chamber, 1989). Carrier gas was helium (99.99% purity) (0.5kg cm-2) and injection temperature 250°C and was split mode. The oven temperature program was set at 70°Cmin-2 with 2 min hold and a ramp of 10°C min-

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1 until 260°C and held for 5 min with column flow of 1 ml/m. The effluent emerging from the column were split using Agilent splitter with makeup gas and the effluents were sent to the EAD and FID (30:1) through a glass wool column of 0.25 mm thickness for the response of antenna to the effluents. One µl of the sample was injected into the injector and traces were recorded using the Agilent Chemstation. The antenna from two days old moths was removed and fitted in the micromanipulator assembly of the electroantennogram and the antennae were kept at close proximity to the stimulus delivery tube described earlier so that the traces of effluents from GC are gently blown on to the antennae after elution in the GC. The traces from FID and EAD were plotted and the matching peaks along with their retention times were measured.

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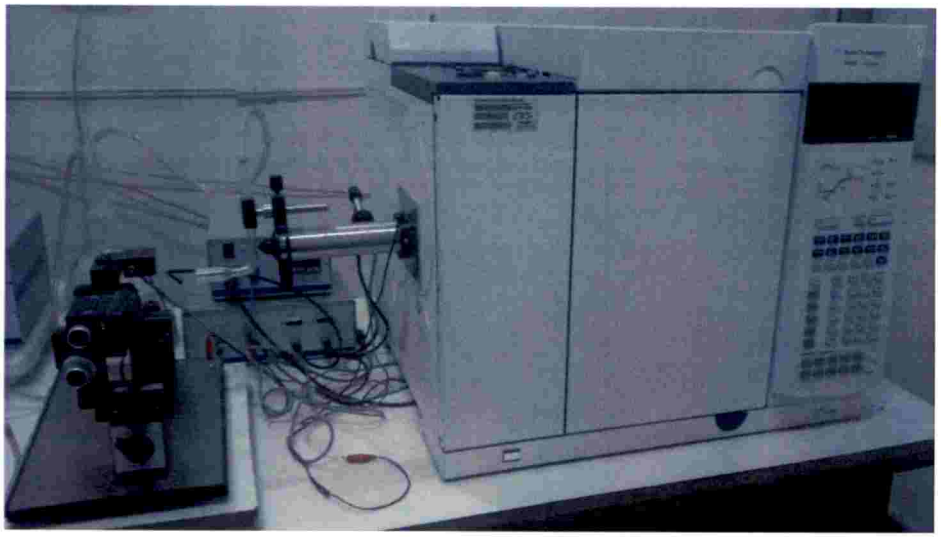

**Plate 5. Electroannegraphy coupled with Gas Chromatography**

**Plate.6 Gas chromatography-Mass-spectrometry (GC-MS)**

# Results and Discussion

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# 4. RESULTS AND DISCUSSION

The study was conducted to know the efficacy of synthetic sex pheromone for the management of legume spotted pod borer *Maruca vitrata.* To understand the female calling and mating behaviour and to study the response of male moths to synthetic lure components.

## 4.1 MASS TRAPPING OF MALE MOTHS OF *M.vitrata*

Survey was conducted in specified areas of Kerala during 2017 Rabi season viz, Periye, Pattambi, Bedakam and instructional farm of College of Agriculture Padannakkad. Damage caused by the insect was assessed and different traps such as funnel trap, water trap, bucket trap and delta trap were installed. One trap was installed per five cents of cropped area. Number of adult moths trapped at weekly intervals were recorded. The details are presented in Table 1.

**Table 1. Results of field experiments to study the efficacy of commercial synthetic pheromone lure**

| Place | No. of plants damaged/plot | No. of larva/plant | Damaged pods/plant | Damaged flowers/plant | No. of traps installed | No. of adults trapped |
|---|---|---|---|---|---|---|
| Periye (Kasaragod) | 150 | 8 | 6 | 2 | 1 | 0 |
| RARS, Pattambi | 120 | 6 | 5 | 1 | 6 | 0 |
| Bedakam(Kasaragod) | 100 | 5 | 4 | 1 | 1 | 0 |
| COA, Padannakkad | 300 | 8 | 5 | 3 | 2 | 0 |

The commercial synthetic pheromone lure used was ineffective in attracting the male moths of *M.vitrata.* This result lead to two questions, viz; whether the insect is actually releasing the sex pheromone for calling the male moths for mating and

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whether the synthetic pheromone lure contains the identified pheromonal molecules. In order to answer these questions, further research work was done.

## 4.2 RESULTS ON BEHAVIORAL STUDIES OF *M.vitrata*

### 4.2.1 Pattern of Adult emergence

Pattern of emergence on *Maruca vitrata* is presented in Table 2 and Table 3.

**Table 2. Pattern of adult emergence of *Maruca vitrata* during 24 hour period**

| Time of emergence (0-24hours) | No. of male moths emerged (1st set) | No. of female moths emerged (1st set) | No. of male moths emerged (2nd set) | No. of female moths emerged (2nd set) |
|---|---|---|---|---|
| 6:00AM | 0 | 0 | 0 | 0 |
| 7:00AM | 0 | 0 | 0 | 0 |
| 8:00AM | 0 | 0 | 0 | 0 |
| 9:00AM | 0 | 0 | 0 | 0 |
| 10:00AM | 0 | 0 | 0 | 0 |
| 11:00AM | 0 | 0 | 0 | 0 |
| 12:00PM | 1 | 0 | 0 | 0 |
| 1:00PM | 5 | 3 | 4 | 0 |
| 2:00PM | 9 | 7 | 7 | 5 |
| 3:00PM | 7 | 3 | 7 | 5 |
| 4:00PM | 10 | 7 | 9 | 8 |
| 5:00PM | 0 | 0 | 0 | 0 |
| 6:00PM | 1 | 0 | 3 | 0 |

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| | | | | |
|---|---|---|---|---|
| 7:00PM | 4 | 6 | 6 | 7 |
| 8:00PM | 0 | 0 | 3 | 6 |
| 9:00PM | 0 | 0 | 0 | 0 |
| 10:00PM | 12 | 22 | 16 | 20 |
| 11:00PM | 4 | 0 | 0 | 2 |
| 12:00PM | 2 | 0 | 1 | 0 |
| 1:00AM | 4 | 5 | 4 | 4 |
| 2:00AM | 8 | 10 | 7 | 9 |
| 3:00AM | 6 | 13 | 8 | 5 |
| 4:00AM | 7 | 4 | 5 | 9 |
| 5:00AM | 0 | 0 | 0 | 0 |

