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Development of Indigenous Pheromone Technology for Monitoring of Spotted Bollworm, *Earias Vittella* Fab (Lepidoptera Noctuidae) on Cotton

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Abstract The present investigation reports the development of an indigenous pheromone technology for monitoring of the spotted bollworm, *Earias vittella* (Fabricius) on cotton. The female produced pheromone blend, comprising of the three components i.e., (E,E)-10,12-hexadecadienal (10), (Z)- 11-hexadecenal (2.0) and (Z)-11-octadecenal (2.0), were synthesized in the laboratory by developing economically viable synthetic routes. The results obtained by electroantennography and Y - tube olfactometer bioassays proved the attractiveness of the three component pheromone blend on *E. vittella* males confirming the laboratory bio-efficacy of the indigenously synthesized blend. The field performance of indigenously developed pheromone lures of E.vittella were initially evaluated in farmers' fields in Kisan nagar village of Mahaboobnagar district in Andhra Pradesh for optimization of effective trap type, dispenser and pheromone lure dosage. Traps baited with the pheromone blend dosage of 3 mg / dispenser were significantly more effective in terms of efficient catching and persistence than dispensers loaded with 1 mg. There was no significant difference between the trap catches in locally manufactured green rubber septa / plastic vial dispensers in comparison to imported rubber septa (Aldrich chemicals). Funnel traps caught significantly more number of males compared to delta sticky traps capturing 3.7 times more males. Based on the results obtained in these study plots, pheromone monitoring trials were conducted in farmers' fields at Dhule, covering 8 ha cotton of crop in collaboration with Mahatma Phule Krishi Vidyalaya (MPKV), Maharashtra. Five traps per hectare were installed in cotton fields and the trapped population of *E. vittella* was monitored every alternate day. The results obtained from the monitoring experiments indicated that maximum moths of *E. vittella* were caught between 45th and 46th M.W in dry land cotton during the kharif season.

Keywords: *E. vittella*, Indigenous Pheromone synthesis, Monitoring, Funnel trap, Dispenser

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Introduction

Cotton is an important cash crop in India, but productivity is very low in comparison to other countries. Several biotic and abiotic factors contribute to low cotton yield, of which insect-pests are the major component. About 184 insect pests have been recorded on cotton in India, causing 30–80% loss in yield, and among them the bollworm complex is a major constraint (Dastur *et al.,* 1960; Sohi, 1964). The spotted bollworms, *Earias insulana* and *Earias vittella* are important pests of cotton along with other bollworms and mainly feed on fruiting parts of the plant, resulting in considerable losses in quantity and quality (Atwal, 1994). As the larvae of these species feed primarily inside the flower buds and bolls of cotton it is difficult to control them by conventional methods. The use of pheromone baited traps to monitor the presence and population level of economically important moth species can be a valuable tool in an Integrated Pest Management Programme (Rosca *et al.*, 1985)). Detection of a pest species and the knowledge of its distribution are crucial for successful IPM strategies. When pheromones are used as a tool to monitor pest populations it results in need based use of pesticides. As cotton cultivation is ridden with the large scale use of insecticides, any reduction in the use of pesticides can make an economic impact in terms of saving on production cost (Tamhanker *et al.,* 2000). Kehat *et al.*, (1981) and El-Mosa (1986) have demonstrated the successful use of the sex pheromone of *E.insulana* as a monitoring tool.

Sex pheromone of *Earias vittella* (Lepidoptera:Noctuidae) was identified by Cork *et al.,* (1986) as a six component mixture hexadecanal, (*Z*)-11-hexadecenal, (*E,E*)-10,12-hexadecadienal, octadecanal, (*Z*)-11-octadecenal, and (*E,E*) -10,12- hexadecadien-1-ol in 1 : 2 : 10 : 2 : 4 : 1 ratio. Field testing in Pakistan showed that a 2 : 10 : 2 mixture of (*Z*)-11-hexadecenal, (*E,E*)-10,12-hexadecadienal, and (*Z*)-11-octadecenal was as attractive to male *E. vittella* moths as the six-component mixture and equal in attractiveness to a virgin female moth. Omitting (*Z*)-11-hexadecenal or (*Z*)-11-octadecenal greatly reduced this attractiveness. In the present study, we report the results of laboratory and field trials carried out to prove the attractiveness of the indigenously synthesized three component pheromone blend of *Earias vittella* as a monitoring tool.

