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Effect of selected plant volatiles on pink bollworm, *Pectinophora gossypiella* (Saunders) in non-Bt and BT cotton

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Abstract

During the study period, eight synthetic plant volatiles viz., cis-3-hexene-1-ol, methyl salicylate, methyl jasmonate, D-limonene, caryophyllene, phenylacetaldehyde, geraniol and farnesol were selected and impregnated in dispenser for slow release of volatile compounds in non-Bt and Bt DCH-32 cotton hybrids under field condition to assess the attractant or repellent nature of the volatile compounds to pink bollworm at Agricultural Research Station, Dharwad farm, Kharif 2019-2020. It resulted non-significant difference in incidence of pink bollworm in both non-Bt and Bt cotton. However, the significant difference was noticed among the treatments in both the hybrids. Among the tested plant volatiles, phenylacetaldehyde and farnesol recorded least larval count and green boll damage in both non-Bt and Bt DCH-32 than the untreated control and registered as repellents. Whereas, D-limonene, cis-3-hexene-1-ol and methyl salicylate recorded significant peak infestation of larval count as well as green boll damage.

Keywords: Cotton, green boll damage, pink bollworm, plant volatiles

Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) emerged as a serious pest of cotton in India and is widely distributed in cotton growing regions viz., tropical America, Africa, Asia, Australia, Egypt, USA and Mexico. The destructive larvae of pink bollworm feeds on flowers, buds, bolls and seeds of cotton plant causing rosetting and malformation of flowers, premature opening and heavy shedding of infested bolls, reduction in fiber length and poor quality of lint due to staining. It accounts yield losses to the extent of 20-90% (Patil, 2003) ^[7]. The development of genetically engineered transgenic cotton targeted the bollworm complex which included American bollworm, *Helicoverpa armigera* (Hubner), spotted bollworm, *Earias vitella* (Fabricious) and pink bollworm. Initially, the Bt cotton technology performed well and offered promising control of bollworm complex until 2009. After 15 years of continuous cultivation of Bt cotton in India, resistance was observed in *H. armigera* and *E. vitella*. Suddenly, pink bollworm has emerged as a major pest problem in recent past (Fand *et al.*, 2019) ^[3]. Most of the strategies deployed by cotton growers to control insect pests rely on routine applications and overuse of synthetic insecticides. This has exerted a severe impact on natural enemies fauna of cotton ecosystem (Kranti *et al.*, 2002) ^[6]. Further, unwise and indiscriminate use of insecticides has evolved resistance in insects. Therefore, there is a need of alternative management strategies to reduce this pest's capacity for genetic resistance to conventional chemical controls (Casagrande, 1987) ^[1]. Therefore, enhancing cotton pest management using plant natural defenses has been described as a promising way to improve the management of insect pests, at the same time as providing environmentally sound pest management options to growers. One alternative pest management strategy is deployment of plant-derived attractants, repellents and antifeedants in a stimulo-deterrent program to push pests away from economic crops at the same time as pulling them to noneconomic part of a production system. In this context, eight synthetic plant volatiles were selected to study the behaviour response of pink bollworm in field condition.

Materials and Methods

A field experiment was undertaken at Agricultural Research Station, Dharwad in *khariif* 2019-20 to study the effect of plant volatiles on the incidence of pink bollworm towards some selected plant volatiles viz., cis-3-hexene-1-ol (T₁), methyl salicylate (T₂), methyl jasmonate (T₃), D-limonene (T₄), caryophyllene (T₅), phenylacetaldehyde (T₆), geraniol (T₇) and farnesol (T₈) in non-Bt and Bt DCH 32 BG-II cotton. The ARS Dharwad is located between 15°N latitude and 76.46° E longitude at an altitude of 678 meters above mean sea level with mean annual rainfall of 922.7 mm. During the growing season, the crop management without pesticides was performed identically as per the package of practice of UAS, Dharwad. The experiment was comprised of nine treatments (eight plant volatiles and an untreated check) which were replicated thrice with a spacing of 90×60 cm and laid out in split-plot design. All the synthetic plant volatiles were procured from Sigma-Aldrich Pvt. Ltd Bangalore, India.

