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Mitigation of Insecticide Resistance in *Helicoverpa armigera* (Hubner) (Lepidoptera:Noctuidae) by Conjunctive Use of Trap Crops, Neem and *Trichogramma chilonis* Ishii in Cotton

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Abstract: Push-pull strategy with conjunctive use of trap crops, neem and *Trichogramma chilonis* Ishii was evaluated against cotton bollworm, *Helicoverpa armigera* (Hubner) under field conditions. Neem seed kernel extract (NSKE) was applied on cotton crop leaving trap crops (okra and pigeonpea) commencing from 46 DAS at weekly interval to increase the pushing of *H. armigera* away from cotton. Application of NSKE on cotton improved the oviposition preference ratio from 1:1.35 and 1:1.40 to 1:3.02 and 1:2.43 on cotton:okra and cotton:pigeonpea, respectively. Egg parasitoid, *T. chilonis* cards were tagged after each application of NSKE on the trap crops. The percent parasitism of eggs of *H. armigera* on cotton sole crop system ranged from 14.8 to 16.4% and that on trap crops was 10.4 to 12.0 and 14.5 to 15.5% on okra and pigeonpea respectively. Cotton (treated with NSKE) + Trap crops (*Trichogramma* released) system recorded the lowest mean egg, larval population and fruiting bodies, boll and locule damage. The synthetic pyrethroids resistance in field survived *H. armigera* at the end of the season was reduced from 85.3-94.0 to 84.8 -92.1%.

Key words: Insecticide resistance management, *Helicoverpa armigera,* neem, *Trichogramma chilonis,* cotton

INTRODUCTION

Helicoverpa armigera (Hubner) (Lepidoptera:Noctuidae) is a serious polyphagous pest, which has emerged as the dominant pest of a wide variety of crops in recent years in India. In earlier days, the management practices included cultural methods and the use of neem products as pesticides. These practices have virtually disappeared and chemical pesticides have taken precedence. Indiscriminate use of pyrethroids had virtually replaced all other insecticides on cotton in South India by 1985[1]. Severe outbreaks of *H. armigera* in Central and Southern regions of India in 1984-85 and in 1987 were attributed to the overuse of pyrethroids[2]. Regular monitoring of field populations since 1993 confirmed high to very high level of resistance (>90%) to synthetic pyrethroids[3]. The removal of selection pressure of pesticides to *H. armigera* by using botanicals and biocontrol agents was found to increase the susceptibility of *H. armigera* to the insecticides. The exclusive use of biocontrol agents viz., HaNPV, Bt and *Trichogramma chilonis* Ishii were found to be less effective compared to IPM approach in controlling *H. armigera*[4]. The low dispersing ability of egg parasitoid, *Trichogramma* is the major constraint in cotton ecosystem[5]. The increasing problems due to continual usage of pesticides and failure of any individual component to check the pest population in cotton have made the adoption of IPM/IRM an imperative and has given urgency to the need to develop ecologically viable and economically feasible alternative technologies[6]. Hence, the possibility of effective utilization of *T. chilonis* through trap crops and use of neem products on cotton to diversify the pest to trap crops was explored.

MATERIALS AND METHODS

A field experiment was conducted on cotton at Agricultural Research Station, Vaigaidam, Tamil Nadu Agricultural University, Tamil Nadu during Summer, 2003 (March–July) with trap crops okra (Arka Anamika) and pigeonpea (APK 1). It was laid out in a Randomized Block Design (10x10 m plot size) with twelve treatments each with three replications. A cotton variety MCU5 was sown (75x30 cm) on one side of the ridge. In each plot having 10 rows of cotton, fifth row was substituted with trap crops, which was sown simultaneously on the other side of the ridge without any loss to cotton cropped area. The trap cropping systems were compared with the cotton sole crop with 10 rows of cotton in each plot. All the plots received recommended agronomic practices of the region except the treatment operations. NSKE 5% was

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applied on cotton leaving trap crops to diversify the pests to trap crops before each release of *T. chilonis* commencing from 46 days after sowing (DAS) at weekly interval upto maturity phase (81 DAS). The release of *T. chilonis* on trap crops (@ 0.625 cc ha^{-1})/cotton sole crop (@ 6.25 cc ha^{-1}) was commenced from one week after the application of NSKE spray (53 DAS) at weekly interval upto the maturity of the trap crop.

Assessment of pests: The bollworm incidence was assessed on the basis of egg, larval population and percent damage on fruiting bodies (squares, flowers and bolls), open bolls, locules and inter locules. Eggs and larvae were counted on 10 randomly selected tagged plants per plot. The total number of fruiting bodies and those damaged by bollworms were counted at ten randomly selected plants per replication. The total number of bolls collected from ten randomly selected plants per plot at each picking was assessed for number of damaged bolls, number of locules damaged, inter locule boring and percentage was worked out. Kapas were picked out at ten days interval from each plot and the yield was expressed in terms of q ha^{-1}.

