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Behavioral Response of *Helicoverpa armigera* (Hubner) Larvae to Flower and Leaf Extracts of African Marigold, Cotton, Okra and Pumpkin

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Abstract

Helicoverpa armigera (Hübner) larvae were more attracted towards the diet treated with flower extracts of pumpkin followed by cotton, okra and African marigold whereas in leaf extracts larvae preferred cotton followed by African marigold, okra and pumpkin treated diet. The TLC analysis of these extracts showed a blend of compounds corresponding to careen, ocimine, methyl benzoate, phenyl acetaldehyde and phenyl ethanol. Attempts were made to correlate the larval behavior with these compounds.
Key words : Attraction, Compounds, Larvae, TLC.

Chemical stimuli from plants play an important role in insect-plant interactions. The insect community and other arthropods depend upon chemical stimuli for survival and reproduction. There is growing evidence that host finding by moths is largely guided by secondary plant metabolites (1), particularly volatile compounds (2). Plant volatiles may act as ovipositional stimulants or deterrents, or may act as communication bridge to locate a suitable host (3). Information about the role of allelochemicals in the host selection by phytophagous pests may prove highly rewarding in devising integrated pest management programs (4). Cotton boll worm or gram pod borer *Helicoverpa armigera* (Hubner) is a polyphagous insect pest attacking more than 182 cultivated and uncultivated species (5). Studies on the orientation responses, including possible disruption of larval orientation, attraction of larva to toxi baits, or cultivation of crop varieties lacking larval attractants (6) could be of great help in formulating insect control strategies. Marigold (7) cotton, okra and cucurbits (8) plants are known to good host of *H. armigera.* Their leaf and flower contains several types of terpenoid, aromatic and aliphatic compounds (9). However, information on the behavioral response of *H. armigera* larvae to leaf and flower extract of these plants is acanty in literature and thus the present study was planned to fill this gap in knowledge.

Methods

The experiments were carried out at the Entomological Research Farm, Punjab Agricultural University, Ludhiana and Insect Molecular Biology Laboratory, Department of Entomology, PAU, Ludhana. African marigold, cotton, okra and pumpkin seeds were sown in Entomological Research Farm in March of 2006-07. The larvae of *H. armigera* were collected from berseem *(Trifolium alexandrium)*, okra *(Abelmoschus esculentus)* and tomato *(Lycopersicon esculentum).* The larvae were reared on semi-synthetic diet (10) in the laboratory at 27 ± 2 C with a 10 : 14 h light : dark photoperiod. Pupae were sexed and kept in separate plastic boxes until ecolosion. For cold extraction, 10 g of different samples (flower and leaf) of African marigold, cotton, okra and pumpkin were crushed in a grinder with liquid nitrogen and were kept in methanol. After that, extracts were filtered and concentrated under vacuum. For partial purification, extracts were transferred to the solid phase column (C_{-18}) and eluted with methanol. Then, these were stored in 4 C for future use. For bioassay tests four troughs were taken (18 cm in diameter and 5 cm in height) at a time. After that diet was treated with African marigold, cotton, okra and pumpkin flower and leaf extracts and offered at equidistance from one another. Diet was treated with four concentrations (1.00, 0.75, 0.50, 0.25 g/ml) of different plant parts (flower and leaf) extract. Then, ten *Helicoverpa larvae* (second instars) were released in the middle of the trough with the aid of a camel hair brush and then covered it with transparent sheet. For different standard chemical compounds, similar experiment was

Table 1. Response of second instar larvae to different plants flower extract treated artificial diet in multiple choice tests. CD (P = 0.05). Concentration— NS, Plants—0.16, Plants × Concentration—NS, # On fresh weight basis, Values parentheses are sq root transformed values ; *10 larvae were released at a time.