Release of plant volatiles

The volatile compound was dissolved in 20 ml paraffin oil (0.2 ml/20 ml of paraffin oil), which was then placed into the glass vial (diameter: 35 mm) i.e. bottle-type HIPV (Host Induced Plant Volatile) dispenser. Bottles of the same type containing 20 ml of pure paraffin oil was used as control. The top of the vial with a polypropylene thread (diameter: 12 mm, length: 70 mm) was exposed to the air, while the end was inserted in to the vial through a hole in the lid and dipped into the volatile compound. Vials were taped with aluminium foil except the lid of the vial to minimize exposure to direct sunlight. So that the vials can dispense the compound at proportionate rate through the polypropylene threads. Before the start of the experiment, the standardization of the amount of release of solvent in the vial without the active compound was done. Further, concentration of the compound solution was adjusted to correspond to the release rate and solution was measured and filled into the vials as and when required based on the evaporation of the volatile compounds (Uefune *et al.*, 2012) [8]. Vials were tied vertically by wire to the central shoot at lower mid canopy when the crop was at 60 Days After Sowing (DAS). Within the field, the vials were spaced at least 5 m between each randomly arranged treatment and the vials were refilled thrice at 15 days interval.

The larval count of pink bollworm and green boll damage due to PBW was recorded from randomly selecting destructive sampling of 20 bolls from each treatment at 90 and 105 DAS.

For this purpose, 20 tender green cotton bolls from each treatment were collected and brought to the laboratory for further observations. Each tender green boll was cut opened along with ridges of the locules with the help of sharp cutter to check the presence of PBW larvae (live or dead). The per cent larval population was calculated by using the following formula.

$$\text{Larval population (\%)} = \frac{\text{No. of larvae in green bolls}}{\text{Total no. of green bolls observed}} \times 100$$

Concurrently, the number of bolls damaged by pink bollworms counted and expressed in terms of per cent green boll damage using the following formula.

$$\text{Green boll damage (\%)} = \frac{\text{No. of damaged green bolls}}{\text{Total no. of green bolls}} \times 100$$

Results and Discussion

The results of the investigation on effect of plant volatiles on pink bollworm (PBW) conducted during *khariif* 2019-20 in Agricultural Research Station Dharwad farm were depicted in Table 1.

At 90 days after sowing

a. Larval count

There was non-significant variation between non-Bt and Bt DCH-32 hybrids in the larval count (3.57 and 3.29 larvae/20 bolls, respectively) and per cent green boll damage (5.16 and 4.52%, respectively) was recorded.

The larval count of PBW ranged between 1.05 to 4.66 per 20 bolls in non-Bt DCH-32. The least larval count of 1.05 per twenty bolls was recorded in phenylacetaldehyde (T₆) followed by farnesol (T₈) (1.42) which were found statistically superior over untreated control (3.26). Meanwhile, the peak larval count of 4.66 per twenty bolls was noticed from the plots impregnated with D-limonene (T₄) which was on par with cis-3-hexene-ol (T₁) (4.52). The similar tendency of volatiles was also noticed in Bt DCH-32 cotton hybrid where the least larval count of 0.98 per twenty bolls recorded in T₆ followed by T₈ (1.18) which were significantly superior over rest of the volatiles in reducing PBW larval count. Meanwhile, D-limonene (T₄) and cis-3-hexene-1-ol (T₁) were recorded greater larval count (4.22 and 4.19).

The mean population from both the entries indicated the similar trend of phenylacetaldehyde (T₆) and farnesol (T₈) where they recorded least PBW larvae (1.02 and 1.30/20 bolls) and found statistically significant over the other treatments.

b. Green boll damage (%)

The mean green boll damage of 5.16 and 4.52% was recorded in non-Bt DCH-32 and Bt DCH-32 due to PBW infestation at 90 DAS. However, the damage between both the genotypes found to be non-significant.

The least green boll damage of 1.85% was noticed in phenylacetaldehyde (T₆) followed by farnesol (T₈) (3.00%) which was significantly least over other volatile treatments including control (4.90%) in non-Bt DCH-32 hybrid. Whereas, the similar trend was also seen in Bt DCH-32 with per cent damage of 1.51 and 2.60 in T₆ and T₈ respectively, due to PBW which were least over rest of the other volatile treatments.

The mean green boll damage due to PBW obtained from both the hybrids ranged between 1.68 to 6.10%. The least incidence (1.68 and 2.80% respectively,) was noticed in T₆ and T₈ which they significantly reduced the pink bollworm damage over rest of the treatments including untreated control.

