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Calling Behavior and Attractive Response of Cardamom Shoot and Capsule Borer *Conogethes punctiferalis* Guenee (Lepidoptera: Pyralidae) to the Female Crude Extract and Synthetic Blend

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ABSTRACT

The calling behavior and attractiveness of *Conogethes punctiferalis* to female crude extract and synthetic blend were investigated. The dose, age, sex of the *C. punctiferalis*, ratio of the compounds and airflow had significant influence over attraction of male shoot and capsule borer in cardamom. The calling of females started 03.45 h after start of scotophase and peak calling was observed 3 h prior to end of scotophase and attraction of male moths to synthetic blend (E-10-hexadecenal and Z-10-hexadecenal) was maximum at 90:10 and followed by 80:20 ratio.

Key words: Cardamom, *Conogethes punctiferalis*, sex pheromone, calling behavior

INTRODUCTION

Shoot and capsule borer, *Conogethes punctiferalis* Guenee (Lepidoptera: Pyralidae) is a serious pest of cardamom and causes more than 70 per cent crop loss (Renuka *et al*., 2006). For management, planters mainly use chemical insecticides which would pose the risk of hazardous problems. Even though eco friendly neem has been evaluated and suggested on different crops (Rajabaskar and Regupathy, 2005; Bhattacharyya *et al*., 2007; Pandey and Tiwari, 2011; Efil *et al*., 2005; Nderitu *et al*., 2008), planters mainly using insecticides for control. Even this should be applied at early stage of infestation and late application may difficult in killing endophytic larva. This makes search for an alternative method to control the pest effectively. Pheromones and other behavior-modifying chemicals found naturally in the environment, offer non-insecticidal alternatives and the sex pheromone of female yellow peach moth, *C. punctiferalis* was first reported by (Konno *et al*., 1980) from Korea as a mixture of (E)-10-hexadecenal and (Z)-10-hexadecenal at 90:10 ratio but less study is available in India. Therefore, a solid understanding of pheromone biology of cardamom shoot and capsule borer will help to develop an alternative method of control. With this view, a study was conducted to evaluate the attractiveness of *C. punctiferalis* to female crude extract and synthetic blend and to find the scope of synthetic blend of yellow peach moth to manage *C. punctiferalis* in cardamom.

MATERIALS AND METHODS

Test insects: The larvae of *C. punctiferalis* collected from cardamom field were reared on the rhizomes of green ginger (*Zingiber officinale*) where, the ginger had been kept into plastic tray (50×25×15 cm) containing moist sand. The filled tray was kept under nylon mosquito net

(2×1×1 m) to collect daily emerged adults and provided with 10% sugar solution from cotton pad. Five pairs of adult moths were allowed to lay eggs on an oviposition substrate which consist of two plastic tea filter (8 cm dia) kept in a opposite position in order to hold the food and covered with white, khada cloth. The substrate was kept inside the oviposition cage and allowed to hang from top of the cage. The adults were provided with 10% sugar solution as food. After 5 days the eggs laid on khada cloth were removed and used for mass culturing. Both male and female were sexed based on pupal marking at posterior abdominal segment (Honda *et al*., 1979).

Calling behavior: Daily emerged virgin females were individually caged in a plastic container (15.5×7.0 cm) and provided with 10% sugar solution. Visual observations were made at 30 min intervals during the scotophase (dark phase), as the insect is reproductively active during this period. A total of 100 females were put under constant observation to find out the time and frequency of calling, starting from 1, 2, 3, 4 and 5 days from emergence.

Pheromone response: The studies were conducted with a wind tunnel as described by Sundararaju *et al*. (1994) with a slight modification. It consists of two plastic boxes connected by a transparent tube (100 cm length and 7.0 cm dia) made up of Mylar film sheet. One of the boxes was used for keeping bait and another for keeping test materials. Each box has a removable lid at the top and two circular holes (6.5 cm in dia) on opposite sides and one end the tube at the bait box was been covered with muslin cloth to confine the insects to the bait box and another end was been free to allow the insect to move toward bait box and make observations. The adults are provided with 10% sugar solution using cotton pad. A portable personal mini fan with an electronic regulator was kept in front of bait box to carry air to test box. Each set of 20 individuals from 1, 2, 3, 4 and 5 days after emergence was tested and the study was replicated for 3 times.

Using adult moths: From mass cultured insects the male and female were sexed based on pupal markings and kept in the separate containers individually. Daily emerged adults were collected in a plastic container and kept. Sex pheromone response was assessed on 1, 2, 3, 4 and 5 days after emergence. Each of 20 females and 20 males were kept in bait and test box, respectively. The same experiment was repeated with male in bait and females in test box and replicated for three times.

