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SYNERGISM OF 1-OCTEN-3-OL WITH SEX PHEROMONE IN LEGUME POD BORER, *MARUCA VITRATA* FABRICIUS (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Sex pheromone components of *Maruca vitrata* include (*E, E*)-10, 12-hexadecadienal (major component), (*E, E*)-10, 12-hexadecadienol and (*E*)-10-hexadecenal (minor components). As increasing field attractancy of these have not been satisfactory world over, an effective blend for field trapping, five blends of sex pheromone components and isomers through electroantennography (EAG) was attempted. Blend that had (Z,E)-10,12-hexadecadienal (100 parts); (E,E)-10, 12-hexadecadienol (5 parts); (Z)-10-hexadecenol (5 parts) and 1-Octen-3-ol (10%) elicited maximum EAG response (P < 0.05). Dose response evaluated at 10 µg, 100 µg, 500 µg and 1000 µg doses through EAG, flight behaviour (wind tunnel) and field experiments revealed that 1000 µg elicited higher electrophysiological and taxis response and male moth catches compared to remaining blends. These findings hint at a possible synergistic action of 1-Octen-3-ol with pheromone components or their isomers.

Key words: Electroantennography; (*E, E*)-10,12-hexadecadienal, (*E, E*)-10,12-hexadecadienol, (*E*)-10-hexadecenal, field attractancy, wind tunnel

The legume pod borer or spotted pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) is a pantropical insect pest of grain legumes infesting reproductive parts and drastically decreasing the yields (Sharma, 1998). Its geographic range extends from northern Australia to East Asia through subSaharan Africa. With the introduction of short duration and determinate growth habit pigeonpea genotypes in recent years, the legume pod borer has become a major limitation. This pest is a major factor responsible for heavy loss in early and medium maturing pigeonpea genotypes (Sahoo, 1995; Shanower *et al.*, 1999). Importance of *M. vitrata* as a pest on grain legumes results from its early establishment and larvae web the leaves and inflorescence and feed inside flowers, flower buds and pods. Vishakantaiah and Jagadeesh Babu (1980) estimated 9 to 51% infestation under Bangalore conditions while 50% avoidable loss on pigeon pea cultivars was reported in Tamil Nadu (Ganapathy, 2010).

Chemical insecticides are widely used for its management, but it results in adverse affects (Sodavy *et al.*, 2000; Alam *et al.*, 2006). Insecticides are ineffective and uneconomical against *M. vitrata* due to typical concealed feeding habits that protect larvae from insecticides (Sharma, 1998). So it is necessary to develop alternate methods like semiochemical based pest management that is effective, efficient, cheap and environmentally safe. Eventhough, several attempts have been made to optimize and develop sex pheromone lures for the management of *M. vitrata* (Hassan, 2007; Downham *et al.*, 2003; Downham *et al.*, 2004), these were not successful. This may be due to the possible existence of polymorphism in blend's composition of *M. vitrata* sex pheromone among populations from different geographical regions (Schlager *et al.*, 2012). An attempt to utilize combination of pheromone and cowpea plant volatile 1-octen-3-ol is made.

MATERIAL S AND METHODS

The legume pod borer culture was established at the rearing chamber of the Biocontrol Research laboratories (BCRL), Pest Control India (PCI) Pvt. Ltd. Sriramanahalli, Bengaluru with larvae collected from infested dolichos bean (*Lablab purpureus*). Insect colonies were maintained on a 12 light (L) : 12 dark (D) with room temperature of 25±2^{o}C and relative humidity 60 ±10%.

Adults mated in rearing cages (60cm×40cm×40cm) were fed on 10% sugar solution. Dolichos bean flower and pod bouquets were placed in cages for oviposition. Eggs were allowed to hatch on boquet, and after 2–3 days collected with a fine camel hair brush into transparent plastic boxes (7cm dia, 12cm height) containing absorbent paper in which larvae subsequently developed. Larvae were reared individually to avoid cannibalism. For the first and second instars, flowers and flower buds were provided as food and from third instars, pods were provided in addition. Fresh food material was replenished daily. Eclosed adults were kept separately for subsequent experimental use or for mating.

Three identified *M. vitrata* sex pheromone components *viz.*, (E, E)-10, 12-hexadecadienal; (E,E)-10,12-hexadecadienol and (E)-10-hexadecenal and their isomers were synthesized at the concentration of 20 mg/ml (2%) at chemical synthesis laboratory of BCRL, PCI Pvt. Ltd. Bengaluru. Following blends with their ratios were used along with control solvent (Hexane) for further bioassays. Bioassays for pheromone blends were conducted using Electroantennography (EAG) and Wind tunnel at Insect Behavior Testing Laboratory (IBTL), BCRL.

