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Pheromone baited biopesticide for control of *Leucinodes orbonalis* Guenee in brinjal plant

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1. ABSTRACT

Leucinodes orbonalis Guenee, Brinjal fruit and shoot borer (BFSB), is the major pest on brinjal world wide. Larvae of this pest cause the damage; which at initial stages adversely affect the shoot growth, and in later stages diminish fruit quality. Spraying of insecticides is the main pest control method. This has been absolutely ineffective due to concealed habit of the larvae. Such a phenomenon apprehends to the development of insecticides resistance which demands continuously increasing doses. Manipulating the insect behavior using semiochemicals could be an opportunity for better management of insect pest. Application of BFSB sex pheromone components (E)-11-hexadecenyl acetate (E11-16: Ac) alone or in combination with (E)-11-hexadecen-1-ol (E11-16: OH) in traps can be used to suppress the populating growth. Goal-oriented interdisciplinary research on semiochemicals for sound administration of BFSB will depend on a better understanding of the key chemical ecology stimuli of relevance to the pest.

2. INTRODUCTION

Eggplant or brinjal plant (*Solanum melongena*) is an economically important plant used in Asian countries, especially in Bangladesh, China, India, Sri Lanka, Pakistan, Philippines and Thailand (1). Distribution of brinjal plant is higher in the areas with hot and humid climate as brinjal is known as a hardy plant (2). China is the largest producer of brinjal while India occupies the position of second largest producer (3). In Bangladesh, brinjal is considered to be the second most produced vegetable after potato (4) and it covers second large extent after curry banana in Sri Lanka (5). Eggplant is available throughout most of the year (6).

Brinjal is a well known vegetable rich in fiber, low in calories and provides a wide range of nutrients, minerals and multivitamins (7-8). Also, it