**(N=160, F=80; M=80)**

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**Table 3. Mean emergence pattern of *M.vitrata* moths during 24 hour period**

| Time of emergence(0-24h) | Mean per cent of male moths emerged | Mean per cent of female moths emerged |
|---|---|---|
| 6:00AM | 0 | 0 |
| 7:00AM | 0 | 0 |
| 8:00AM | 0 | 0 |
| 9:00AM | 0 | 0 |
| 10:00AM | 0 | 0 |
| 11:00AM | 0 | 0 |
| 12:00PM | 0.625 | 0 |
| 1:00PM | 5.625 | 1.875 |
| 2:00PM | 10 | 7.5 |
| 3:00PM | 8.75 | 5 |
| 4:00PM | **11.875** | **9.375** |
| 5:00PM | 0 | 0 |
| 6:00PM | 2.5 | 0 |
| 7:00PM | 6.25 | 8.125 |
| 8:00PM | 1.875 | 3.75 |

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| | | |
|---|---|---|
| 9:00PM | 0 | 0 |
| 10:00PM | **17.5** | **26.25** |
| 11:00PM | 2.5 | 1.25 |
| 12:00AM | 1.875 | 0 |
| 1:00AM | 5 | 5.625 |
| 2:00AM | **9.375** | **11.875** |
| 3:00AM | **8.75** | **11.25** |
| 4:00AM | 7.5 | 8.125 |

**(N=160 F=80; M=80)**

Both the sexes were observed throughout the day for emergence (N=160, Female=80 and Male=80). It was observed that 30 per cent of moths emerged during photophase (6am-5pm) and 69.68 per cent of moths emerged during scotophase (6pm-5am).

The moths emerged during photophase had a gender distribution of 36.25 per cent males and 23.75 per cent females whereas during scotophase it was 63.12 per cent males and 76.25 per cent females.

Peak emergence of males was observed at 4pm with 11.88% during photophase but for females, it was 9.38 per cent during scotophase. There was peak emergence at 10 pm for females with 26.25 per cent but for males, it was 17.5 per cent. Another small peak of emergence was observed during 2-3am (scotophase) with 18.12 percent for males and 23.12 per cent for females.

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In males, lowest emergence was recorded with 5.63 per cent at 1pm (photophase) and 2.5 per cent at 6pm during scotophase, whereas for females least emergence recorded with 1.25 percent at 1pm of photophase and 11pm of scotophase.

The study conducted by Huang and Peng (2001) on adult emergence pattern revealed that emergence occurred throughout the day which is found to be similar with the present study. However, disparity was observed in emergence pattern of both sexes. They reported that about 31 per cent males and 55 per cent females emerged during scotophase, which is peaked at 3-5am and 1-3 pm respectively. The cause for this disparity in emergence sequence was not understood exactly in insects. (Thornhill and Alcock, 1983). However, it has been demonstrated in few lepidopteran insects and represents an evolutionary strategy to promote mating between individuals from distinct population (Uematsu and Morikawa, 1997).

Luo *et al.* (2004) revealed that the daily emergence rhythm for *Liriomyza huidobrensis* (Blanchard) and *Liriomyza sativae* Blanchard is strongly influenced by temperature. As temperature increases, the emergence period are shortened. However, the peak emergence for females was more than males during photophase. In another study conducted by Lu *et al.* (2007a) showed that *M.vitrata* emergence was seen throughout the day under 14L: 10D condition in which 73 per cent of males and 86 per cent of females emerged during scotophase.

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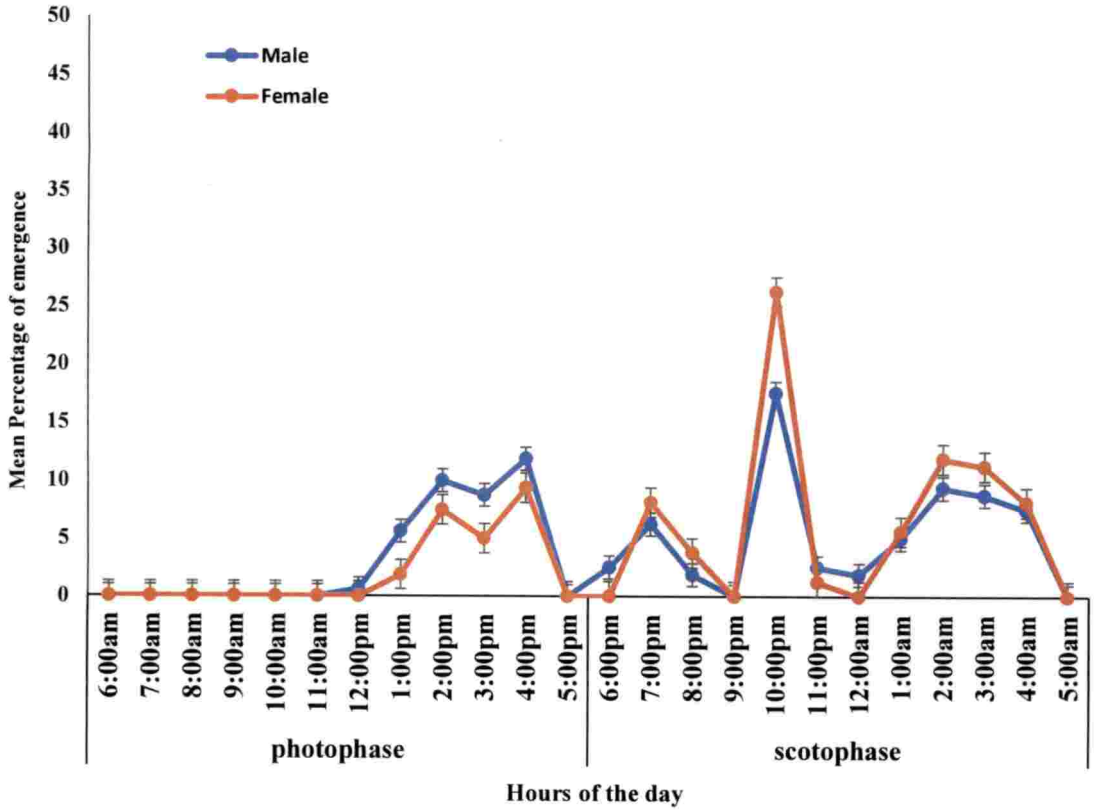


**Fig.1 Mean emergence of *Maruca vitrata* male and female moths (N=160)**

The biotic cause for this disparity among emergence sequence not understood exactly in insects. (Thornhill and Alcock, 1983). However, it has been demonstrated in few lepidopteran insects and represents an evolutionary strategy to promote mating between individuals from distinct populations (Uematsu and Morikawa, 1997).

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One more study conducted by Lu *et al.* (2007a) showed that *M.vitrata* emergence was seen throughout the day under 14L: 10D condition in which 73% of males and 86 % of females emerged during scotophase.

### 4.2.2 Female calling behaviour of *Maruca vitrata*

Calling behaviour of *M.vitrata* female moths showed a specific pattern. There was restless flying initially and later, the moths bend their abdomen in curved manner (Plate 7). Antennal up and down movements were also exhibited as a sign of calling. In response to female calling, males buzzed their wings (Plate 10). The typical sign of calling was that female extruded its pheromone gland in and out during calling period. (Plate 8). The pheromone gland extruded by females was considered as standard clue for calling position and sex pheromone releasing time (Baker and Carde, 1979; Conner *et al.*, 1980 and Webster and Carde, 1982).