At 105 Days After Sowing

a. Larval count

The mean PBW larval population of 4.26 and 3.82 per twenty bolls were noticed in non-Bt DCH 32 and Bt DCH-32 cotton, respectively and the difference was non-significant.

The least larval count of 1.03 and 1.26 per twenty bolls was noticed in the plots installed with phenylacetaldehyde (T₆)

and farnesol (T_8) which were found to be repellent as they recorded significantly less population over untreated control (4.00) in Bt cotton. The highest larval count was observed in D-limonene (T_4) (5.36/20 bolls) in non-Bt cotton.

The mean larval population of PBW obtained from non-Bt and Bt cotton ranged between 1.07 to 5.13 larva per twenty bolls. Phenylacetaldehyde (T_6) (1.03) and farnesol (T_8) (1.26) recorded significantly least larval count over rest of the treatments in Bt cotton. Whereas, D-limonene (T_4) (5.36) and cis-3-hexene-1-ol (T_1) (5.30) recorded highest number of larvae per twenty bolls which were significantly higher than the untreated control (4.25) in non-Bt DCH-32 cotton and found to be attractant for them. The larval count of PBW was higher in non-Bt DCH-32 than Bt DCH-32 cotton hybrid throughout the period of investigation and the difference was found to be non-significant.

b. Green boll damage (%)

The mean green boll damage of 6.31 and 5.58% was recorded at 105 DAS in non-Bt DCH-32 and Bt DCH-32 cotton and the difference between both of them were found to be non-significant.

The significant variation existed in the PBW damage among the different volatile treatments in both the entries. The green boll damage in non-Bt DCH-32 ranged between 1.90 to 8.00% whereas, in Bt DCH-32 it was to the extent of 1.60 to 7.20%. However, in both the entries least green boll damage of 1.90 and 1.60% was recorded from phenylacetaldehyde (T_6) in both the non-Bt and Bt cotton hybrid, respectively.

The similar trend of least damage on T_6 and T_8 was confirmed with the mean damage obtained from both the non-Bt and Bt cotton which was found to be 1.75 and 3.00% and statistically significant over rest of the treatments. Whereas, the other volatile treatments recorded the green boll damage which was significantly more than the untreated control (5.25%). Throughout the period of investigation, the pink bollworm infestation in terms of green boll damage was non-significantly higher in non-Bt DCH-32 than the Bt DCH-32 cotton hybrid.

Among the different plant volatiles, phenylacetaldehyde (T_6) registered significantly least larval count of 1.08 and 1.03 per twenty bolls. However, it was on par with farnesol (T_8) (1.54 and 1.26/ twenty bolls) in both non-Bt and Bt cotton,

respectively. Whereas highest larval incidence 5.01 and 4.90 per twenty bolls were noticed in D-limonene (T_4) followed by cis-3-hexene-1-ol (T_1) (4.91 and 4.82) and methyl salicylate (T_2) (4.88 and 4.75/ twenty bolls) in both non-Bt and Bt cotton, respectively. The similar trend was noticed in green boll damage in plots treated with phenylacetaldehyde (T_6) and farnesol (T_8) recorded least damage. Whereas, highest green boll damage noticed in D-limonene (T_4) followed by cis-3-hexene-1-ol (T_1) and methyl salicylate (T_2) in both non-Bt and Bt DCH-32 cotton (Table 2). Successful amplification of these volatiles in cotton hybrids can be achieved through identification and induction of a gene responsible for their production and can be bait with other pheromones to enhance their efficacy to trap the adult moths. The present findings are supported by Flint *et al.* (1977) [4] who reported that field plots of cotton treated with methyl salicylate and untreated control had significantly greater infestations of *P. gossypiella* than similar plots treated with phenylacetaldehyde. Further, they said that phenylacetaldehyde possesses oviposition deterrence against PBW. Das *et al.* (2007) [2] observed electroantennogram (EAG) response of female gelechiid moth, *Phthorimaea operculella* towards 27 plant volatiles and found that hexanal, farnesol, α -myrcene, citronellol, phenylacetaldehyde, eugenol and α -pinene elicited the least response. Meanwhile, cis-3-hexene-1-ol, hexanal, myrcene, S-limonene, phytol and carvacrol elicited greater responses. Phytophagous insects use the particular green odour blend of their host plants to locate suitable feeding and oviposition sites (Visser *et al.*, 1979) [9]. Further study by Ibrahim *et al.* (2018) [5] reported that a blend of volatile compounds from leaves, flowers and bolls comprising major constituent as caryophyllene representing odour for cotton plant acts as an orientation cue for the *P. gossypiella* which is in agreement with the present outcomes.