Using female moth crude extract: The sex pheromone was extracted by clipping the abdominal tips (terminal two to three segments) of four day old virgin female moths into 50 µL of n-hexane and left to soak for about 60 min at room temperature. Female moths were put back in the dark before clipping to enhance the pheromone yield, this darkness exposure was started six hour after light on and clipping was done after 3 h darkness.

Using synthetic blend: The synthetic pheromone of yellow peach moth (E-10-hexadecenal and Z-10-hexadecenal) was obtained from the Chemtech; The Netherland, was used to study the pheromone response of *C. punctiferalis* at different ratio. Each one micro gram of blend (E-10-Hexadecenal and Z-10-Hexadecenal) was prepared using hexane at different ratio 100: 0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90, and 0:100 and impregnated with rubber septa. The adult making upwind flight and crossing more than 50%, length of wind tunnel was considered as successfully attracted. The experiment was conducted with airflow and without airflow and repeated for three times.

Statistical analysis: The percent data were subjected to statistical analysis adopting randomised block design. The percentage values were transformed to arc sine $\sqrt{\text{percentage}}$, using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) then separated the mean values of treatments.

RESULTS AND DISCUSSION

Calling behavior: Females of *C. punctiferalis* exhibited two types of calling behavior postures which are somewhat similar to those of jasmine bud worm *Hendecasis duplifascialis* Hampson (Suganthi *et al.*, 2006), *Earias insulana* Boisduval (Tamhankar, 1995) and carob moth, *Ectomyelois ceratoniae*, Zell (Soofbaf *et al.*, 2007) one with the wings fully raised approximately 45° to the body and with frequent wing vibration and occasional walking, the another one is keeping the wings close to the body and remaining stationary. During both calling postures, the ovipositor was extruded beyond the abdomen tip.

The age at which *C. punctiferalis* initiated calling varied considerably (Table 1) from day 2 to 5 following emergence and there was no calling on the first scotophase following emergence. The percent calling was increased with age of the females and maximum was in four day-old females (97.5%) (Table 1). The earliest initiation of calling was 03.45 h after the start of scotophase and extended upto 15.00-17.30 h. Peak calling was observed three hrs prior to end of scotophase. Once the calling behavior started, this posture continued for 2 h. This behavior was more or less common to other pyralids like *E. insulana* (Tamhankar, 1995) and jasmine bud worm *Hendecasis duplifascialis* Hampson (Suganthi *et al.*, 2006). Age had a significant influence over calling behavior and this may be related to differences in reproductive maturity, as noted by Kanno (1979) in rice stem borer *Chilo suppressalis* and in *E. ceratoniae* by Soofbaf *et al.* (2007).

Upwind flight: In the wind tunnel, female kept in test box showed considerable variation in attraction of males. Attraction varied to an extent of 5.33-22.33 males with airflow and 1.33 to 15.33 males without airflow and it was highest in four day old females; and lowest in one day old females; the extent being 89.32 and 29.32%, respectively with airflow and 61.32 and 5.32% without airflow (Table 2). Then male kept in the test box showed that attraction was 20.0-42.68 and 12.0-13.32% with and without airflow respectively whereas, blank showed 10.68 and 6.68% response. Usually after several unsuccessful attempts, the male entered the cylinder to reach at the opening of opposite box from which the pheromone of virgin females was dispersed. Some males flew away once but were attracted again. When testing with male in bait box attracted the females and it was reported as cross attractiveness (Konno *et al.*, 1981; Honda, 1986). *Abdominal crude*

Table 1: Calling behaviour of virgin adult female of *C. Punctiferalis*

Age of virgin female (days)	Mean No. of females initiated calling	Females initiated calling (%)
0 (1 h after emergence)	0.8	4.5^{e} (12.12)
1	4.3	21.5^{d} (27.62)
2	10.5	52.5^{c} (46.43)
3	16.3	81.5^{b} (64.62)
4	19.5	97.5^{a} (81.09)
5	4.8	24.0^{d} (29.32)

Values in the parentheses are arc sine $\sqrt{P}$, where P is % moth attracted, Means followed by same letter(s) in a column are not significantly different by DMRT (p = 0.05)

Table 2: Laboratory bioassay test against pheromone response of *C. punctiferalis* (adult moths)