Electrophysiological responses of male *M. vitrata* antennae to synthetic blends were recorded using Syntech EAG system. 10 µl blend was placed on the filter paper (6 cm length and 5 mm dia; Whatman No. 1) strip inside a glass Pasteur pipette (5.75, Length-overall 145.0 mm; tip 47.0 mm) used for stimulus delivery. This was connected to the stimulus controller by silicone rubber tubing. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the antenna by injecting the vapour phase of the chemical stimuli through a polyesterene tube along with the continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the test antenna. Before antennaectomy, moths were maintained at 6^0 C for 1 min to make them inactive in plastella cups (dia 10 cm). Antennae were incised using microscissor. Each recording was replicated five times. Best performed blend was tested at four doses *viz.*, 0.5, 5, 25 and 50 µl to obtain 10, 100, 500 and 1000 µg, respectively to find out the dose-variation response.

The blend having the best electrophysiological response from EAG, was further tested to determine the behavioural effects in a wind-tunnel in above mentioned doses to find out the dose-variation response. Flight behaviour of male to synthetic pheromone blends was recorded. A wind tunnel of 80x30x30 cm calibrated to 25 cm s^{-1}, 5 lux (light intensity), 24±1^0C and 65±5 % RH in a room separated from the *M. vitrata* culture was used in these studies. Observations on number of moths taken to flight and number of moths reaching the zone where pheromone source kept was recorded.

An experiment was carried out to know the field efficacy of doses of best performing blend at Kakol village (13° 11' 26 20'' N; 77° 31' 10 50'' E), Bengaluru Rural district in dolichos bean field during October-November, 2014. Lures used in the experiment were of polyethylene vial dispensers. Since the dose 0.5 µl (10 µg) has not elicited better response in EAG and wind tunnel, it was not considered for field trapping studies. So, doses *viz.*, 5, 25, 50 µl of 2% solution were used to make 100, 500 and 1000 µg respectively, were used in the field trapping experiment with control under Randomized Complete Block Design (RCBD). Experiment was replicated five times. Commercially available Wota-T water traps were used for trapping at 0.8m height from the ground level. Within the field, individual traps were positioned 10 m apart. Trap catches were counted daily and captured moths were removed. Data were pooled at weekly intervals for analysis. Mean data were compared using one-way ANOVA and the means were separated using Tukey's HSD test using IBM SPSS (version 21).

RESULTS AND DISCUSSION

EAG responses (mV) of male *M. vitrata* to isomeric blends are summarized in Table 1. Among the blends tested, combination of (Z,E)-10,12-hexadecadienal; (E,E)-10,12-hexadecadienol; (Z)-10-hexadecenol and 1-Octen-3-ol (Blend D) elicited significantly higher EAG ($F_{4, 20}$=14.50; P =0.0001) response than original components. On contrary, Adati and Tatsuki (1999) showed that the failure of attraction in male *M. vitrata* was caused possibly by the geometrical isomers of E10, E12-16: Ald. The addition of *EZ, ZE*, or ZZ isomers to lures containing the synthetic pheromone and the crude extract antagonized their attractancy to male moths. Studies by Downham et al. (2003) suggested that when EAG responses to a range of mono unsaturated hexadecenal isomers were compared, the *E* isomers of 10-, 11-, and 12-hexadecenal gave the highest response and they also noticed that tetradecenyl acetate isomers elicited lower EAG responses from male *M. vitrata* compared with the hexadecenal isomers. The results of current study corroborate with the above findings.

Blend D revealed that addition of 1-Octen-3-ol, an identified cowpea floral volatile evoked higher response than blend C which lacked this volatile. Probably 1-Octen-3-ol acts as synergist with the pheromone

Table 1. EAG responses of male *M. vitrata* to isomeric blends

Sl. No.	Blend Number	EAG Response (mV) (mean±SE)
1	A	1.064±0.14 [bc]
2	B	0.662±0.35 [cd]
3	C	1.465±0.36 [ab]
4	D	1.716±0.36 [a]
5	E	0.331±0.24 [d]
	Df	4, 20
	F	14.50
	P	0.0001

components. Results by Arx *et al.* (2011) are in agreement with our results. Comparison of the host plant headspace profiles showed that 14 EAG active plant volatiles including 1-Octen-3-ol to which European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller) responded. They showed that male moths perceived and responded to host plant volatiles as olfactory cues and males can discern places where the likelihood of encountering females is higher. 1-octen-3-ol and CO_2 acted synergistically in attracting significantly greater numbers of male *Aedes taeniorhynchus* (Wiedemann) and *Anopheles* spp. (Takken and Kline, 1989).

Among doses of best performed blend (Blend D) tested (10µg, 100µg, 500µg and 1000 µg), 1000 µg had significantly higher EAG response than remainng doses (R^2=0.9734; P=0.03) (Fig. 1.). These results are in agreement with Jyothi *et al.* (2008), who showed that dose-dependent increase in EAG response was observed with increasing doses of synthetic pheromone blend of *Aproaerema modicella* (Deventer) (Gelechiidae: Lepidoptera).