Preference ratio: The preference ratio of pests on cotton and trap crops were worked out by using the following formula:

$$PR = \frac{\text{Population of pests on trap crop}}{\text{Population of pests on cotton}}$$

Assessment of effectiveness of *T. chilonis* on trap crops/cotton: The *Trichogramma* cards were tied to the bottom of the leaves by stapling randomly on trap crops/cotton. On fourth day after each release, 20 eggs of *H. armigera* were collected from each plot and brought to the laboratory for observing the emergence of parasitoid and the percentage of parasitisation was calculated.

Monitoring of resistance frequency in the field population of *H. armigera*: The resistance frequency of F_1 field population of *H. armigera* before first spray and F_1 field survived population at the end of the crop to synthetic pyrethroids was monitored using Discriminating Dose (DD) assays and the per cent resistance was calculated by using the formula given by Regupathy and Dhamu[7].

RESULTS AND DISCUSSION

Effectiveness of push-pull strategy with conjunctive use of trap crops, neem and *T. chilonis* against *H. armigera* in cotton: The most robust crop-protection package was obtained when trap cropping, neem on cotton and restricted release of *T. chilonis* on trap crops were combined (Table 1). In cotton sole crop (untreated check), the mean egg and larval population was 27.4 and 24.6 per ten plants, respectively and percent damage was 29.9, 38.7 and 36.5 on fruiting bodies, boll and locule basis respectively. Conjunctive use of trap crops, NSKE application on cotton and release of *T. chilonis* on trap crops reduced the incidence of *H. armigera* in cotton. Cotton (NSKE treated) + pigeonpea (*T. chilonis* released) treatment was superior and effected 43.1 and 54.9% reduction of eggs and larvae and 56.5, 64.1 and 72.3% reduction of fruiting bodies, boll and locule damage respectively. It recorded a maximum yield of 14.4 q ha^{-1} compared to 6.3 q ha^{-1} in cotton sole crop (untreated). This was on par with cotton (NSKE treated) +okra (*T. chilonis* released) treatment, in which the percent reduction of eggs and larvae was 47.5 and 50.8, respectively and 55.9, 63.3 and 70.4% reduction of fruiting bodies, boll and locule damage, respectively.

The other treatments when arranged in descending order according to efficacy followed as cotton (NSKE treated) + pigeonpea (*T. chilonis* unreleased) > cotton (NSKE treated) + okra (*T. chilonis* unreleased) > cotton sole crop (NSKE treated and *T. chilonis* released) > cotton sole crop (NSKE treated) > cotton (NSKE untreated) + pigeonpea (*T. chilonis* released) > cotton (NSKE untreated) + pigeonpea (*T. chilonis* unreleased) > cotton (NSKE untreated) + okra (*T. chilonis* released) > cotton (NSKE untreated) + okra (*T. chilonis* unreleased) > cotton sole crop (*T. chilonis* released) > cotton sole crop (untreated).

The trap cropping, NSKE application on cotton and release of *T. chilonis* on trap crops did not bring about any impact on the inter locule damage, but inter locule damage was considerably reduced in all the NSKE treated plots irrespective of cropping system and release of *T. chilonis* on trap crops. Application of NSKE on cotton resulted in 43.5 to 58.5% reduction in inter locule damage compared to cotton sole crop (untreated check).

Diversification of *H. armigera* by restricted application of NSKE on cotton towards trap crops: Application of NSKE on cotton diversified the *H. armigera* towards untreated trap crops. The egg and larval preference of *H. armigera* on cotton:okra increased from 1:1.35 to 1:3.02 and 1:1.12 to 1:2.45 towards okra, respectively. The egg and larval preference on cotton: pigeonpea increased from 1:1.40 to 1:2.43 and 1:1.27 to 1:2.64 towards pigeonpea, respectively (Table 2).