Conc (gm/ml) #	No. of larvae observed feeding on diet containing extracts of flower* Cotton	African marigold	Okra	Pumpkin	Mean
0.25	2.33 (1.82)	1.33 (1.52)	1.67 (1.63)	4.67 (2.39)	2.50 (1.84)
0.50	2.33 (1.82)	1.00 (1.38)	1.67 (1.63)	5.33 (2.51)	2.58 (1.84)
0.75	2.33 (1.82)	1.33 (1.52)	1.33 (1.52)	5.00 (2.45)	2.50 (1.83)
1.00	1.33 (1.52)	1.67 (1.61)	1.33 (1.52)	5.67 (2.58)	2.50 (1.81)
Mean	2.08 (1.75)	1.33 (1.51)	1.50 (1.57)	5.17 (2.48)	

carried out with three different concentrations (90, 60 and 30%).

Proper care was taken to keep the room devoid of any other olfactory stimulus that might interfere with the orientational responses of the insects. Then movement of the larvae was continuously monitored for 15 min. Distribution of the larvae in each sector of the trough was recorded at the end of each observation period. The experiments on various aspects were carried out umder room conditions at temperature ranging from 27 to 30 C and at 65 to 70% RH. All tests were replicated three times, using 10 larvae per replicate. For TLC, Silica 254G plates (E. Merck, Germany) were used for the separation of different compounds present in test samples after activation at 110 C for 45 min. Two types of mobile phases viz., phase A-chloroform : methanol : acetic acid (65 : 25 : 4) and phase B—hexane : ethyl ether : acetic acid (9 : 10 : 1), were used for separation. Thereafter plates were placed in UV chamber and followed by iodine chamber for identification. The spots were marked a marker and compared with standard compounds.

Results and Discussion

The results of the bioassays with the second instar larvae of *H. armigera* under multiple choice conditions showed that more number of larvae showed oriented movement towards pumpkin flower extract treated artificial diet than that towards cotton, okra and African marigold extracts treated diet at all the concentrations (Table 1). The mean numbers of larvae at different concentrations were statistically at par with each other. However, maximum numbers of larvae were attracted towards pumpkin flower extract, which were significantly higher than on diets treated with all other flower extracts followed by cotton, okra and African marigold. Further, response to later two treatments was at par with each other. The interaction between the concentrations and plants was non significant.

The orientated response of larvae towards surface treated diet with leaf extracts of different plants (Table 2) showed that more number of larvae were attracted towards diet treated cotton leaf extract and it was significantly higher than response to diet treated with other plant leaf extracts. The response of larvae towards diet with cotton leaf extract was followed by that towards. African marigold, okra and pumpkin extract treated diet. The response was significantly dif-

Table 2. Response of second instar larvae to different plant leaves extract treated artificial diet in multiple choice tests. CD (P = 0.05). Concentration-NS, Plant-0.16, Plants × Concentration-0.32, # On fresh weight basis, Values parentheses are sq root transformed values, *10 larvae were released at a time.

Conc (g/ml)#	No. of larvae observed feeding on diet containing extracts of leaves* Cotton	African marigold	Okra	Pumpkin	Mean
0.25	4.67 (2.38)	2.00 (1.71)	2.67 (1.91)	0.67 (1.27)	2.50 (1.82)
0.50	4.67 (2.38)	2.33 (1.82)	2.67 (1.91)	0.33 (1.14)	2.50 (1.81)
0.75	5.33 (2.51)	2.33 (1.82)	2.00 (1.71)	0.33 (1.14)	2.50 (1.79)
1.00	6.33 (2.71)	3.00 (1.99)	0.67 (1.27)	0.00 (1.00)	2.49 (1.74)
Mean	5.25 (2.49)	2.41 (1.84)	2.00 (1.70)	0.33 (1.14)	

Table 3. Chemical compounds identified in flower extracts of African marigold, cotton, okra and pumpkin.