Materials and methods

Insect culture

Field collected cotton bolls (infested) were maintained in the laboratory insectary until pupation at 27º C ± 1º C, a 14:10 (L: D) photoperiod and 70 % relative humidity. The pupae were segregated by gender and maintained separately in adult cages. The adults after emergence were fed on 10% honey solution. The three components of *E.vittella* pheromone blend - (*E, E*)-10, 12-hexadecadienal, (*Z*)-11-hexadecenal and (*Z*)-11-octadecenal in the ratio of 10:2: 2 (as confirmed by Cork *et al.*) were synthesized in the laboratory by developing economically viable synthetic routes (Yadav *et al.*, 1989).

Electroantennogram Recording Technique (EAG)

The antennal receptivity of *E.vittella* males (3- 4 day old) to indigenously synthesized female produced pheromone blend was determined by Electroantennogram Recording Technique (EAG). For electrophysiological recordings, Syntech EAG (Syntech, Hilversum, The Netherlands) was employed and the procedure for the preparation of stimulus pipettes and methodology is described in detail in our earlier paper (Jyothi *et al.,* 2008). The three component pheromone blend was diluted in n-hexane (HPLC grade) to obtain different concentrations (0.5µg to 5µg). Fifty micro liters of the solution to be tested was transferred to a 2 cm^2 filter paper and after complete evaporation, the filter paper was inserted in to 15 cm Pasteur pipette. EAG responses were recorded from five male moths individually to different concentrations of the synthetic blend. ‘

Olfactometer Bioassays

The orientation response of *E.vitella* males to indigenously synthesized 3 component pheromone blend was assessed in a Y-tube olfactometer following the experimental procedures describer earlier Prasuna *et al* 2008). The Y-tube olfactometer (stem, 12 cm; arms, 7.5 cm at 60° angle; internal diameter 1cm Analytical Research Systems, Florida , USA) consisted of two odour-bearing chambers of 4 cm length & 1cm diameter; (one for the test sample and the other for control or reference) one on each of the arms. An air delivery unit (Syntech model) was connected to the two arms of ‘Y’ tube to draw purified air to pass through the odour sources in the ‘Y’ tube. Airflow through each of the olfactometer arms was maintained at 250 ml / min.

E.vitella pheromone blend diluted in n-hexane (5µg- the dose which elicited maximum EAG response) was applied to Whatman filter paper of the size 1 x 2–cm and placed in one of the 'Y' tube chambers against the air stream and with a control (equal volume of HPLC-grade hexane) in the other. A single male (3-4 day old) was introduced into the Y-tube at the entrance of the stem so that it can make a choice between the test odour and the control. The behaviour of the males was classified as one of the three categories, choosing between hexane or the pheromone or no-choice (individuals which had not made a choice for either odour source within 5 min of crossing the start line). Each experiment was replicated three times using 50 insects and the olfactometer arms were flipped around (180°) to minimize positional effect after testing of 10 insects. Per cent attractancy was calculated according the formula described earlier (Prasuna *et al*., 2008)

Field experiments for optimization of dispenser, lure dosage and trap type

The field performance of the indigenously developed pheromone lures of *E.vittella* were initially evaluated in cotton fields at Kisan nagar village, Shadnagar mandal of Mahaboob Nagar district, Andhra Pradesh. Two study plots comprising of one hectare each of cotton crop were selected. The effectiveness of pheromone blend dose, trap design (funnel and delta sticky traps) and dispenser type was examined in these study plots. A variety of factors affect the attractiveness of pheromone based lures, such as pheromone dose, release rate, blend of active volatiles and dispensers. Since pheromones are volatile in nature, slow release formulations have been developed so that an effective release rate is maintained over several days. Controlled releases of pheromone lures have included cotton dental wicks, rubber septa, polyethylene vials, laminates, hollow fibers, membranes and polymeric systems. Hence, in the study plot I, lure dosage, longevity and efficacy of three commercially available dispensers was evaluated. Observations on moth catches were recorded at weekly intervals over two month's period. Three different types of dispensers procured from local market i.e., rubber septa and plastic vials with lids purchased from Abhishek Enterprises, Hyderabad and rubber septa from Aldrich Chemicals, USA (Catalog No.Z10072-2) were selected. 100 µl of hexane (HPLC grade) containing 1 mg and 3 mg dose of the 3 component pheromone blend along with equivalent weight of antioxidant - Butylated hydroxy toluene (BHT) was impregnated on to individual dispensers. After complete evaporation of the solvent, the dispensers were sealed in plastic sachets and stored at 0-4^{O} C after proper labeling until field use.