The calling behaviour for one to eight day old moths were observed individually throughout scotophase (6pm-5am). Since there is no report of calling during photophase in previous studies (Lu *et al.*, 2007b; Wen *et al.*, 2009) in the current study the observations were restricted to scotophase.

A study on biology of *M. vitrata* reported that adults live up to 8 days (Chaitanya *et al.*, 2012) which was in line with the current study where they lived up to 6-8 days. Hence, the observation were taken from one day to eight-day-old moths. Calling pattern of one to eight day old moths are represented in (Table 5, 6, 7, 8, 9, 10, 11 and 12). Calling was initiated during 2$^{nd}$ hour of scotophase and reached maximum at 6$^{th}$ hour of scotophase in third day old moth (Table 7).Calling females of

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*Maruca vitrata* were correlated with their age. There was no calling observed during the first hour of scotophase irrespective of the moth age (Fig. 2, 3, 4, 5, 6, 7, 8 and 9). Single calling peak was observed at sixth hour for the moths of all age groups except for 3 day old where additional peak of calling at $5^{th}$ and $8^{th}$ hour of scotophase were observed.

Similar trend of calling were recorded in all age groups of moths. There was minimum per cent of calling in initial hours and gradually increased with time. However, there was a decrease after $7^{th}$ hour of scotophase (Fig. 2, 3, 4, 5, 6, 7, 8 and 9). Peak calling of 43.5 per cent was recorded in 3-day-old moths during sixth hour of scotophase, which was statistically significant from 4-day-old moths. The female calling percentage was maximum during $6^{th}$ hour of scotophase for one to eight day old females (14.58; 39.67; 43.75; 26.25; 16.67 ; 11.67; 5.42 and 2.92 per cent respectively ($F_{9,20}$= 73.71, P=0.01; $F_{9,20}$= 60.43, P=0.01; $F_{9,20}$= 278.26, P=0.01; $F_{9,20}$=32.94, P=0.01; $F_{9,20}$ =35.52; $F_{9,20}$ =15.46, P=0.01; $F_{9,20}$=17.43, P=0.01 and $F_{9,20}$=23 P=0.01 for one to eight days respectively) (Table 4).

**Table 4. Age of female moths and corresponding percent of calling at $6^{th}$ hour of scotophase**

| Age of the female moths | Per cent females calling at $6^{th}$ hour of scotophase |
|---|---|
| One day old | 14.58 |
| Two day old | 39.67 |
| Three day old | **43.75** |
| Four day old | 26.25 |
| Five day old | 16.67 |
| Six day old | 11.67 |
| Seven day old | 5.42 |
| Eight day old | 2.92 |

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In one-day-old female moths, maximum calling occurred during 6th hour (11:00 pm) with 14.58 per cent and least being during 2nd hour (7:00pm) of scotophase (Table 5). However, in second day old moths, maximum mean percentage was recorded during 6th hour with 31.67 per cent, and least being 7.5 per cent at 2nd hour of scotophase (Table 6).

In three-day-old moths, the peak calling occurred during 6th hour with 43.75per cent, which was on par with 8th hour of scotophase whereas minimum percentage of calling recorded during 2nd hour of scotophase with 7.5 per cent (Table 7). In fourth day old moths, maximum calling with 26.25 per cent was recorded during 6th hour of scotophase and minimum per cent of 5.42 in 2nd hour of scotophase (Table 8).

Calling behaviour of five-day-old moths was recorded maximum during 6th hour of scotophase with 16.67per cent, which was on par with 7th hour and least percentage recorded during 2nd hour with 1.67 per cent (Table 9). In 6-day-old female moths maximum calling occurred with 11.47 per cent at 6th hour of scotophase and least being 2.5 per cent during 2nd hour of scotophase (Table 10).

The calling percentage drastically reduced in seven-day-old females with 5.4 per cent during 6th hour of scotophase and 1 per cent at 2nd hour of scotophase (Table 11). For the eight-day-old females, it was 2.9 per cent during 6th hour and 1.67 per cent during 4th hour of scotophase (Table 12).

Females of *Maruca vitrata* showed clear calling sequence where the percentage of calling moderately increased in younger females *i.e.*15 and 30 per cent for one and two day old moths respectively. Among all the age groups, highest peak of calling with 44 per cent was observed in 3-day-old moths. There was gradual reduction from fourth, fifth, sixth, seventh and eighth day old moths (26.25; 16.67; 11.67, 5 and 3 per cent).

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The result obtained in the study is in contradiction to the earlier studies where there was positive relation between calling and age. The pattern of calling is different in other moth species such as *Sesamia nonagrioides* (Schal and Carde, 1986), *Grapholitha molesta* (Baker and Carde, 1979) and *Holomelina lamae* (Babilis and Mazomenos, 1992) where with increase in age there was increased calling percentage. This dissimilarity might be due to the physiological difference in different species.

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**Plate7. Calling posture of female moths with curved abdomen**

**Plate 8. Calling female extruding its pheromone gland**

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**Plate 9. Male moth exposing its hair pencil**

**Plate 10. Wing buzzing in male moth towards calling female**

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**Table.5 Calling behaviour of one-day-old *M.vitrata* female moths**

| Time of calling (scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 1.3 | 1.67 |
| 8:00PM | 4.3 | 5.42 |
| 9:00PM | 7 | 8.75 |
| 10:00PM | 9 | 11.17 |
| **11:00PM** | **13** | **14.58** |
| 12:00AM | 10.3 | 12.92 |
| 1:00AM | 7.3 | 8.75 |
| 2:00AM | 2.6 | 3.33 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

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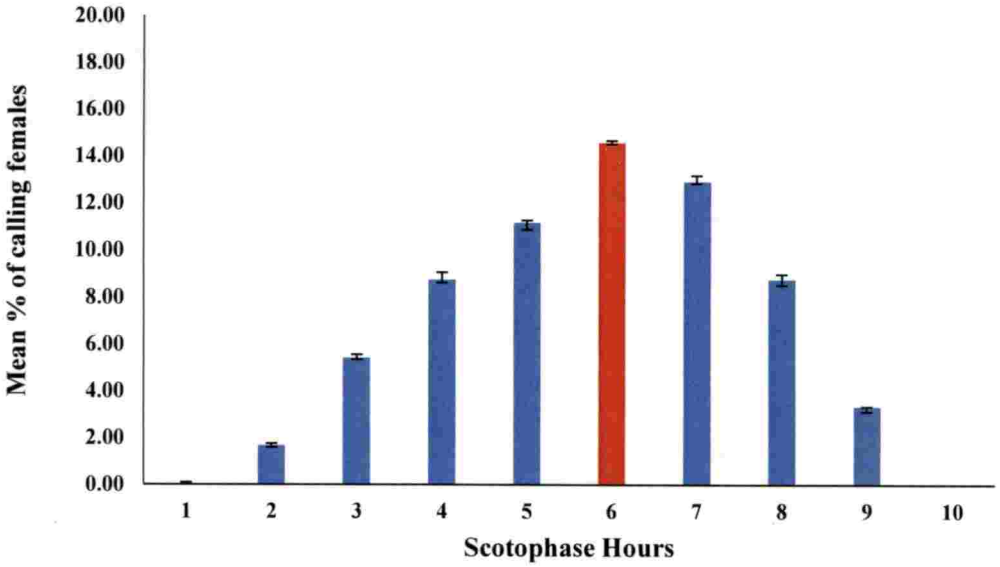
**Fig.2 Calling behaviour of one-day-old *Maruca vitrata* female moths during scotophase**