The results of the present study has greater importance in host odour enhancement or synergism of sex attraction in the development of pheromone-based lures and the modification of insect behaviour through induced plant volatiles has greater significance and opportunities in future breeding programmes. The present investigation may also provide elementary knowledge on potentiality of volatile compounds which can be utilised in formulating ecofriendly management practices in future.

Table 1: Effect of plant volatiles on pink bollworm in non-Bt DCH-32 and Bt DCH-32 cotton

Tr. No.	Treatments	No. of PBW larvae/20 bolls						Green boll damage (%)					
		90 DAS			105 DAS			90 DAS			105 DAS		
		Non-Bt	Bt	Mean	Non-Bt	Bt	Mean	Non-Bt	Bt	Mean	Non-Bt	Bt	Mean
1	Cis-3-hexene-1- ol	4.52 (2.24) ^a	4.19 (2.17) ^{abc}	4.36 (2.20) ^a	5.30 (2.41) ^a	4.82 (2.31) ^{ab}	5.06 (2.36) ^a	6.30 (14.53) ^a	5.60 (13.64) ^{abc}	5.95 (14.09) ^a	7.80 (16.21) ^a	7.00 (15.22) ^{ab}	7.40 (15.72) ^a
2	Methyl salicylate	4.48 (2.23) ^a	4.16 (2.16) ^{abc}	4.32 (2.20) ^a	5.27 (2.40) ^a	4.75 (2.29) ^{ab}	5.01 (2.35) ^{ab}	6.25 (14.47) ^a	5.50 (13.56) ^{abc}	5.88 (14.01) ^a	7.75 (16.21) ^a	6.90 (15.22) ^{ab}	7.33 (15.72) ^a
3	Methyl jasmonate	4.32 (2.20) ^{ab}	3.85 (2.08) ^{abc}	4.09 (2.14) ^a	5.02 (2.35) ^{ab}	4.52 (2.23) ^{ab}	4.77 (2.29) ^{ab}	5.80 (13.93) ^{abc}	5.10 (13.05) ^{bcd}	5.45 (13.49) ^a	7.40 (15.56) ^{ab}	6.60 (14.88) ^{ab}	7.00 (15.22) ^a
4	D-limonene	4.66 (2.22) ^a	4.22 (2.17) ^{abc}	4.44 (2.20) ^a	5.36 (2.39) ^a	4.90 (2.32) ^{ab}	5.13 (2.36) ^a	6.40 (14.65) ^a	5.80 (13.93) ^{abc}	6.10 (14.29) ^a	8.00 (16.42) ^a	7.20 (15.52) ^{ab}	7.60 (15.97) ^a
5	Caryophyllene	4.41 (2.20) ^{ab}	4.09 (2.14) ^{abc}	4.25 (2.17) ^a	5.19 (2.39) ^a	4.63 (2.26) ^{ab}	4.91 (2.33) ^{ab}	6.15 (14.35) ^{ab}	5.40 (13.35) ^{abc}	5.78 (13.85) ^a	7.60 (16.42) ^a	6.85 (15.53) ^{ab}	7.23 (15.98) ^a
6	Phenyl acetalddehyde	1.05 (1.24) ^d	0.98 (1.22) ^d	1.02 (1.23) ^c	1.10 (1.26) ^c	1.03 (1.23) ^c	1.07 (1.25) ^c	1.85 (7.63) ^f	1.51 (7.05) ^f	1.68 (7.34) ^d	1.90 (10.30) ^d	1.60 (9.63) ^d	1.75 (9.96) ^c
7	Geraniol	4.00 (2.12) ^{abc}	3.81 (2.08) ^{abc}	3.91 (2.10) ^a	4.98 (2.34) ^{ab}	4.47 (2.23) ^{ab}	4.73 (2.29) ^{ab}	5.75 (13.87) ^{abc}	5.00 (12.92) ^{cd}	5.38 (13.39) ^a	7.40 (15.78) ^a	6.50 (14.88) ^{ab}	6.95 (15.33) ^a
8	Farnesol	1.42 (1.39) ^d	1.18 (1.30) ^d	1.30 (1.34) ^c	1.66 (1.47) ^c	1.26 (1.33) ^c	1.46 (1.40) ^c	3.00 (9.94) ^e	2.60 (9.27) ^e	2.80 (9.61) ^c	3.20 (10.26) ^d	2.80 (9.63) ^d	3.00 (9.94) ^c