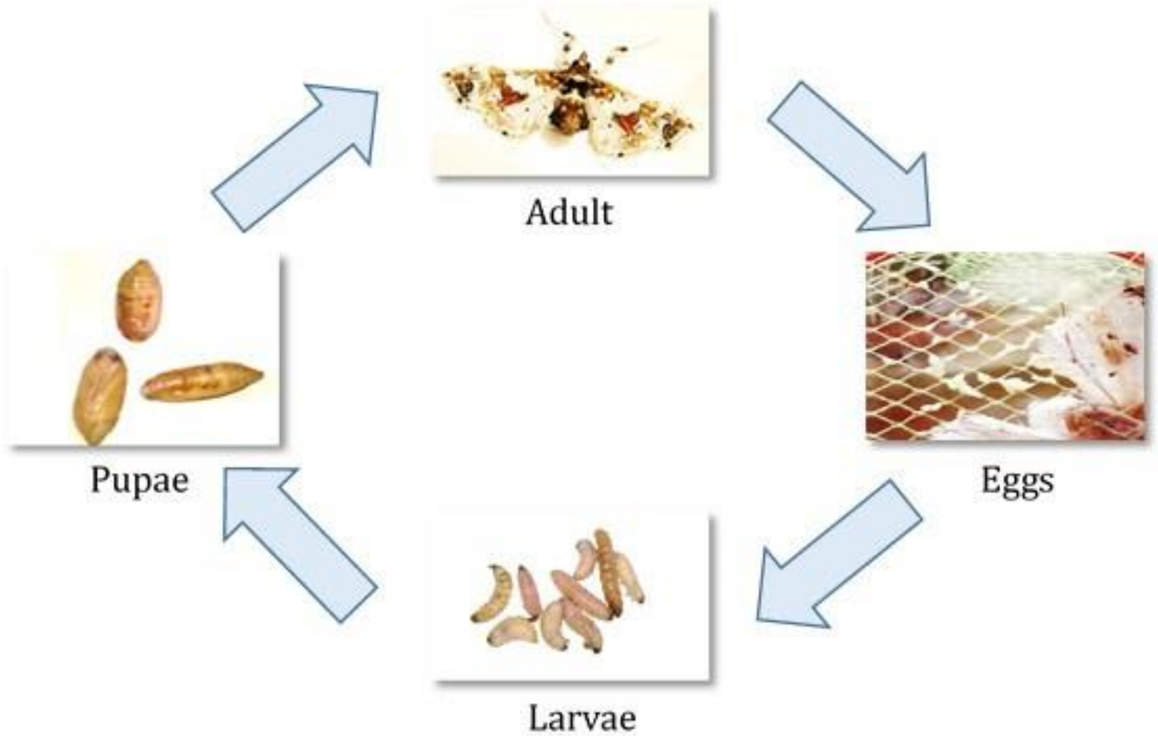

Figure 1. Life cycle of *Leucinodes orbonalis* Guenee.

contains various bioactive secondary metabolites which provide significant health benefits for the consumers. The high content of the phenolic compounds, mostly chlorogenic acid and anthocyanins are highly associated with bioactive properties of the brinjal plant (9-10). Due to these factors, brinjal plant is considered among the top 10 vegetables in terms of oxygen radical absorbance capacity (11). Besides its nutritional and agronomical values, brinjal plant has potential medicinal, pharmaceutical and excellent therapeutic properties especially antioxidant, anti-inflammatory, cardioprotective, anti-obesity and anti-diabetic properties (12).

In recent years, production of eggplant is under imminent threat due to the increased management cost of insect pest complex *Leucinodes orbonalis* Guenee which is considered to be the key insect pest that attacks brinjal plant (2). The impacts of this pest lead to the loss of quality and yield of the crop (3). At present, farmers are completely dependent on chemical insecticides to control this pest (1). Farmers in general used to spray insecticides regularly to control the borer larvae (13). Indiscriminate use of synthetic chemicals cause many unwarranted problems. These problems include pest resistance, secondary pest outbreak, pest resurgence, elimination of beneficial fauna, different human health hazards and environmental pollution (3-4). Certain behavioral chemicals could be employed to reduce the pesticidal load in the environment. Researches on possible management tactics using semiochemical strategies have led to many operational management programs. One such program is the utilization of semiochemical-baited traps and inhibitors to suppress the BFSB population in order to protect vulnerable host plants. This paradigm shift in management strategy addresses almost all of the biosafety concerns arises due to the indiscriminate use of synthetic chemicals and plays a key role in combatting insect pests of high value and damage of sensitive crops (14-15). As a part of this study, scientists recognized a system to monitor the adult population of the pest. Working with insects from China, they identified (E)-11-hexadecenyl acetate (E11-16: Ac) to be the main element of the female sex pheromone. They suggested that this pheromone was attractive to male moths (6). Subsequently, they validated the presence of E11-16: Ac using insects from Sri Lanka and found trace amounts of (E)-11-hexadecen-1-ol (E11-16: OH) as well (6). This review gives a brief account on the general concepts, definitions, chemical and physical properties of semiochemicals, and the applications of semiochemicals from BFSB (especially sex pheromone) for better management of this serious pest on brinjal.

3. NATURE AND EXTENT OF DAMAGE

Larval feeding in fruits and shoots is the reason for the damage to brinjal crop (16). The larva bores into petioles and midribs of large leaves at the early stage of the plant's growth (17) and subsequently lead to drooping and withering of the young shoots (18). Feeding activities of the larva is confined to the shoot of the crop at its early stage and it causes wilting and dieback of the branch terminals; this adversely affects the fruity bearing capacity of the host plant (19). Larvae of this pest alone is responsible for 12-16% of damage to shoots (20). At a later stage, when the plant bears flower buds, flowers, and fruits the larva bores into the flower buds and fruits through the calyx without leaving any visible signs of infestation (17). Larval feed creates tunnels inside the fruits and at the same time leaves large exit holes when moving to the next phase of its life cycle, the pupae (19). Larvae while feeding inside the fruit, block their boreholes with frass in order to protect them from natural rivals and frequently applied insecticides (6). This leads to additional infection by certain types of bacteria which further deteriorates the fruits (21) which can adversely affect the market price of the fruit and make it unfit for consumption (22). Larvae may cause fruit damage as high as 95 percent in commercial plantings (23). Further, it reduces up to 80 percent of the content of Vitamin C in brinjal fruit (24).

