Effect of Leaf Cuticular Wax Extract of Wild *Gossypium* spp. on Behaviour of *Helicoverpa armigera* (Hübner)

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ABSTRACT

Cuticular wax extracts were obtained in hexane from eight wild species of Gossypium and sprayed on G. hirsutum var. MCU 9 plants that are susceptible to attacks of Helicoverpa armigera (Hübner). The extracts adversely affected oviposition and feeding and was highly toxic to neonate larvae of H. armigera. Helicoverpa moths laid fewer eggs on MCU 9 plants treated with the extracts from G. raimondii, G. anomalum, G. davidsonii and G. arboreum compared to the untreated control. The extracts from G. anomalum, G. raimondii and G. davidsonii significantly affected egg hatch and were found to be more toxic to neonate larvae than the extracts of other wild species. Leaf area consumption was reduced more on MCU 9 plants treated with extract of G. raimondii than with the extracts of other wild species. The extracts also affected the activity of Chrysoperla carnea and Trichogramma chilonis. Leaf cuticular wax extracts of the susceptible MCU 9 did not stimulate the oviposition of H. armigera moths when sprayed on wild species of Gossypium. Gas chromatographic profiles of cuticular wax extract of wild species indicated that tetradecanoic acid, 3-eicosanic and hexa decanoic acid detected from G. anomalum, 1,3,11, trimethyl and caryophyline in G. raimondii and naphthalene from G. davidsonii may be responsible for inhibition of oviposition and feeding in H. armigera.

Introduction

Incorporation of factors that reduce or eliminate insect pest populations into cultivated plant species is an efficient and economic approach to the management of crop insect pests. It is now generally believed that chemicals are the basis for many plants' defense against insect attack and that the chemicals defense system are composed of not one but a spectrum of chemical compounds (Alborn, 1988). Although plant breeders have been successful in producing single gene resistant material, little is known about plant chemicals imparting resistance in plants. To become more specific in breeding efforts, breeders must know how the behaviour and biology of the insect pest is influenced by the plants defense chemicals and how it can be modified to obtain an increased and more durable resistance.

Helicoverpa armigera rank high among the most destructive crop pest in India (Sachan and Yadav, 1991). The wild species of *Gossypium* are not preferred for oviposition (Mohite, 1995) compared to the cultivated species.

The chemicals and ultrastructure characteristic of plant cuticular surfaces can affect many aspects of insect behaviour such as orientation, movement, oviposition and feeding (Bernays *et al.*, 1976; Chapman and Bernays, 1989; Espelie *et al.*, 1991). Variations in these characteristics may result in variable plant resistance to insect attack. If the insects do not like the plant surface, they may exhibit non-acceptable behaviour and move away from the leaf surface (Bernays *et al.*, 1985; Stoner, 1990; Eigenbrode *et al.*, 1991). In this paper, the role that the leaf surface wax from wild species of *Gossypium* may play in the resistance to *H. armigera* is examined.

Material and Methods

Surface cuticular wax extract from leaves of eight wild species of *Gossypium* was bioassessed against *H. armigera*.

Extraction of cuticular wax

The extraction of leaf cuticular wax was done following the method of Jackson *et al.* (1984). Fresh leaves (100 g) of wild and cultivated species of *Gossypium* were harvested and washed thoroughly with 500 ml of hexane for 30 sec. The whole leaf wash was then filtered through a filter paper. The filterate was evaporated down to 15 ml before being decanted into weighed glass vials for further evaporation, leaving a yellow residue. The vial was re-weighed. The yellow residue was diluted with 0.025 percent Triton X100 in distilled water and Triton X100: Acetone (1:3) and sprayed at 300 ppm.

Effect of leaf cuticular wax extracts (LCWE) of wild Gossypium on the oviposition behaviour of H. armigera

Individual month old susceptible MCU 9, potted cotton plants with six leaves were sprayed with 6 ml of

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acetone-water solution of the leaf extracts of the eight wild species. One control plant received acetone water and another (absolute control) was sprayed with water alone. The treated plants were kept inside a 120x90x90 cm cage. Newly emerged *H. armigera* moths were released at the rate of 5:10 (male: female) at 17.00 hours. The number of eggs laid per treatment were counted three days after release. The experiment was conducted in a completely randomized block design (CRBD) with three replications.