For optimization of dispenser and pheromone lure dosage studies, funnel traps were uniformly selected as trapping device (mini field experiments conducted in the laboratory indicated the performance of funnel traps to be superior over the sticky traps). The trial area was designed in three replicated blocks. The rule of the thumb for trap height in cotton as suggested by Tamhanker *et al.* (2005) is followed which is 60 cm above ground level in the early season and 15 cm above the crop canopy in the late season. The position of traps inside each block was rotated weekly to minimize the possible position effect on trap catch efficiency. The moths caught were counted weekly for a period of two months. Data from the moth catches in different types of dispensers was analyzed by analysis of variance (ANOVA).

In the study plot II of Kisan Nagar trap catch efficacy of two types of commercially available traps i.e., plastic funnel trap and sticky delta trap (purchased from Pest control India, Hyderabad) were evaluated for their effectiveness in capturing male moths in order to confirm our earlier mini field studies. Green rubber septa baited with 3 mg of the 3 component pheromone blend was uniformly used in these studies. Five replicates of two types of traps were placed in the field with a minimum of 40 m apart. Traps were periodically rotated to minimize the possible position effect on trap efficiency. Weekly counts of males caught in the two types of traps were recorded and the data were log transformed and analyzed by analysis of variance.

Pheromone Monitoring Trials

The monitoring trials were conducted in 8 ha of cotton crop (Hirsutum variety) in the farmers' fields of Nagaon village on kharif cotton. Monitoring traps were installed two months after sowing at the on set of the flowering stage. Five funnel traps per ha baited with 3 mg of the 3-component pheromone blend in rubber septa dispensers were installed in the fields and monitored the trapped population of *E. vittella* on every alternate day. The percentage of infested squares / bolls was evaluated by counting the total and attacked number of squares / bolls in a sample of twenty randomly collected plants at weekly intervals.

Results and discussion

EAG bioassays

The EAG data in Figure 1 represents corrected EAG values (synthetic pheromone blend minus control). The synthetic blend evoked significant olfactory responses in the male antenna confirming the bio-efficacy of the indigenously synthesized pheromone blend. A dose dependant increase in EAG activity was observed with increasing doses of synthetic pheromone blend with maximum depolarization at 5 µg dose (Fig. 1). Tamhanker *et al*. (1989) reported a similar concentration dependant response of both *E.insulana* & *E.vittella* males to female sex pheromone and an increase in the level of response with an increase in pheromone concentration in olfactometer bioassays.

Olfactometer bioassays

The arm with pheromone blend odour was preferred by males compared to the other arm with hexane. Indeed, 90 % of the males selected the chamber containing the female synthetic pheromone compared to the hexane chamber. Analysis of variance by ANOVA gave p-values of 1.04^{-06} which meant that *E.vitella* males were attracted by pheromone blend odour with a very highly significative difference compared to control proving the efficacy of the indigenously synthesized pheromone blend.

Optimization of effective dispenser, lure dosage and trap

The data on the field performance of various dispensers loaded with 1mg and 3 mg of pheromone blend is depicted in Table I. Trapping efficiency of locally manufactured rubber septa dispensers were equally effective in attracting the males to traps in comparison to imported rubber septa dispensers. *E.vittella* males trapped in all the three types of dispensers loaded with 3 mg of the pheromone blend is depicted in Fig 2. There was no significant difference between the moth catches in locally manufactured green rubber septa / plastic vial dispensers in comparison to imported rubber septa ($P = 0.5$; $F = 0.71$; F crit $= 5.14$). All the three types of dispensers loaded with 3 mg of pheromone blend gave a consistent trap catches for at least 3 – 4 weeks; while trap catches with 1 mg loadings started to decline from the 3rd week onwards in all the three types of dispensers (Table 1). Similarly Kehat *et al*. (1989) reported that dosage of 3 mg / trap effectively attracted *E.insulana* males when released from polyethylene vials but not from rubber dispensers. However, our results indicated efficient attraction of males with rubber dispensers. Rubber septa was also found as an effective dispenser for trapping the males of gram pod borer & rice yellow stem borer whose pheromone blends constitute aldehydes similar to *E.vittella* pheromone blend (Yadav *et al*., 2003; Prasad *et al*., 2010). Rubber septa have been the most widely used substrate for the controlled release of many insect pheromones (Butler and McDonough, 1981; Weatherston, 1989).