EAG experiments clearly indicated that blend D had significantly higher response. The behavioral assays in wind tunnel conducted with this blend indicated that significant differences were noticed in male moths responding to all doses except at 10µg (Table 2). Significantly higher taxis response was observed when 1000 µg of synthetic blend was compared with the remaining doses ($F_{3,16}$=39.33; P=0.0001). In contrast, Mondhe (2001) reported no significant differences in response levels between the 3-component *M. vitrata* lure and the hexane solvent (control). Downham *et al.* (2003) showed that male moths responded to a range of synthetic blends were weaker. This may be due to the fact that in the present study isomers of these components coupled with 1-Octen-3-ol, a possible synergist were used. Similarly, a blend of the four host plant volatiles (1-hexanol, 1-octen-3-ol, (Z)-3-hexenyl acetate and (E)-â-caryophyllene) mixed at a ratio attracted significantly more males than any single compound and the study suggested that 1-octen-3-ol served as a synergist (Arx *et al.*, 2011).

In the field experiment conducted with blend D at different doses, 1000 µg trapped 5.80 ±1.35 (mean±S.E) mean number of moths per trap significantly higher than remaining doses ($F_{3,16}$= 3.453, P=0.042) (Fig. 2.). Similar study conducted at Benin, West Africa by Downham *et al.* (2003, 2004) found good attraction of male *M. vitrata* moths to a 3-component synthetic pheromone blend (EE10,12-16:Ald), (EE10,12-16:OH) and (E10-16:Ald) in the ratio of 100:5:5 (Benin blend). But the same blend never caught moths efficiently in Taiwan (0 and 0.3±0.3

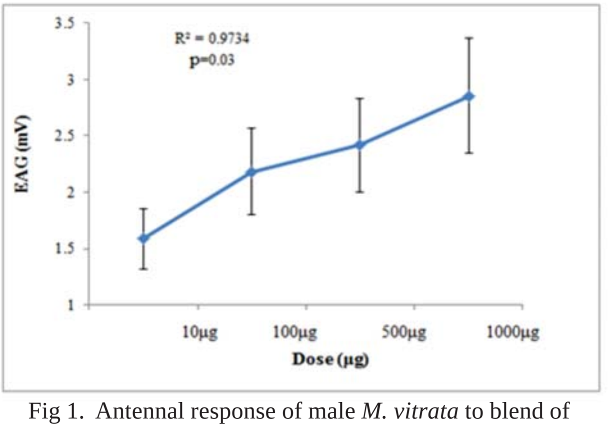

Fig 1. Antennal response of male *M. vitrata* to blend of female synthetic pheromone and 1-Octen-3-ol

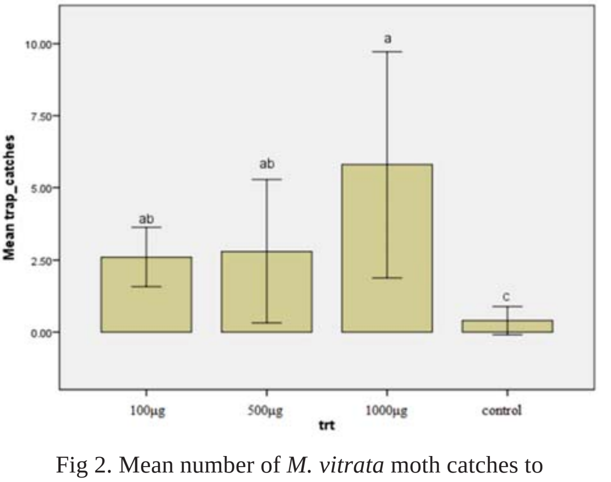

Fig 2. Mean number of *M. vitrata* moth catches to doses of Blend D

Table 2. Relative taxis response of *M. vitrata* moths to different doses of Blend D

Sl. No.	Doses (µg)	*Mean taxis response (%)
1	10	22.32[c]
2	100	41.33[b]
3	500	44.66[b]
4	1000	89.40[a]
Df		3, 16
F		39.33

* mean values with different letters in a column are significantly different by one-way ANOVA with Tukey's Post-hoc test

males at 120 and 180 cm, respectively in delta trap) (Schläger *et al.*, 2012). These findings lead to the conclusion that there is a possible polymorphism in the blend composition of the *M. vitrata* sex pheromone among populations from geographical regions. Moth catches in this study appears to be less, possibly due to experiment being conducted in farmer's fields where they apply insecticides as per their schedule.

Biological laboratory assays (EAG and wind tunnel) and field tests, revealed that the blend consisting of a (Z,E)-10,12-hexadecadienal; (E,E)-10,12-hexadecadienol; (Z)-10-hexadecenol and 1-Octen-3-ol proved to be the most effective for catching moths. This study reports the possible synergism of 1-Octen-3-ol with the pheromone components/isomers of *M. Vitrata*. Based on moth trapping it would be important to use a pheromone concentration of 1mg in order to optimize attraction under field conditions.

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