9	UTC	3.26 (1.94) ^{bc}	3.12 (1.90) ^c	3.19 (1.92) ^b	4.50 (2.24) ^b	4.00 (2.10) ^b	4.25 (2.17) ^b	4.90 (2.78) ^{cd}	4.20 (1.82) ^d	4.55 (2.30) ^b	5.70 (3.81) ^c	4.80 (2.65) ^c	5.25 (3.23) ^b
	Mean	3.57 (2.02)	3.29 (1.95)	3.43 (1.98)	4.26 (2.18)	3.82 (2.08)	4.04 (2.13)	5.16 (3.12)	4.52 (2.27)	4.84 (2.70)	6.31 (4.54)	5.58 (3.66)	5.94 (4.11)
		SE.m±	CD at 5%		SE.m±	CD at 5%		SE.m±	CD at 5%		SE.m±	CD at 5%	
	Main plot	0.06	0.25		0.05	0.20		0.37	1.61		0.42	1.81	
	Sub plot	0.09	0.18		0.08	0.16		0.42	0.85		0.53	1.09	
	Sub plot @ same level of MP	0.12	0.25		0.11	0.23		0.59	1.20		0.75	1.53	
	Main plot @ diff. level of SP	0.39	0.97		0.35	0.83		2.01	5.52		2.48	6.51	

DAS- Days After Sowing, UTC- Untreated control, MP- Main plot, SP- Sub plot, NS: Non-Significant

*Figures in parentheses are $\sqrt{x+0.5}$ transformed values

**Figures in parentheses are arc sine transformed values

Means followed by same alphabet in a column do not differ significantly (0.05) by DMRT

Table 2: Overall mean of larval count and green boll damage of pink bollworm

Tr. No	Treatments	Larval count (No. /20 bolls)			Green boll damage (%)		
		Non-Bt	Bt	Mean	Non-Bt	Bt	Mean
1	Cis-3-hexene-1- ol	4.91 (2.33) ^a	4.82 (2.31) ^a	4.86	7.05 (15.39) ^a	6.30 (14.53) ^{ab}	6.68
2	Methyl salicylate	4.88(2.32) ^a	4.75 (2.29) ^a	4.81	7.00 (15.34) ^a	6.20 (14.41) ^{ab}	6.60
3	Methyl jasmonate	4.67 (2.27) ^a	4.52 (2.24) ^a	4.59	6.60 (14.88) ^a	5.85 (13.99) ^{ab}	6.23
4	D-limonene	5.01 (2.35) ^a	4.90 (2.32) ^a	4.95	7.20 (15.56) ^a	6.50 (14.76) ^a	6.85
5	Caryophyllene	4.80 (2.30) ^a	4.63 (2.26) ^a	4.71	6.88 (15.19) ^a	6.13 (14.32) ^{ab}	6.50
6	Phenyl acetaldehyde	1.08 (1.25) ^b	1.03 (1.24) ^b	1.05	1.88 (7.87) ^b	1.56 (7.16) ^d	1.72
7	Geraniol	4.49 (2.23) ^a	4.47 (2.23) ^a	4.48	6.58 (14.85) ^a	5.75 (13.87) ^{ab}	6.16
8	Farnesol	1.54 (1.43) ^b	1.26 (1.33) ^b	1.40	3.10 (10.14) ^b	2.70 (9.45) ^c	2.90
9	UTC	3.88 (2.09) ^a	4.00 (2.12) ^a	3.94	5.30 (13.30) ^a	4.50 (12.24) ^b	4.90
	Mean	3.92	3.82	3.87	5.73	5.05	5.39
	S.E.m±	0.09	0.14		0.79	0.69	
	CD at 0.05	0.27	0.41		2.38	2.08	
	CV (%)	7.59	11.61		10.08	9.43	

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