55

**Table 6. Calling behaviour of two-day-old *M.vitrata* female moths**

| Time of calling(scotophase hours) | Average no of females called ( N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 6 | 7.5 |
| 8:00PM | 11.6 | 14.58 |
| 9:00PM | 16.3 | 20.42 |
| 10:00PM | 15 | 24.58 |
| **11:00PM** | **23.6** | **31.67** |
| 12:00AM | 23 | 29.58 |
| 1:00AM | 19.3 | 22.50 |
| 2:00AM | 12.3 | 15.42 |
| 3:00AM | 0.6 | 0.83 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

56

**Fig.3 Calling behaviour of two-day-old *Maruca vitrata* female moths during scotophase**

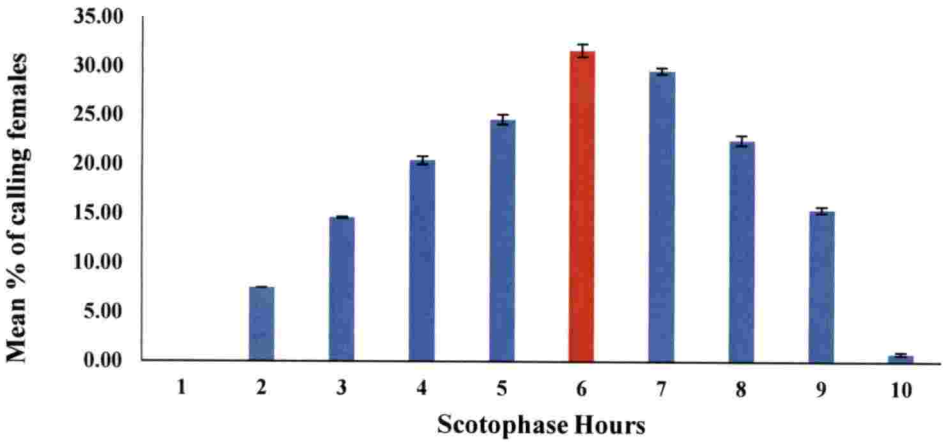


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**Table 7. Calling behaviour of three-day-old *M.vitrata* female moths**

| Time of calling(scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 6 | 7.50 |
| 8:00PM | 10.3 | 12.92 |
| 9:00PM | 20.3 | 23.33 |
| 10:00PM | 31.6 | 39.58 |
| **11:00PM** | **35** | **43.75** |
| 12:00AM | 33 | 38.33 |
| 1:00AM | 34.3 | 40.00 |
| 2:00AM | 17 | 21.25 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

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**Fig.4 Calling behaviour of three-day-old *Maruca vitrata* female moths during scotophase**

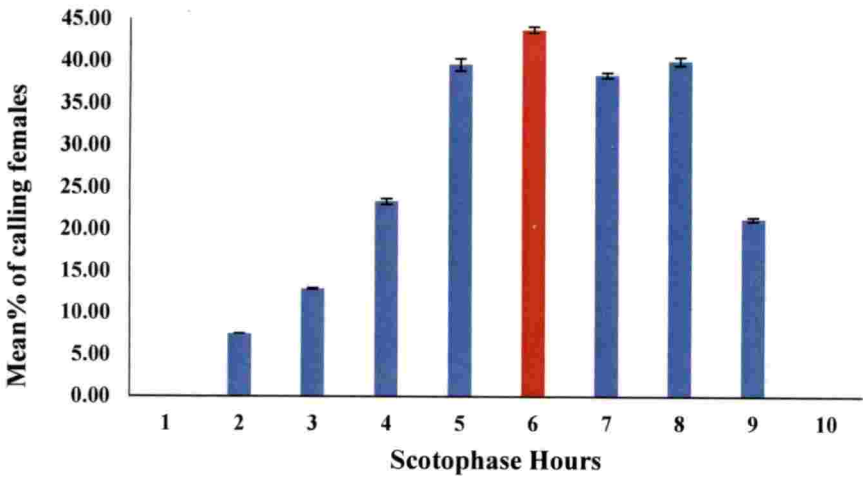

**Table8. Calling behaviour of four-day-old *M.vitrata* female moths** 59

| Time of calling(scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 4.3 | 5.42 |
| 8:00PM | 7.6 | 9.58 |
| 9:00PM | 10.3 | 12.92 |
| 10:00PM | 17.6 | 22.08 |
| **11:00PM** | **21** | **26.25** |
| 12:00AM | 15.6 | 19.58 |
| 1:00AM | 13 | 15 |
| 2:00AM | 8 | 10 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

60

**Fig.5 Calling behaviour of four-day-old *Maruca vitrata* female moths during scotophase**

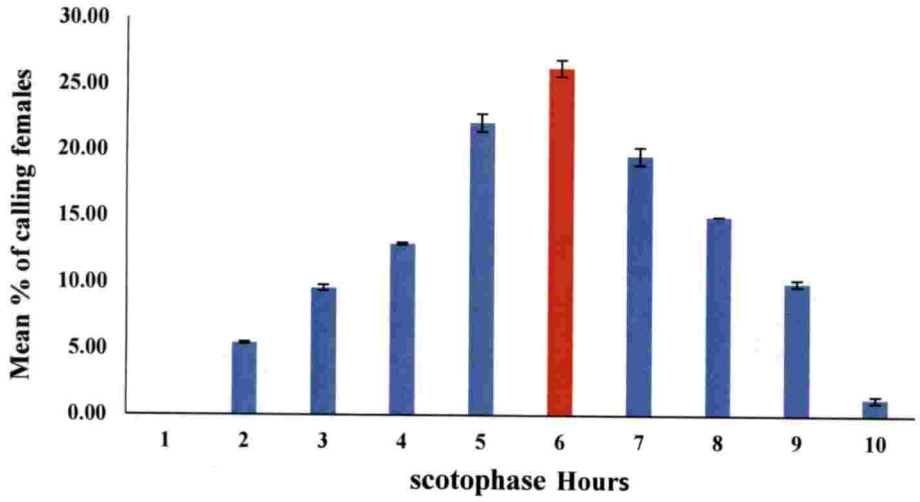

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**Table 9. Calling behaviour of five-day-old *M.vitrata* female moths**

| Time of calling (scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 1.3 | 1.67 |
| 8:00PM | 6 | 5.83 |
| 9:00PM | 77.3 | 8.75 |
| 10:00PM | 10.3 | 12.92 |
| **11:00PM** | **13.33** | **16.67** |
| 12:00AM | 11.33 | 14.17 |
| 1:00AM | 8.66 | 10.42 |
| 2:00AM | 6.6 | 7.08 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