4. LIFE CYCLE OF BFSB

Same as other members of the Pyralidae family, *L. orbonalis* also goes through four stages of growth: egg, larva, pupa and adult (Figure 1). Egg: A female moth lays the eggs singly or in batches where the number varies from 80-253 (2) during the later part of the night to early part of the morning. It normally lays eggs on shoots, flower buds, near the peduncle of fruits and on the lower surface of leaves. The egg has an incubation period of 3-8 days (25). Larva: The full-grown larvae measure 15 - 18 mm. The larval period is the longest (25) and it lasts 12 - 22 days (2). Larvae pass through 5 larval instars (26). Larval feeding in the fruits and shoots causes damage to the brinjal crop (16). Pupa: The matured larvae come from the infested shoots and fruits for pupate. Then the larvae transform into inactive silken cocoons in plant debris or the dried plant parts which fall to the ground (19). Depending on the temperature pupal period lasts 6 to 17 days (19). Adult: The adult is identified as a small white moth which has brown spots on forewings (2). Moths do not feed on eggplant. Following the emergence young adults are found either on the lower surfaces of the leaves or hidden under the leaves within the plant canopy (19). All major activities from feeding; and mating to finding a place to lay eggs will take place during the night hours (27). It takes 10 to 14 days for an adult to gain full maturity and 22 to 55 days complete the overall life cycle (2).

5. SEMIOCHEMICALS

Semiochemicals are defined as chemicals that are emitted by living organisms which could induce a response either behavioral or physiological in other individuals. Semiochemical communication is divided into two broader classes: (a) pheromones mediated intraspecific communication which happens between individuals of the same species (b) allelochemicals mediated interspecific communication among individuals of different species (28).

The large variety of volatile organic compounds (VOCs) are synthesized and emitted from plants in response to insect attacks (29-30) which is specific to the certain phytophagus insect (31). Plant semiochemicals also produce a wide range of behavioral responses in insects (32). Moreover, a variety of VOCs are released by insects in the external environment which elicit a particular

Table 1. Single compound pheromone

Pheromone	Insect species	References
Trans-10-*cis*-12-hexadecadien-1-ol	*Bombyx mori*	69
(3E, 5E, 7E, 9E) 6-8-diethyl-4-methyl-3, 5, 7, 9-dodecatetraene	*Carpophilus antiquus*	70
(3R, 4S) 4-methyl-3-heptanol	*Leptogenys diminuta*	71
2(E)-(4-methyl-3-pentenylidene)-butanedial	*Caloglyphus polyphyllae*	72
(2S)-butyl (7Z)-tetradecenoate	*Theresimima ampellophaga*	73
(E)-9-oxodec-2-enoic acid	*Aphis mellifera*	74

reaction in receiving individuals of the same species (33). Insects possess a high sensitive olfactory system that could detect and discriminate certain volatile semiochemicals with high degree of specificity and selectivity (34-35).

Use of semiochemicals in crop protection while reducing the application of chemical pesticides is a novel approach for global pest management. Semiochemicals are considered as safe and eco-friendly molecules due to their natural origin and low persistency in the environment. (36-37). Contrary to chemical insecticides, insects find it difficult to develop resistance against volatile semiochemicals as semiochemicals work by a non-toxic mode of action through modifying the pest's behavior (38).