Days after emergence	Sex in test box	No. of moths attracted (with air current)	Moths attracted (%)	No. of moths attracted (with out air current)	Moths attracted (%)
1	F	5.33	29.32^{gf} (32.78)	1.33	5.32^{g} (13.27)
2	F	9.67	38.68^{f} (38.45)	2.00	8.00^{f} (16.39)
3	F	17.67	70.68^{d} (57.21)	14.67	58.68^{b} (49.99)
4	F	22.33	89.32^{a} (70.94)	15.33	61.32^{a} (51.54)
5	F	19.33	77.32^{b} (61.56)	10.33	41.32^{c} (40.00)
1	M	10.67	42.68^{e} (40.78)	3.33	13.32^{de} (21.39)
2	M	6.33	24.79^{h} (29.86)	3.00	12.00^{e} (20.25)
3	M	5.00	20.00^{i} (26.55)	3.33	13.32^{de} (21.39)
1,2,3,4,5	F	18.33	73.32^{c} (58.90)	3.67	14.68^{d} (22.51)
Blank		2.67	10.68^{j} (19.05)	1.67	6.68^{g} (14.93)

Values in the parentheses are arc sine$\sqrt{P}$, where P is percent moth attracted, Means followed by same letter (s) in a column are not significantly different by DMRT (p = 0.05), F: Female, M: Male

Table 3: Laboratory bioassay test against pheromone response of *C. punctiferalis* (crude extract)

Female equivalent	No. of males attracted (with air current)	Male moths attracted (%)	No. of males attracted (with out air current)	Male moths attracted (%)
1	6.33	28.87^{e} (32.50)	2.33	15.53^{e} (23.20)
3	8.67	57.80^{d} (49.49)	4.67	31.13^{d} (33.91)
5	10.33	68.87^{c} (56.09)	5.33	35.53^{c} (36.59)
10	12.33	82.20^{b} (65.05)	7.67	51.07^{b} (45.61)
20	14.33	95.53^{a} (77.87)	10.33	68.87^{a} (56.09)
Blank	1.33	8.87^{f} (17.30)	0.33	2.20^{f} (8.30)

Values in the parentheses are arc sine$\sqrt{P}$, where P is percent moth attracted, Means followed by same letter (s) in a column are not significantly different by DMRT (p = 0.05)

extract: Attraction was considerably influenced by airflow into wind tunnel and it was extended from 6.33 to 14.33 males and in the absence airflow attraction was 2.33 to 10.33 male moths (Table 3). Whereas, in the blank 0.33-1.33 male moths were observed. Twenty female equivalent quantities attracted the highest number of males with airflow though the activity was detected even with one female equivalent (Table 3). When the pheromone titer of crude extract is more, the attraction was also high. This may be due to the dose of sex pheromone dispersed into the environment.

Synthetic blend: The laboratory evaluation of synthetic blends (E-10-hexadecenal and Z-10-hexadecenal) in rubber septa attracted 0.33-12.33 male moths (Table 4). The highest response was observed in 90:10 ratio (82.20%) which was on par with 80:20 ratio (80.00%). The dose, age, sex of the *C. punctiferalis* , ratio of the compound and airflow had significant influence over attraction of male shoot and capsule borer in cardamom.

Cardamom is being an export oriented crop the use of pesticide should be strictly avoided for the prosperity of cardamom industry hence, alternative and eco friendly pest management strategies is need for it's sustainability. The use of pheromone for monitoring of *H. armigera* (Malik *et al*., 2003), *Pectinophora gossypiella* Saunders (Attique *et al*., 2000), *Chilo supperassalis* (Cork and Basu, 1996) and etc., were successful in IPM programme, therefore the synthetic blend can be further test verified in field condition and could be used for monitoring or mass trapping of *C. punctiferalis* in cardamom plantation.

Table 4: Bioassay to test against pheromone response of *C. punctiferalis* to synthetic blend

Blend ratio	No. of males attracted (with air current)	Male moths attracted (%)	No. of males attracted (with out air current)	Male moths attracted (%)
100:0	0.33	2.2^{e} (8.30)	0	0^{d} (0.02)
90:10	12.33	82.20^{a} (65.05)	6.67	44.47^{a} (41.82)
80:20	12.00	80.00^{a} (63.44)	6.33	42.20^{a} (40.51)
70:30	8.33	55.53^{b} (48.18)	0.33	2.20^{e} (8.30)
50:50	2.33	15.53^{c} (23.20)	1.67	11.13^{b} (19.47)
10:90	0.67	4.47^{d} (12.13)	0.33	2.20^{e} (8.30)
20:80	0.33	1.70^{e} (7.40)	0	0^{d} (0.09)
30:70	0.67	4.47^{d} (12.13)	0	0^{d} (0.21)
Blank	0.33	2.20^{e} (8.30)	0	0^{d} (0.09)

Values in the parentheses are arc sine $\sqrt{P}$, where P is percent of moth attracted, Means followed by same letter (s) in a column are not significantly different by DMRT (p = 0.05)

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