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**Fig.6 Calling behaviour of five-day-old *Maruca vitrata* female moths during scotophase**

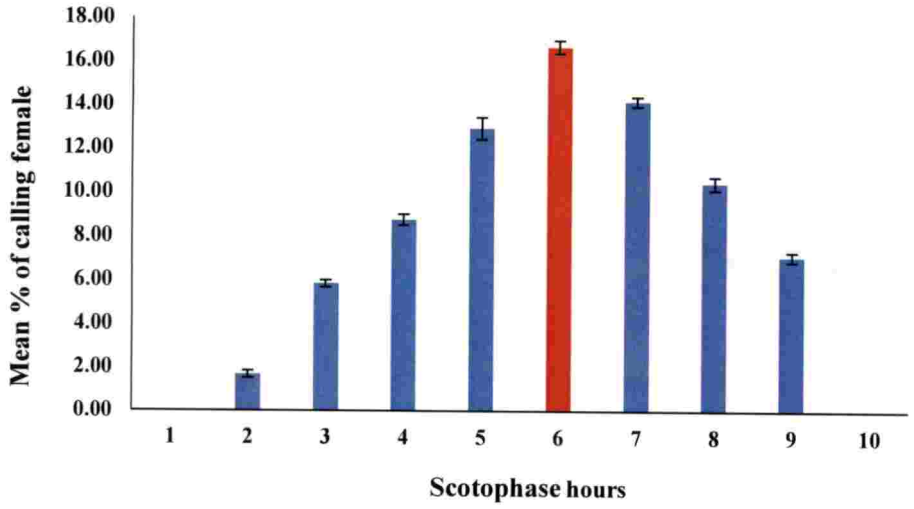

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**Table.10 Calling behaviour of six-day-old *M.vitrata* female moths**

| Time of calling(scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 0.3 | 2.5 |
| 8:00PM | 3.6 | 4.17 |
| 9:00PM | 5.33 | 5.83 |
| 10:00PM | 7 | 9.17 |
| **11:00PM** | **15** | **11.67** |
| 12:00AM | 11.6 | 7.92 |
| 1:00AM | 9 | 5.42 |
| 2:00AM | 3.3 | 4.17 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

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**Fig.7 Calling behaviour of six-day-old *Maruca vitrata* female moths during scotophase**

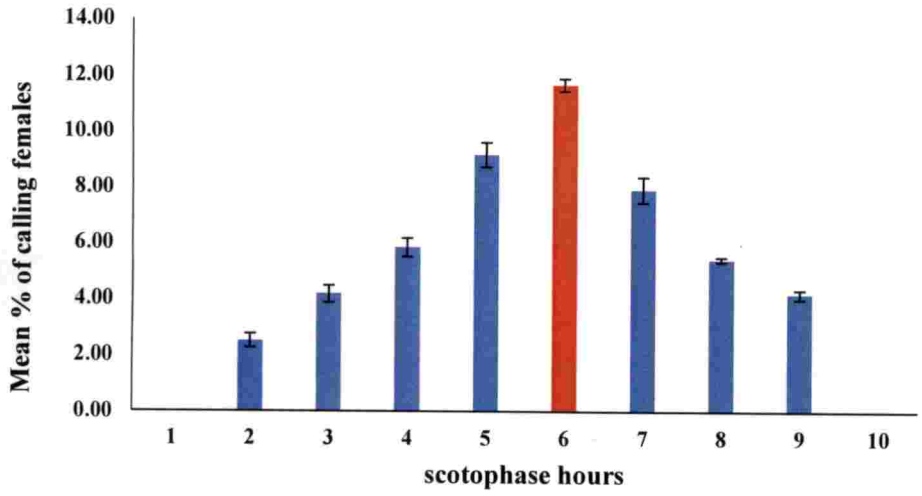

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**Table.11 Calling behaviour of seven-day-old *M.vitrata* female moths**

| Time of calling (scotophase hours) | Average no of females called (N=60) | Mean per cent of calling females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 0.3 | 0 |
| 8:00PM | 1.6 | 1.25 |
| 9:00PM | 3.6 | 2.92 |
| 10:00PM | 5.33 | 3.75 |
| **11:00PM** | **6.66** | **5.42** |
| 12:00AM | 3 | 2.50 |
| 1:00AM | 2.6 | 1.67 |
| 2:00AM | 2 | 0.42 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

**Fig.8 Calling behaviour of seven-day-old *Maruca vitrata* female moths during scotophase**

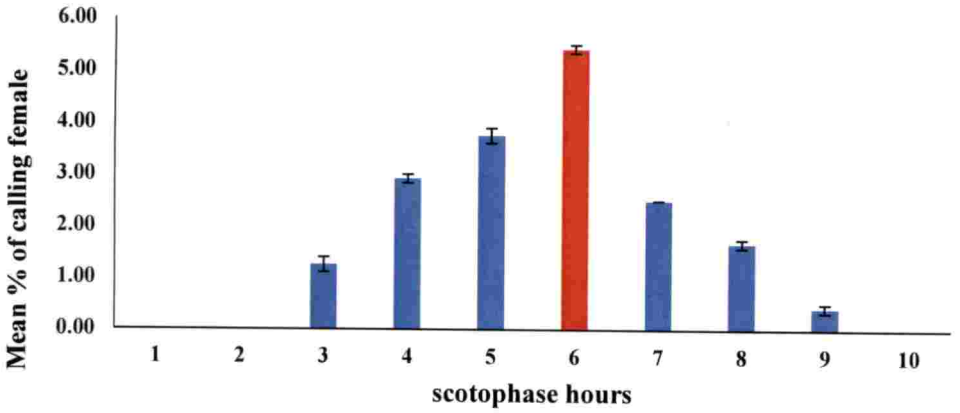

**Table 12. Calling behaviour of eight-day-old *M.vitrata* female moths**

| Time of calling(scotophase hours) | Average no of females called (N=60) | Mean per cent of calling Females |
|---|---|---|
| 6:00PM | 0 | 0 |
| 7:00PM | 0 | 0 |
| 8:00PM | 0 | 0 |
| 9:00PM | 1.6 | 1.67 |
| 10:00PM | 2.3 | 2.08 |
| **11:00PM** | **2.6** | **2.92** |
| 12:00AM | 1.6 | 1.67 |
| 1:00AM | 1.6 | 0.42 |
| 2:00AM | 0.3 | 0 |
| 3:00AM | 0 | 0 |
| 4:00AM | 0 | 0 |
| 5:00AM | 0 | 0 |