Pheromone lures used in monitoring traps should ideally release an effective dose of pheromone at a constant rate throughout the trapping period

(Vrkoc *et al.*, 1988; Wall, 1989). Residual analysis of field exposed pheromone lures (4 week) loaded with 3 mg of the pheromone blend in the laboratory indicated the presence of the major component(E, E) – 10, 12 - hexadecadienal only. The decline in trap catches as depicted in Table 1 may be due to the absence of miner components in the blend. We reported similar decline in trap catches of groundnut leaf miner pheromone lures after 3 weeks of field exposure due to the absence of minor components (Yadav *et al.*, 2001). To ensure the availability of the pheromone blend in adequate amounts for optimal attraction throughout the experimental period, lures were replaced with fresh ones every 21 days (3 weeks).

Average number of males trapped per trap per week in the funnel and delta sticky traps are depicted in Fig 3. The results indicated that funnel traps are more effective in capturing the *E.vittella* males (almost 3.7 fold more) than sticky traps (ANOVA Single factor $P = 8.57^{-07}$; $F= 69.32$; F crit $= 4.60$). Since the moth entry is restricted in case of the delta trap, it may have interfered with the pheromone plume resulting in reduced trapping of the attracted insects. Also, limited trapping capacity, dust and probably the scales of the trapped insects may have interfered with the trapping. Kehat *et al*. (1989) during their monitoring trials with *E.insulana* in Israel reported funnel traps baited with synthetic pheromone or with virgin females to be more effective than water traps. Funnel traps, being much easier to handle than either water traps or delta sticky traps and with their larger trapping capacity are preferable and therefore selected for *E.vittella* monitoring trials.

Monitoring trails

The results of monitoring trials are presented in Fig. 4. Moth catches were not recorded in most of the traps up to 3 weeks of pheromone trap installation. Catches were almost zero up to 37th MW except for 1-2 catches of males in 2-3 traps. However, the activity of the *E. vittella* started from the 38th MW with a mean number of 5-6 moths / trap / week. A gradual increase in trap catches was observed in all the traps with a peak record of 19 moths / trap / week during 45th and 17 moths / trap / week 46thstandard weeks respectively. There after, a steady decline in trap catches was observed with a minimum trapping of 1.5 moth /trap/week during the 49th MW and the moth catches were almost zero by the end of 50th M W. Larval damage amongst the squares / bolls was not significantly noticeable up to 36th MW (Fig. 4). Build up of larval population started from 37th MW which peaked to a maximum of 5.5% during 45th & 46th standard weeks significantly correlating with the maximum number of insects caught in pheromone traps. Pazhanisamy & Deshmukh (2011) reported a similar peak activity period of *E.vittella* male moths from 39th to 45th

in the range of 8.15 to 11.15 male moths / trap / week during the field trials conducted in kharif 2004-2005 at Cotton Research unit, Akola, Maharashtra. Pheromone monitoring trials of spotted bollworm conducted in Pakistan indicated a positive correlation between pheromone trap catches & number of larvae per plant (Qureshi and Ahmed, 1991).

In conclusion monitoring trials reported here provided information on appropriate time for trap placement for monitoring of *E.vittella* and the peak activity period of male moths along with data on crop damage. The results of the present study demonstrated that 3 mg of the indigenously synthesized 3-component pheromone blend of *E.vittella* impregnated on to locally available green rubber septa / plastic vial dispensers along with equal quantity of antioxidant (BHT) in a funnel trap can be effectively employed to monitor the males of *E.vittella*.

Table 1. Trap catch data of *E.vittella* males caught with different pheromone dispensers (1mg & 3 mg) aged 1- 4 weeks

S.No	Dispenser type	Pheromone dose	No. of insects trapped/trap/week				
			1st Week	2nd Week	3rd Week	4th Week	Total
1	Rubber septa (Aldrich)	1 mg	10.0 ±0.75	12.3± 1.3	6.9 ± 0.3	3.00 ± 1.1	32.2± 1.6
		3 mg	14.0 ± 1.1	10.0± 1.5	11.4 ± 1.5	9.2 ± 2.0	44.6±0.85
2	Rubber septa (local)	1 mg	10.6 ±1.6	11.0 ± 0.5	9.6 ± 2.0	2.3 ± 0.5	33.5± 0.5
		3 mg	14.2± 1.3	12.6± 2.5	13.5 ± 1.7	11.2 ± 0.85	51.5± 1.1
3	Plastic vial (local)	1 mg	13.3 ± 3.1	9.5 ±2.0	8.6 ± 1.1	3.3 ± 1.5	34.7± 0.25
		3 mg	11.3 ± 3.2	14.6 ± 3.4	10.5± 3.0	13.3 ± 1.5	49.7± 0.85