Effect of LCWE on egg hatch of H. armigera

Ten eggs of *H. armigera* were attached to the third leaf of the MCU 9 cultivar with gum solution. These leaves were dipped in the 300-ppm solution of LCWE individually and excess liquid allowed to drain off. The leaf was then kept in petri dish (10cm diameter) lined with moistened tissue paper. The number of hatched and unhatched eggs was recorded. The experiment was conducted in CRBD with three replications.

Toxicity of LCWE to neonate larvae of H. armigera

Leaf disc 8.51 cm in diameter from the susceptible cultivar MCU 9 were individually dipped in LCWE of wild species and placed in petri dishes lined with filter paper. After the acetone had evaporated, 10 neonate larvae were released onto each disc, using a fine camel-hair brush. The experiment conducted in CRBD with three replications. Larval mortality was recorded 24 and 48 hours after the treatment. The leaf area consumed by the larvae was measured after 48 hours, using automatic leaf area meter (Lincon 3000 area meter).

Effect of LCWE on parasitization by Trichogramma chilonis

Leaves from the third position of susceptible cultivar MCU 9 were individually dipped in the LCWE of wild species of *Gossypium* and kept in petri dishes lined with moistened filter paper. Twenty-five eggs of *H. armigera* were transferred onto the treated leaf surface using a camel hair brush. A two cm strip of Corcyra eggs parasitised by *T. chilonis* about to hatch were kept inside the petri dishes. The percent parasitisation of *H. armigera* eggs was recorded 48 hours after the treatment.

Effect of LCWE on predation by Chrysoperla carnea

Third leaf of MCU 9 was treaded with LCWE as above and kept in petri dishes lined with moistened filter paper. Twenty five eggs of *H. armigera* were transferred to each leaf using a fine hair brush. One second instar grub of *C. carnea* was released in each petri dish. After 24 hours, the number of eggs on the leaf surface was counted and percentage of eggs devoured was calculated.

Volatile profile

The residues of LCWE of wild species of *Gossypium* dissolved in either hexane or methylene chloride were injected into a coupled Hewlett Packard 5890 GC/MSD interfaced with a GC/MSD Chemstation with an NBS 49K mass spectral library containing mass spectra of over 40,000 compounds. A fused silica capillary column (100mm x 0.2mm) with a cross-linked methyl silicon phase (HP) was used.

Helium was used as the carrier gas. The temperature programme was 40^{o}C to 250^{o}C at a 5^{o}C rise per minute with a 2-minute solvent delay. The injector transfer line and ion sources were set at 230^{o}C and 220^{o}C respectively. Mass spectral data obtained during the assay were compared with the mass spectral of compounds available in the Chemstation NBS 49K library.

Results and Discussion

Effect of LCWE on oviposition behaviour of H. armigera

Hexane extracts of leaf cuticular wax of eight wild *Gossypium* species when applied to susceptible cultivar MCU 9 plant leaves influenced oviposition behaviour of *H. armigera* significantly (Table 1). The highest number of eggs were laid on the untreated control (30.7) and on leaves treated with Triton X100: Acetone (29.0) and the mean lowest number (13.0) were laid on the plant treated with extract of G. *raimondii*, which was not significantly different from the number of eggs laid on the plants treated with the extract of G. *anomalum*, *G. arboreum* and *G. davidsonii*. Significant differences did not exist among treatments. The LCWE inhibited the oviposition of *H. armigera* by 26 to 55 percent compared to the Triton100:Acetone control. Johnson and Severson (1984) and Severson *et al.,* (1983) reported that resistant tobacco TI 1112 had low levels of several leaf surface chemicals that contribute to ovipositional non-preference.