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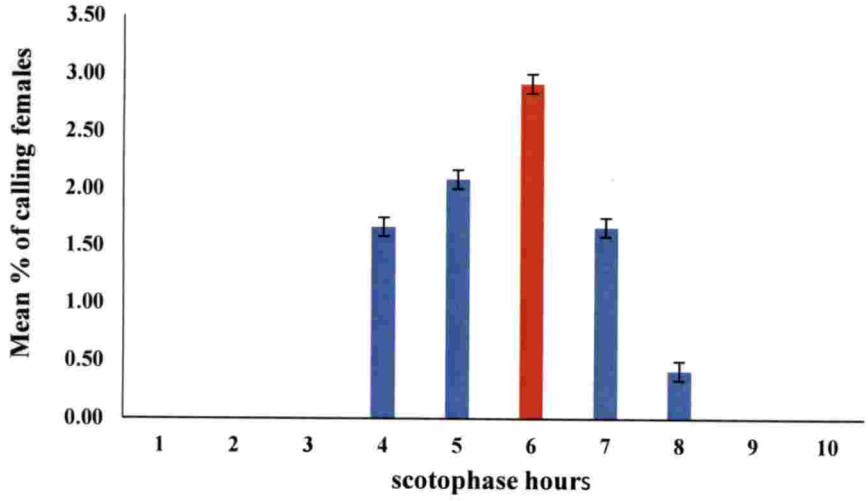
**Fig.9 Calling behaviour of eight-day-old *Maruca vitrata* female moths during scotophase**



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### 4.2.3 Mating of *Maruca vitrata*

In present study, 15 pairs of active moths were used to know the mating frequency over the age and time. The response of *Maruca vitrata* males to calling females were signalized by constant antennal swing, movement of their head in circular manner, rubbing of legs and exposed hair pencil (Plate 9). Before commencement of mating, males advance towards females from sideways and over the body and get close by walking. Whereas female settled down at the base of substrate wall. Once paired, they pose their body in opposite direction (Plate 11).

Highest mating frequency was occurred in 2nd and 3rd day old moths during 11-12th hour and 12-13th hour respectively. A similar result was obtained for the study conducted by Huang and Peng (2001) and Lu *et al.* (2007a) on *Maruca vitrata* where highest mating frequency was found on 3rd night after emergence.

**Table 13. Details of mating pattern of *Maruca vitrata***

| Age of moths | No of pairs observed | No of pairs mated | Time of pairing | Duration of mating | Percentage of mating |
|---|---|---|---|---|---|
| One day old | 15 | 0 | 0 | 0 | 0 |
| Two day old | 15 | 2 | 11:15pm | 45 min | 16.6 |
| Three day old | 15 | 4 | 12:15 | 60 min | 26.6 |
| Four day old | 15 | 1 | 1:00pm | 45 min | 6.6 |
| Five day old | 15 | 0 | - | - | 0 |
| Six day old | 15 | 0 | - | - | 0 |
| Seven day old | 15 | 0 | - | - | 0 |
| Eight day old | 15 | 0 | - | - | 0 |

**Plate 11. Mating position of *Maruca vitrata* moths**

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### 4.3 Electroantennography (EAG) studies

The EAG studies was conducted to know the response male moths to synthetic lure along with hexane stimulus and air. The result indicated that the solvent hexane response was 0.05mv and the response to PCI Lure was 0.05mv. Since response was similar for all the three i.e. PCI lure, solvent hexane and air which is 0.05 mv, no clear distinction in physiological response for pheromone lure could be discerned from the figures 10,11 and 12.

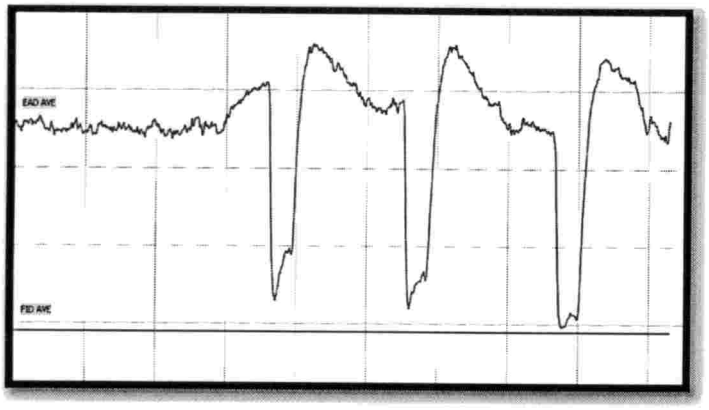


**Fig.10)EAG Response of *M.vitrata* male moth to synthetic lure**

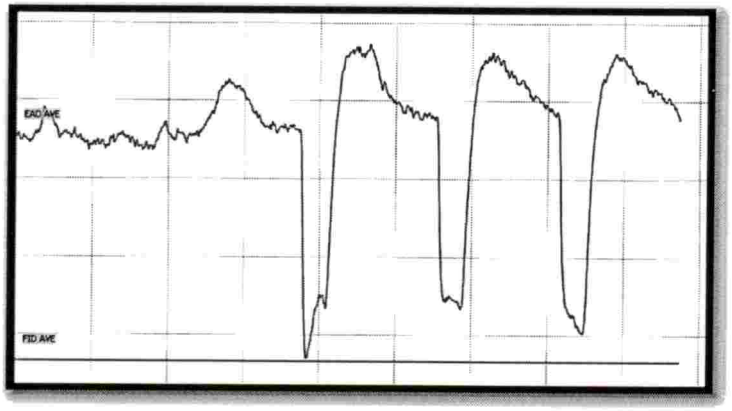


**Fig.11)EAG Response of *M.vitrata* male moth to Hexane solvent**

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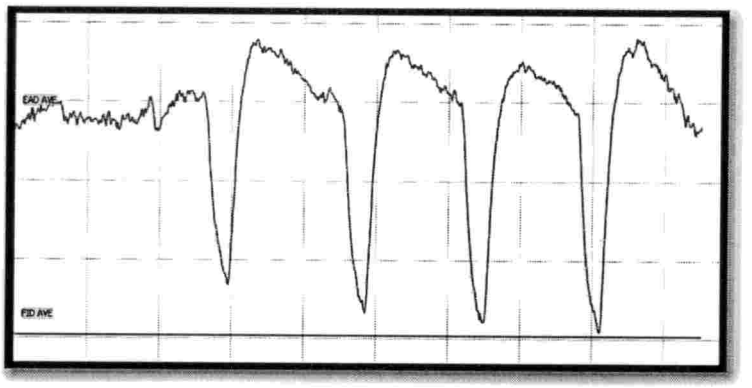


**Fig.12 EAG Response of *M.vitrata* male moth to Air**

### 4.3.1 GC-EAD Response with synthetic lure

Results of GC- MS analysis revealed that a major peak was found at 16.42 min of retention time.E,E-10, 12-Hexadecadienal with a isomeric purity of 89% (Fig.13) was the compound identified and in GC, it showed retention time of 17.7 min but the compound was the same. The difference in retention time for the chemical was because of the difference in the flow rate used in MS and GC.