6. CHEMICAL AND PHYSICAL PROPERTIES OF SEMIOCHEMICALS

Chemical characterization of semiochemicals are complicated due to its biologically active nature at very low concentration in the environment (39). The number of carbons in the carbon chains of semiochemicals varies from zero to forty five. The molecular weight of VOCs generally falls in the range of 50–200 Daltons and has an appreciable vapor pressure under ambient conditions (40). Scientists suggest that the volatile pheromones naturally exploited in insect communication have 5 to 20 atoms of carbon with molecular weights ranging from 80 to 300 (41).

Differences in degrees of saturation, isomerism, cyclic structure and aromaticity are the reasons for the chemical diversity of the category. Physical properties such as chemical nature, volatility, solubility and lifetime of the molecules decide the efficiency of semiochemical substances in chemical communication. Effectiveness of the pheromone is controlled by temperature, which increases the diffusion of molecules in the air. Efficiency in the integrated pest management is affected by the stability of these volatile compounds as well (35).

7. PLANT VOLATILES

Plant VOCs are usually lipophilic liquids with high vapor pressures (42-43). They characterize the chemical landscape of various ecosystems wherein they mediate intraspecific and interspecific interactions (44). In some plants, these volatile compounds are the main compounds that are responsible for the attraction of insects, while in other plants attraction and recognition involve a blend of compounds released in specific ratios (45-46). In response to particular host plant cues insects either produce or release sex pheromones; and chemicals from host plants often synergistically enhance the response of an insect to sex pheromones (32). These bioactive compounds belong to different classes of VOCs and many are secondary metabolites mainly represented by terpenoids, phenylpropanoids or benzenoids, amino acid derivatives, fatty acid derivatives and green leaf volatiles (35, 47, 48).

8. PHEROMONES AND ALLELOCHEMICALS

Crop protection through pheromone technology is gaining importance in the present-day demand for safe, pesticide-free products (49).

Table 2. Multi compound pheromone

Pheromone	Insect species	References
Ethyl 4-methyloctanoate Ethyl 4-methylheptanoate 4-methyloctanoic acid	*Oryctes rhinoceros*	75
Phenol p-Cresol 2, 6-dichlorophenol	*Rhipicephalus appendiculatus*	76
Cis-2-isopropenyl-1-methylcyclobutaneethanol *Cis*-3, 3-dimethylcyclohexaneethanol Cis-3, 3-dimethylcyclohexaneacetaldehyde *Trans*-3, 3-dimethylcyclohexaneacetaldehyde	*Anthonomus grandis*	77

A pheromone of any insect is characterized by four different factors: carbon number, functional group, the arrangement of double bonds, and the ratio between major and minor compounds (50). Most of these components are straight-chain structures with an even number of carbon atoms and 10-18 carbons in length. They have a functional group at the end of the molecule that is either an aldehyde, an alcohol or an acetate. Finally, they have either one, two, or three double bonds at various positions on the molecule in either the Z or E geometric configuration (51). Moreover, pheromone could be a single compound or a multi compound mixture. Some examples for single compound pheromone are shown in Table 1 and multi compound pheromone in Table 2. Allelochemicals are organic compounds produced by members of one species that influence the behavior of members of another species (52). The allelochemicals are classified into synomones, kairomones and allomones depending on the benefits for sender and receiver (53).