Effect of LCWE on eggs hatchability

The hexane extract of LCW of the wild species also significantly influenced eggs hatch. The percentage of unhatched eggs was highest (57 percent) with the extract of G. *raimondii* followed by the extract of *G. raimondii* and *G. davidsonii* (53 percent each) (Table 1). On the plant treated with Triton X100:Acetone, 6.67 percent did not hatch while on the untreated control, all the eggs hatched. This suggested that the LCWE of the wild species inhibited the hatching of eggs.

Toxicity to neonate larvae and leaf area consumption

The neonate mortality recorded 24 hours after the application of LCWE indicated that the extract of *G. raimondii* was highly toxic (37 percent mortality) followed by the extract of *G. davidsonii* (30 percent), *G. harknesii* and *G. stocksii* (27 percent each). Solvent alone gave 3.3 percent mortality. Mortality recorded 48 hours after treatment revealed that the extract of G. *raimondii* was highly toxic to neonate larvae (87

percent mortality) while G. stocksii extract gave 80 percent and *G. davidsonii* 77 percent mortality.

The maximum leaf area consumption was 1.07 sq.cm. on the untreated control. The leaf area consumption was least (0.47 sq.cm) with the extract of *G. raimondii* and *G. davidsonii*. These extracts caused 87 and 77 percent mortality, respectively. This suggested that the LCWE of *G. raimondii* and *G. davidsonii* were highly toxic and reduced the feeding by larvae.

Toxicity to third instar larva of H. armigera

Hexane extracts of LCW of *G. raimondii* were highly toxic to third instar larvae of *H. armigera*, causing 57 percent mortality, while the extract of *G. stocksii* and *G. davidsonii* caused 53 and 50 percent mortality of third instar larvae, respectively, 48 hours after application.

Effect of LCWE on the activities of natural enemies

Parasitization by T. chilonis

The highest parasitization of 57 percent of *H. armigera* was recorded with the extracts of *G. arboreum*, *G. armourianum* and *G. stocksii*. This was not significantly different from the value recorded with the solvent treated (53 percent) and untreated control (63 percent). The lowest parasitization recorded was with the extracts of *G. raimondii* and *G. anomalum* (33 percent each). This suggests that some of the LCWE can influence parasitization.

Predation by C. carnea

Predation of eggs by *C. carnea* was highest with the extract of G. *armourianum* (57 percent) followed by *G. harknesii* and *G. stocksii* (47 percent each). Predation was 67 and 57 percent eggs/day by grubs on leaves of the untreated and solvent treated controls, respectively. Some LCWE, therefore, influenced predators significantly.

Effect of LCWE from susceptible MCU 9 when applied to leaves of wild species of Gossypium

When a leaf surface wax extract of susceptible cultivar MCU 9 was applied to leaves of wild species of *Gossypium*, significantly more eggs were laid on *G. anomalum* (9) and *G. raimondii* (8), than on other species where the number varied from 3 to 4.

Jackson *et al.*, (1984) found in cage bioassay, that when the cuticular compounds from green leaves of susceptible tobacco (NC 2326) were removed and sprayed on leaves of tobacco resistant to budworm (TI 1112), oviposition was stimulated on the non-preferred TI 1112. However, when LCWEs of susceptible MCU 9 were sprayed on the resistant non-preferred wild species of *Gossypium*, they did not stimulate oviposition of H. *armigera*. This clearly indicates that the allelochemicals in certain wild species of *Gossypium* inhibit oviposition.

Analysis of leaf surface wax

A gas chromatographic profile of cuticular wax of wild species suggested that tetradecanoic acid, haxe decanoic acid and 3-elcosanic from *G. anomalum*, 2,6,10 dodecatrien 1; 1,3,11 trimethyl in *G. raimondii* and napthalene phenol 2 C 1-1-dimethyl ethyl-5 methyl in *G. davidsonii* may be involved in non-preference for oviposition by *H. armigera* and for toxicity to larvae of *H. armigera*. Bicyclo (7-2.0) unde-4-ewe, heptadecane 2-6,10,15 tetramethyl, nonacosane and pentacosane present in the LCWE may be implicated in the stimulation of oviposition on MCU 9 (Table 2). Sivasuramanian (1992) concluded that caryophyllen and undecanane from wild species were responsible for non-preference for oviposition of pink bollworm.