In previous studies the Pheromonal compounds of *M.vitrata* identified were (E, E)-10, 12-hexadecadienal; (E, E)-10, 12-hexadecadienol and (E)-10-hexadecenal (Adati and Tatsuki, 1999; Downham *et al.,* 2003). In the study of Downham *et al.,* sufficient number male moths could be collected from the field, but in the present study, no catches were obtained. This disparity might have occurred due to geographic variability among *M.vitrata* population.

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The commercial lure containing one mg of *EE* 10, 12-16: Ald, *EE* 10, and 12-16: OH and *E*10-16: Ald in 100:5:5 (Benin blend) was found effective in trapping *M.vitrata* male moths during the experiments in Benin. However, this commercial blend composition did not trap any male moth at different regions of Taiwan, (Schlager *et al*., 2012), Thailand or Vietnam (Srinivasan *et al* 2013). These information lead to the conclusion that there was possible polymorphism in the blend composition of *M. vitrata* sex pheromone among populations from different geographical regions.

Mondhe (2001) tested the 3-component *M. vitrata* pheromone and natural female ovipositor extracts. He reported that for synthetic blend, most of the male moths were unresponsive (> 80 percentage), but a few flew upwind and hovered in front of the pheromone source (< 10 percentage each).

Adati and Tatsuki, first reported the major pheromone component (*E, E*)-10, 12-hexadecadienal (EE10, 12-16: Ald) from female ovipositor washings of *M. vitrata*. They confirmed that only 99 percent isomerically pure EE10, 12-16: Ald to be an electroantennogram-active component in the extract from female *M. vitrata* abdominal tips. They also detected the corresponding alcohol (*E, E*)-10, 12-hexadecadienol (EE10, 12-16: OH) in female ovipositor extracts at 3-4 per cent of EE-10, 12-16: Ald, as a minor component. Experiment conducted by Wang *et al*. (2015) showed that males of *Maruca vitrata* elicited electroantennogram response to primary sex pheromone component EE 10, 12-16 Ald. There were significant differences among the tested males with different day-ages. Third day male moth after emergence showed higher antennal response. Similarly, in wind tunnel experiment male after emergence elicited major response during third day old and found to decline in later age.

Unmole (2009) reported that males of *M. vitrata* did not respond to any NRI lures (Natural Resource Institute) made up of EE, 10, 12-16: Ald. alone or in

combination with two minor components like EE 10, 12-16: OH and E 10-16: Ald. in Mauritius, which were effective in Benin, Ghana and Burkina Faso. When the traps were tried in Mauritius, no response was found to synthetic lure but males were attracted to caged virgin females. This lead to postulate that the pheromone released by virgin females were not similar to any of the synthetic lures. Results indicated that the *M. vitrata* in Mauritius could represent another geographically distinct population from those in Benin, Ghana and Burkina Faso.

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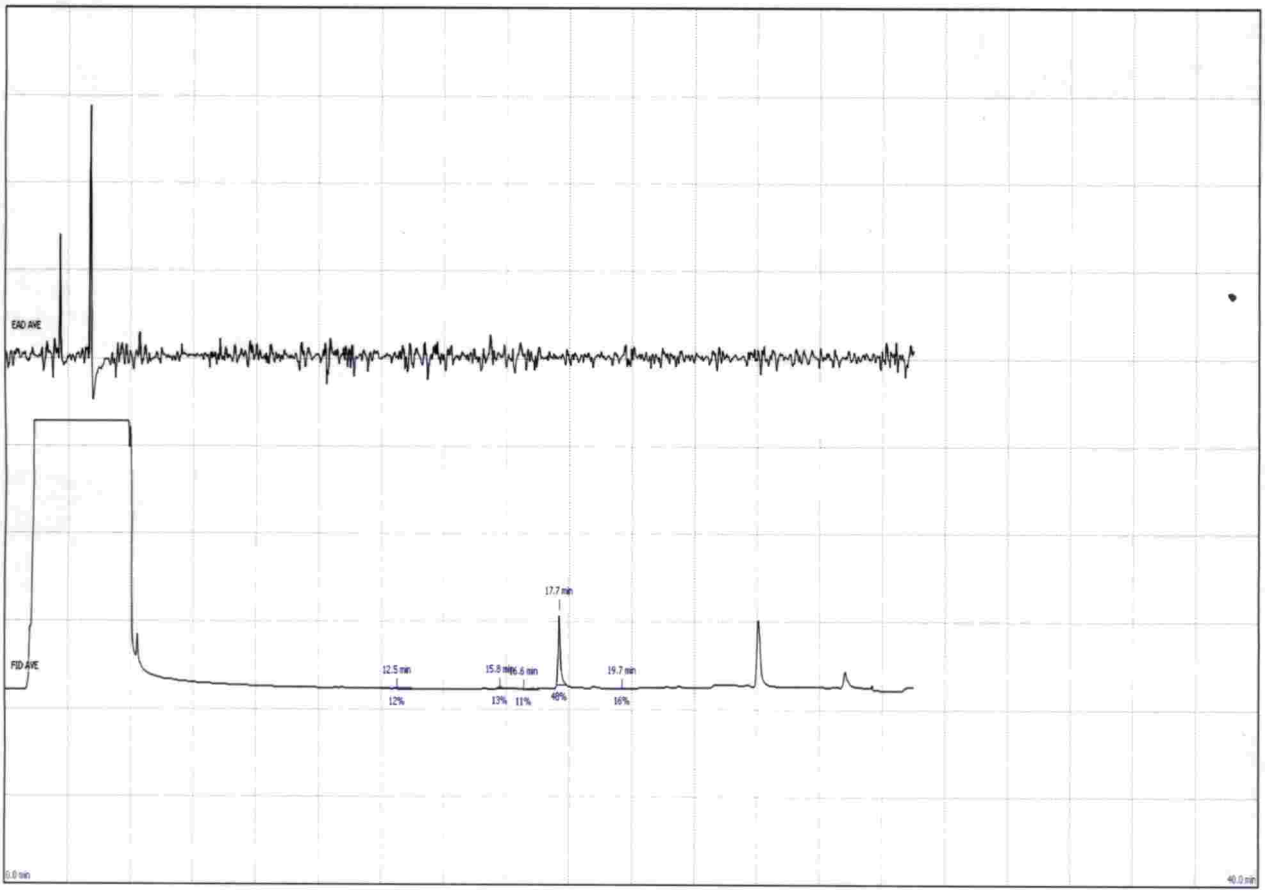
**Fig 13. GC-EAD response of Male *M.vitrata* to commercial lure**



But Schlager *et al.*, (2015) showed that no significant differences in the relative amounts of three compounds previously reported as components of the female sex pheromone in extracts of pheromone glands of female *M. vitrata* from colonies originating in Taiwan, Thailand, Vietnam, or Benin. Furthermore, in the Behavioural assays, *M. vitrata* males from Taiwan and Benin responded similarly to females from both regions and to the corresponding gland extracts. So, they argued that there is no geographical variation in sex pheromone blends among test insect populations from West Africa and Asia. On the other hand, Periasamy *et al.*, (2015) reported the presence of putative subspecies in *M.vitrata* in Asia and Sub Saharan Africa based on mitochondrial cytochrome oxidase 1(CO1) gene sequences, which may be a reason for non-response.