Standard chemicals	African marigold	Cotton	Okra	Pum-pkin
Careen	+	-	-	-
Pinene	-	-	-	-
Phenyl ethanol	+	-	+	-
Limonin	+	+	+	-
Phenyl acetaldehyde	+	-	+	-
Linalool	+	-	-	-
Methyl benzoate	+	-	-	-
Caryophylline oxide	-	-	-	-
Hexenyl acetate	-	-	-	-
Ocimine	+	+	-	-

ferent from each other towards diets treated with extracts for African marigold, okra and pumpkin. The concentrations did not play any significant role in larval attraction. The mean number of larvae at different concentrations was statistically at par with each other. However the interaction between the plant and concentration was significant with maximum number of larvae being attracted towards diet having cotton (1.00 g/ml) extract, which was significantly higher than all the other combinations of extracts except for the cotton leaf extract impregnated diet at 0.75 g/ml.

A number of chemical compounds tentatively identified from different plant part (flowers and leaves) extracts of okra, cotton and pumpkin were observed to be similar (Tables 3 and 4). The okra flowers and leaves contained the chemicals which were also present in *T. erecta* while the cotton leaves contained caryophyllene oxide which was not present in any of the plants evaluated. The Rf values of compounds isolated from leaves and flowers extract of pumpkin

Table 4. Chemical compounds identified in leaves extracts of African marigold, cotton, okra and pumpkin.

Standard chemicals	African marigold	Cotton	Okra	Pum-pkin
Careen	-	-	-	-
Pinene	+	-	-	-
Phenyl ethanol	+	-	-	-
Limonin	-	-	-	-
Phenyl acetaldehyde	-	+	-	-
Linalool	-	-	-	-
Methyl benzoate	-	-	+	+
Caryophylline oxide	-	+	-	-
Hexenyl acetate	+	-	-	-
Ocimine	-	-	-	-

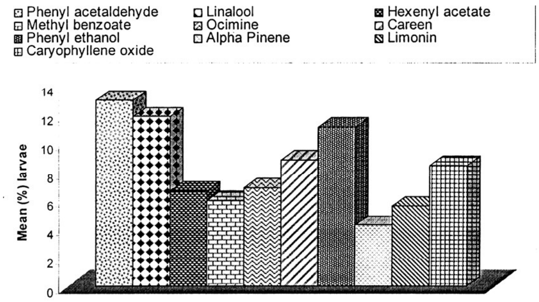

Figure 1. Mean number of *Helicoverpa armigera* feeding on diet containing different standard compounds.

revealed the presence of a complex of unidentified compounds except the one that had Rf value similar to that of methyl benzoate.

In bioassay using standard compounds, *H. armigera* larvae were more attracted towards diet incorporated with phenyl acetaldehyde whereas it was the minimum towards pinene incorporated diet (Fig. 1). Behavior of larvae of *H. armigera* towards different concentrations of different standard compounds was not uniform. Some compounds (Phenyl acetaldehyde, hexenyl acetate, ocimine and caryophyllene oxide) showed greater larval orientation at lower concentrations as compared to higher concentrations while some (linalool, phenyl ethanol, careen, methyl benzoate, limonin and pinene) showed greater larval orientation at higher concentrations as compared to lower ones.

Response of second instar larvae of *H. armigera* elicited higher oriented response (Table 2) towards cotton leaf extract as compared to okra, marigold and pumpkin leaf extract, possibly due to the presence of phenyl acetaldehyde, caryophyllene oxide and other compounds (Table 4). Similarly, larvae were more attracted towards pumpkin flower extract (Table 1) than to cotton, okra and marigold flower extracts, which might be due to the presence of more kairomonal compounds in comparison to others.

Hartlieb and Rembold (11) identified kairomonal components of pigeonpea leaves. These components contain a characteristic mixture of sesquiterpenes and pure alpha-bulnesene, which was oberved to elicit orientational responses of *H. armigera* larvae. Cantelo and Jacobson (12) isolated phenylacetaldehyde from the volatiles of corn silk, which attracted *H. zea* and *Pseudoplusia includens.*

So, different phytochemicals play an important role in host plant selection by helping the female to locate their host plants or different feeding sites on a plant.

However, further study is required to isolate these different chemicals and also it is important to know the electro physiological response of larvae to different plant parts extract. This will not only increase our understanding about host plant selection but also provide a basis for synthesis of phytochemicals or their analogues, which may be devising a push-pull (13) kind of strategy for its management.

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