9. FEMALE SEX PHEROMONES OF BFSB

Female sex pheromones are significant element of insect pest management programs and they are largely used to monitor and mass-trap the male insects (15, 22). The first group of Scientists Zhu *et al*. (1987) reported (E)-11-hexadecenyl acetate (E11-16: Ac) as the key component of BFSB sex pheromone in China (6) and the presence of this component was identified from the sex pheromone glands of BFSB in Sri Lanka (15). In addition to that, scientists have identified trace quantities of (E)-11-hexadecen-1-ol (E11-16: OH). However, E11-16: Ac when used alone or in combination with E11-16: OH attracted significantly high numbers of male moths in India and Bangladesh. Since E11-16: OH alone showed no attraction at any concentration, it was reported to be “not active” when used alone (54-55). Later, Gunawardena *et al.* (1989) and Attygalle *et al.* (1988) identified the sex pheromone of BFSB in Sri Lanka (56) and some trace quantities of (E)-11-hexadecen-1-ol (E11-16: OH) (57). Later on Kong *et al.* (1990) also identified E11-16: Ac as the key component of BFSB sex pheromone (58). Moreover, Srinivasan *et al*. (2000) also reported the sex pheromone of BFSB found in India. They used Synthetic sex pheromone components of (Z)-11-hexadecenyl acetate and (Z)-11-hexadecen-1-ol, at a series of different concentrations alone, or in combination with (Z11-16: Ac): (Z11-16: OH) at different ratios. They were evaluated using water trough traps for moth (*L. orbonalis*) attraction. This was done for monitoring pest incidence in brinjal in a field experiment conducted in Tamil Nadu, India. Component Z11-16: Ac at a concentration of 300 mg resulted in the highest number of moths (86) trapped, while component Z11-16: OH showed no attraction at any concentration. Among the (Z11-16: Ac): (Z11-16: OH) combinations, a concentration ratio of 100: 50 mg showed the highest number of moths (33) trapped (59). Cork *et al*. (2001) also identified and confirmed (*E*)-11-hexadecenyl acetate (E11-16: Ac) as the major pheromone component with 0.8 to 2.8 percent of the related (*E*)-11-hexadecen-1-ol (E11-16: OH) from the extracts prepared using female pheromone glands through the insects collected from India and Taiwan (6).

10. MANAGEMENT OF BFSB USING PHEROMONE

There are many semiochemical-based pest control techniques such as mass trapping, mating disruption and attract-kill. Pheromone traps are commonly used for monitoring and management of BFSB due to its easy handling, species-specificity and sensitivity. Mass trapping or attract and kill technique are most commonly used in pheromone traps to eliminate sufficient amounts of adults and reduce the damage caused by larvae. (60-61). Mating disruption causes communication interference and disorientation between both sexes, thus delaying or preventing the reproduction of insects. Mating disruption is the technique where enough amount of pheromone is discharged into a pest habitat to reduce sexual attraction towards the opposite sex. Sex pheromone is the key component for mating disruption, and this can be achieved by using a high concentration of synthetic sex pheromones at regular intervals throughout the field where specific insect pests need to be controlled. When high concentration of synthetic sex pheromone saturates in the area, abilities of male insects to locate the natural pheromone plume emitted by females are disrupted, thus it prevents the mating and fertilization of the female pest (62).

Studies conducted in Bangladesh and India using pheromone-baited traps showed that high concentrations of E11-16: Ac alone or low concentrations of mixtures of E11-16: Ac and E11-16: OH (10: 0.5 or 10: 1) attract large numbers of male moths (59). In the trials conducted in India, blends of E11-16: OH between 1 and 10% caught more male *L. orbonalis* than E11-16: Ac alone. On white rubber septa at 1000 µg dose, addition of 1% E11-16: OH to E11-16: Ac was proved to be more attractive to male *L. orbonalis* than either 0.1 or 10% E11-16: OH (6). Srinivasan (2008) reviewed the brinjal pest management strategies in South East Asia and reported that significant reduction in the pest damage on brinjal plant can be achieved when BFSB sex pheromone traps based on with the combination of (E)-11-hexadecenyl acetate and (E)-11-hexadecen-1-ol which can continuously trap the adult males (22). As well as Cork *et al*. (2005) reported mass trapping was successful for BFSB (63). Thus, in South Asia damage caused to brinjal fruits was significantly reduced and the yield was increased when BFSB sex pheromone traps were deployed as a component of IPM (19, 64). Therefore, the usage of semiochemicals has proven to be useful in BFSB management as it significantly reduced the cost of longer running monitoring period. Furthermore, semiochemical-baited traps proved to have higher potential for suppressing the BFSB population.