In conclusion, the commercial synthetic pheromone lure of *M. vitrata* provided by M/s Pest Control India Private Limited, Bangalore did not elicit any response in the adult moths of the test insect in field studies conducted at Kasaragod and Palakkad districts in Kerala as well as in laboratory studies involving Electroantennogram and GCMS coupled EAD. However, mating behaviour studies showed that females of *M. vitrata* was calling the males, which elicit specific response in the male moths. Presence of Pheromonal compound in this species was confirmed in previous studies. So, the exact reason for the absence of response to commercial synthetic lure has to be elucidated in future studies for developing an effective commercial pheromone for the legume spotted pod borer *M. vitrata*.

# Summary

# 5. SUMMARY

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A study was conducted know the efficacy of synthetic sex pheromone of legume pod borer *Maruca vitrata* (F.) (Lepidoptera; Pyralidae) at College of Agriculture, Padannakkad. Also investigated the female calling and mating behaviour of the adult moths of *M. vitrata.* Response of commercial lure to male moths of the test insect was studied in Electroantennogram at ICAR-NBAIR, Bangalore. The major chemical compound in the commercial lure was identified and its response to male moths was investigated.

The commercial synthetic pheromone lure of legume pod borer *M.vitrata* from M/s Pest control India Private Limited, Bangalore was tested at four different cowpea fields for its efficacy in attracting and trapping the male moths. The lure failed to attract not even a single moth during the studies. Subsequently, the mating behaviour of the species was undertaken in detail.

The adult emergence of *M.vitrata* observed throughout the day and night (N=160, Female=80 and Male=80). It was observed that 30% of moths emerged during photophase (6am-5pm) out of which male moths constituted 36.25% and female moths constituted 23.75%. During scotophase (6pm-5am), 69.68% moths emerged, out of which 63.12% was males and 76.25% was females. The emergence found to peak at $5^{th}$ and $9^{th}$ hour of scotophase.

Highest mean percentage of emergence of male moths was 11.88% at 4pm (Photophase) whereas it was 9.4 % for the females. At 10 pm (Scotophase), 26.25% of female moths and 17.5% of the male moths emerged. Another small peak of emergence was observed during 2-3am (scotophase) with 18.12% for males and 23.12% for females.

During calling period female extrudes its pheromone gland beyond the abdominal tip. Female calling behaviour of one to eight day old moths was observed

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throughout the scotophase. A single calling peak was observed in all aged moths at 6th hour of scotophase except for three old day moths, which showed additional peaks of calling at fifth, seventh and eighth hour of scotophase. For three-day-old moths, 43.5% was the mean percent of calling at 6th hour of scotophase. The mating behaviour of 1-8 day old *M.vitrata* moths involving 15 pairs throughout the scotophase showed maximum mating percentage of 26.2 % and 16.6 % for 3 day old and 2 day old moths respectively and least was seen in 4-day-old moths with 6.6%.

Electroantennogram study was conducted to know the response of 3-day-old male moths of *M.vitrata* to synthetic lure obtained from PCI Pvt. Ltd., Bangalore. Results indicated that response to the stimulus was 0.05mv only, which was the same for the solvent hexane and the air. This showed that there was no distinct physiological response for synthetic pheromone lure. For further confirmation GC-MS analysis was done and the compound E, E-10, 12-Hexadecadienal was identified at a retention time of 16.42 min. This is the major pheromonally active compound identified in previous studies. In GC-EAD analysis, the response of *M.vitrata* male moths to E, E-10, 12-Hexadecadienal was checked by matching EAD and FID peaks but without any response.

The commercial synthetic pheromone lure of *M. vitrata* provided by M/s Pest Control India (P) Limited, Bangalore did not elicit any response in the adult moths of the test insect in field studies as well as in laboratory studies involving Electroantennogram and GCMS coupled EAD. However, mating behaviour studies showed that females of *M. vitrata* were calling the males, which elicit specific response in the male moths. Presence of Pheromonal compound in this species was confirmed in previous studies. Therefore, the exact reason for the absence of response to commercial synthetic lure is to be elucidated in future studies for developing an effective commercial pheromone for the legume spotted pod borer *M. vitrata*.

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# Abstract

# MASS TRAPPING OF COWPEA POD BORER *Maruca vitrata* (F.) (LEPIDOPTERA; PYRALIDAE) USING SEX PHEROMONES

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*by*

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**Abstract of the Thesis**

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# ABSTRACT

A study was conducted know the efficacy of synthetic sex pheromone of legume pod borer *Maruca vitrata* (F.) (Lepidoptera: Pyralidae) at College of Agriculture, Padannakkad. Also investigated the female calling and mating behaviour of the adult moths of *M. vitrata.* Response of commercial lure to male moths of the test insect was studied in Electroantennogram at NBAIR, Bangalore.

The commercial synthetic pheromone lure of legume pod borer *M.vitrata* from M/s Pest control India (P) Limited, Bangalore was tested and found that it failed to attract not even a single moth. Subsequently, the mating behaviour of the moth was undertaken in detail to investigate whether pheromones are involved in the mating process of the insect. 30 per cent of moths emerged during photophase (6am-5pm) out of which male moths constituted 36.25% and female moths constituted 23.75%. During scotophase (6pm-5am), 69.68% moths emerged, out of which 63.12% was males and 76.25% was females. The emergence found to peak at 5$^{th}$ and 9$^{th}$ hour of scotophase.

During calling period female extrudes its pheromone gland beyond the abdominal tip. Female calling behaviour of one to eight day old moths was observed throughout the scotophase. A single calling peak was observed in all aged moths at 6$^{th}$ hour of scotophase except for three old day moths, which showed additional peaks of calling at fifth, eighth and 9$^{th}$ hour of scotophase. For three-day-old moths, 43.5% was the mean percent of calling at 6$^{th}$ hour of scotophase. The mating behaviour of 1-8 day old *M.vitrata* moths involving 15 pairs throughout the scotophase showed maximum mating percentage of 26.2 % and 16.6 % for 3 day old and 2 day old moths respectively. Electroantennogram study conducted to know the response of 3-day-old male moths to synthetic lure obtained from PCI, Bangalore showed that response to the stimulus was 0.05mv only, which was the same for the solvent hexane and the air.

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This showed that there was no distinct physiological response for synthetic pheromone lure. For further confirmation, GC-MS analysis was done and the compound E, E-10, 12-Hexadecadienal was identified at a retention time of 16.42 min. This is the major pheromonally active compound identified in previous studies. In GC-EAD analysis, the response of *M.vitrata* male moths to E, E-10, 12-Hexadecadienal was checked by matching EAD and FID peaks but without any response. So, the exact reason for the absence of response to commercial synthetic lure is to be elucidated in future studies for developing an effective commercial pheromone for the legume spotted pod borer *M. vitrata*.