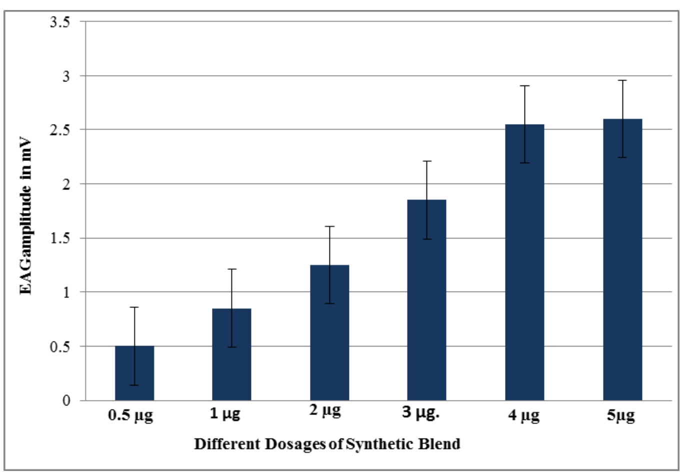

Fig. 1. Electroantennogram Responses of *E.vittella* Males Towards Different Dosages of Female Produced Synthetic Pheromone Blend

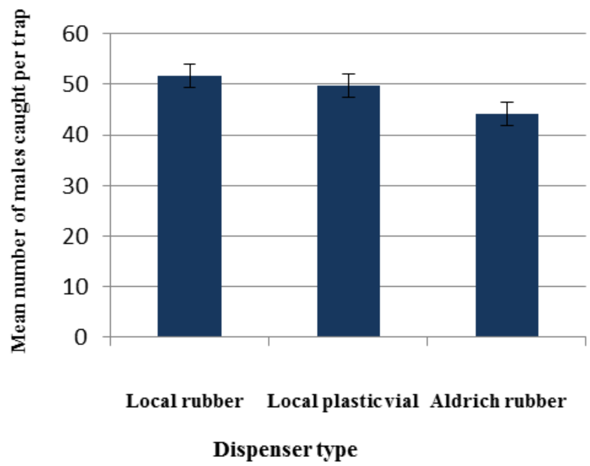

Fig. 2. Mean number of males of *E.vittella* caught by funnel traps baited with 3 mg of 3-component synthetic blend

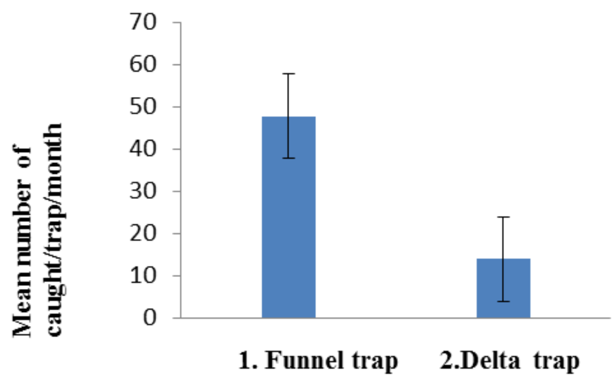

Fig. 3. Mean number of *E.vittella* males caught in funnel and delta sticky traps baited with green rubber septa dispensers impregnated with 3 mg of the 3 - component synthetic blend

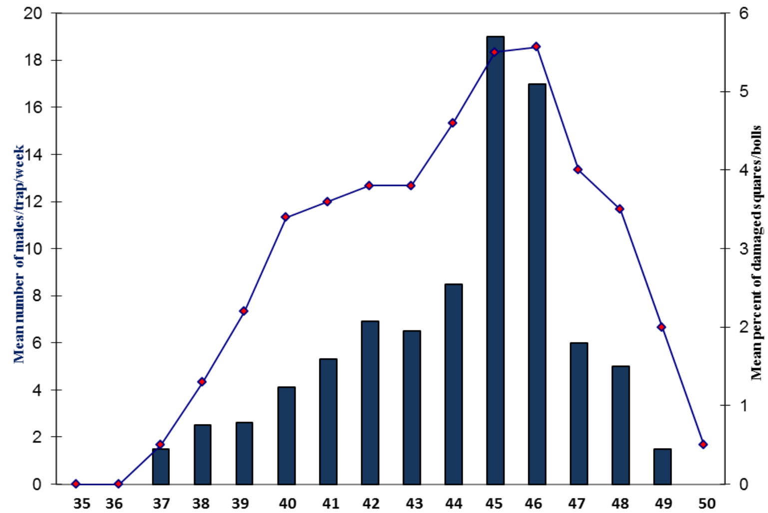

Fig. 4. Pheromone monitoring trials of *E.vittella* conducted in farmers fields of cotton at Dhule, Maharashtra during kharif 2005-2006

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