There is an urgent need for further chemical analysis of natural deposits on cotton leaves. It might be possible to establish a cause and effect relationship between a component of natural deposits on cotton leaves and bollworm resistance and to explore its use in pest management.

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Table 1. Effect of leaf cuticular wax extract (LCWE) of wild *Gossypium* spp. on oviposition and feeding behaviour of *H. armigera.*

Leaf cuticular wax extract	Eggs laid per plant	Unhatched eggs	Neonate larval mortality	Leaf area consumed	3rd Instar larval mortality	Parasitis-ation	Predation
	No.	%	%	Sq. cm.	%	%	%
G. anomalum	14.7 cd	56.7 a	70.0abc	0.51 ef	36.7 a	33.3 c	33.3 c
G. raimondii	13.0 d	53.3ab	86.7 a	0.47 fg	56.6 a	33.3 c	36.7 c
G. harknesii	20.0 bc	36.7bc	66.7bcd	0.62 de	43.3 a	53.3ab	46.7bc
G. arboreum	19.0 bcd	36.7bc	60.0 cd	0.73 c	40.0 a	56.7ab	40.0 c
G. palmeri	20.7 bc	33.3 c	50.6 d	0.75 c	33.3 a	53.3ab	43.3bc
G. davidsonii	16.0 bcd	53.3ab	76.7abc	0.47 g	50.0 a	43.3 c	36.7 c
G. armourianum	21.33 b	36.7bc	70.0 bc	0.68 cd	43.3 a	56.6ab	56.6ab
G. stocksii	19.33 bc	26.7 c	80.0 ab	0.74 c	53.3 a	56.7ab	46.7bc
Triton X100:Acetone	29.0 a	6.7 d	13.3 e	1.00 b	6.7b	53.3ab	56.7ab
Untreated control	30.7 a	0.0 e	0.0 f	1.07 a	0.0c	63.3 a	66.7 a

*Means of three replications. Means followed by a common letter are not significantly different at 5% level using Duncan's Multiple Range Test.

Table 2. Leaf culticular wax profile of wild species of Gossypium.

Compounds		Quality percentage				
		Gossypium				
	R.T.	*anomalum*	*raimondii*	*davidsonii*	*armouruanum*	*hirsutum*
1,Benzene 1,2,dimethoxy-4-[2-propenyl]	6.08				58	
Bicyclo (d.2.0) unde-4-ene	6.41					93
Caryophyliene	6.61		53			
Spiro (2,4) teptane	6.65			45		
1,3,3-methatriene	6.72				60	
11[dimethyl ethyl]5-methyl-phenol	8.02				60	
Azabicyclo, azobi, nonene	8.18				62	
Diethenyl, methyl cyclohexane	9.88				72	
Cyclohexane 4(-1,5,dimethyl 1-4-hexadiene)	10.55			43		
Phenol,2-(1,1-dimethyl ethyl-5-methyl)	10.70			52	58	
9-dimethyl, cycloun decane	11.50					
Napthalene	11.23			62		
1,2,Benzene dicarboxylic acid buty1	11.67	38				
Tetradecanoic acid	12.14	95				
Hexadecanoic acid	12.20	93				
9-Eicosane (E)	13.47	70				
3-Eicosane (E)	13.97	89				
Cyclopropane	21.48	25				
9-octadecanoic	22.39	64				
Cyclopentadecanone	22.47	70				
2,6,10,dodecartrien 1,-01,3-11 trimethyl	22.65		50			
Heptadecane 2,6,10,15-tetramethyl	27.01					91
Nonacosane	27.25					93
Pentacosane	27.94					93
1,2,benzenedi-2-carboxylic acid-2-me	29.21					62

Source: Mohite, 1995.