11. PHEROMONE APPLICATION AND TRAP DESIGNS

Behavioral manipulation to control the BFSB by means of semiochemical applications has proved to be far effective as there is very little risk of target species developing resistance to the active ingredient, a common shortcoming experienced among the conventional insecticides (65). Furthermore, semiochemicals are deployed in discrete point sources whereas conventional insecticides are often applied as cover/blanket sprays over the entire crop. This enables growers to avoid contamination of food crops as it reduces the likelihood of non-target organisms coming into contact with the compound (66). Insects such as BFSB which has extremely diverse adaptations such as hidden and protected lifestyles in adult stage and concealed habits in the larval stage cannot be easily controlled with cover sprays of insecticides. Yet the use of pheromones to control is advantageous, since it aims at the mobile adult life stage, and works to obstruct oviposition (61). For the effective management of insect pests, mass trapping and mating disruption has been successfully practiced and sometimes biocontrol technologies were also used for this purpose. These approaches reduce insecticide usage and facilitate conservation of natural enemies. This method has proved to be one of the pest control method in which the insect does not become resistant (62). The sex pheromone of BFSB confuses the male adult for mating and thus prevents fertilized egg production by trapping significant number of male moths, which results in reduction of larval and adult population (67).

The type of trap used to catch the adult correlates with the number of pests caught. So it is a challenge for entomologists to develop a more efficient trap design for *L.* ***orbonalis.*** The aim of selecting a trap design should be to catch the maximum number of adults at any given time. Cork *et al.* (2003) have used delta and wing traps baited with synthetic female sex pheromone of *L. orbonalis* and found that they retain ten times more moths than either spodoptera or uni-trap designs. Water and funnel traps have also proved to be effective as delta traps (64). However, the trap design that would attract increased numbers of insects will vary from one location to the other (22). It requires detailed investigation to know why different traps are effective in different regions for the same pest.

Moreover, a positive correlation was established between the number of trap catch and pheromone release rate (6). Further, in addition to pheromone loading, the use of corrective dispenser is also very important to succeed in mass trapping. Dispensing rate of release of pheromone varies with the technology being used. Hence, the nature of the dispenser used influences the responses of male moths to pheromone traps (68). These semiochemical compounds have complex biological activity. A slow release devise ensuring controlled release of biologically active VOCs are required to monitor and protect its dispersion in the environment. These sensitive molecules also need protection from degradation by UV light and oxygen. Therefore, it is important to advance further studies on estimation of release-rate from commercialized or experimental slow-release devices (41). Costly equipments are required for extraction and chemical characterization of semiochemicals. Plenty of work on electrophysiological and behavioral bioassay are required for the development of new synthetic pheromone blends (39).

12. CONCLUSION

Leucinodes orbonalis Guenee is considered to be a serious pest of brinjal distributed throughout South-East Asia. The life cycle and the nature of the pest has made brinjal cultivation to be worst in the region. Semiochemicals approach can destroy the pest population without harming the nature. (E)-11- hexadecenyl acetate (E11- 16: Ac) is identified and reported as the major component of BFSB sex pheromone. Reviews shows that use of sex pheromone traps for BFSB attracted a significantly higher number of male moths. This shows the potential effectiveness of sex pheromone in pest control strategies. Most common phenomenon in semiochemical based pest management of BFSB is the mating disruption technique which manipulates the insect behavior to reduce its population growth.

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Abbreviations: BFSB: brinjal fruit and shoot borer; VOCs: volatile organic compounds; *L. orbonalis*: *Leucinodes orbonalis*; IPM: integrated pest management; UV: ultra violent; (E11-16: Ac): (E)-11-hexadecenyl acetate; (E11-16: OH): (E)-11-hexadecen-1-ol; Z-E: is a IUPAC preferred method of notation describing the absolute stereochemistry of double bonds in organic chemitry

Key Words: *Leucinodes orbonalis* Guenee, Semiochemicals, Pheromones, Insecticides, Chemical Ecology, Review

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