Parasitization of *H. armigera* by *T. chilonis* on trap crops and cotton: The percent parasitization of eggs of *H. armigera* by *T. chilonis* on okra and pigeonpea ranged

Table 1: Effect of push-pull strategy with conjunctive use of trap crops, neem and *T. chilonis* on bollworm incidence (Vaigaidam, Summer 2003)

Treatments	1#	2*	3#	4*	5*	6*	7*	8*	9*	10*	11*	12*	13
T_1 Cotton (NSKE treated)+	14.4	47.5	12.1	50.8	13.2	55.9	14.2	63.3	10.8	70.4	10.1	47.1	14.1^{ab}
okra (*T. chilonis* released)	(3.8)^{ab}	(43.5)^{b}	(3.5)^{ab}	(45.4)^{c}	(21.2)^{a}	(48.3)^{a}	(22.1)^{a}	(52.7)^{a}	(19.1)^{a}	(57.0)^{b}	(18.4)^{b}	(43.3)^{c}	
T_2 Cotton (NSKE treated)+	13.8	49.6	13.3	45.9	15.3	48.8	16.1	58.4	12.4	66.0	9.5	50.3	13.7^{ab}
okra(*T. chilonis* un released)	(3.7)^{a}	(44.7)^{a}	(3.7)^{bc}	(42.6)^{d}	(22.9)^{b}	(44.3)^{c}	(23.6)^{b}	(49.8)^{b}	(20.6)^{b}	(54.3)^{c}	(17.8)^{ab}	(45.1)^{b}	
T_3 Cotton (NSKE untreated)+	23.8	13.1	19.7	19.9	22.2	25.7	27.3	29.5	26.1	28.5	17.9	6.3	8.8^{cd}
okra (*T. chilonis* released)	(4.9)^{d}	(21.2)^{g}	(4.4)^{ef}	(26.4)^{h}	(28.0)^{e}	(30.4)^{g}	(31.4)^{d}	(32.8)^{e}	(30.7)^{e}	(32.2)^{g}	(25.0)^{cd}	(14.4)^{gh}	
T_4 Cotton (NSKE untreated)+	24.2	11.7	21.9	10.9	25.1	16.1	33.4	13.7	30.0	17.8	18.1	5.2	8.2^{cd}
okra (*T. chilonis* un released)	(4.9)^{de}	(19.9)^{h}	(4.7)^{g}	(19.3)^{j}	(30.0)^{f}	(23.6)^{h}	(35.3)^{f}	(21.7)^{g}	(33.1)^{f}	(24.9)^{I}	(25.1)^{cd}	(13.2)^{h}	
T_5 Cotton (NSKE treated)+	15.6	43.1	11.1	54.9	13.0	56.5	13.9	64.1	10.1	72.3	7.9	58.5	14.4^{a}
pigeonpea (*T. chilonis* released)	(4.0)^{b}	(41.0)^{d}	(3.3)^{a}	(47.8)^{a}	(21.1)^{a}	(48.7)^{a}	(21.9)^{a}	(53.2)^{a}	(18.4)^{a}	(58.2)^{a}	(16.2)^{a}	(49.9)^{a}	
T_6 Cotton (NSKE treated)+	15.0	45.3	11.6	52.9	14.2	52.5	15.8	59.2	12.8	65.0	8.2	57.1	12.5^{ab}
pigeonpea (*T. chilonis* un released)	(3.9)^{ab}	(42.2)^{c}	(3.4)^{ab}	(46.6)^{b}	(22.1)^{ab}	(46.4)^{b}	(23.4)^{b}	(50.3)^{b}	(20.9)^{b}	(53.6)^{c}	(16.6)^{a}	(49.0)^{a}	
T_7 Cotton (NSKE untreated)+	25.8`	5.8	16.3	33.7	18.9	36.8	25.5	34.1	23.8	34.8	16.0	16.2	9.5^{c}
pigeonpea (*T. chilonis* released)	(5.1)^{def}`	(13.9)^{I}	(4.0)^{d}	(35.5)^{f}	(25.7)^{cd}	(37.3)^{e}	(30.3)^{cd}	(35.7)^{d}	(29.1)^{d}	(36.1)^{e}`	(23.5)^{c}	(23.7)^{e}	
T_8 Cotton (NSKE untreated)+	26.2	4.4	19.0	22.8	20.5	31.4	29.7	23.3	24.4	33.2	17.0	10.9	8.9^{cd}
pigeonpea (*T. chilonis* un released)	(5.1)^{ef}	(12.0)^{j}	(4.4)^{e}	(28.4)^{g}	(26.9)^{de}	(34.1)^{f}	(33.0)^{e}	(28.8)^{f}	(29.5)^{de}	(35.1)^{f}	(24.3)^{cd}	(19.3)^{f}	
T_9 Cotton (both NSKE	19.5	28.8	14.0	43.1	17.9	40.1	24.0	37.9	21.2	42.2	10.8	43.5	13.1^{ab}
and *T. chilonis* treated)	(4.4)^{c}	(32.4)^{e}	(3.8)^{c}	(41.0)^{e}	(25.0)^{c}	(39.3)^{d}	(29.3)^{c}	(38.0)^{c}	(27.3)^{c}	(40.5)^{d}	(19.2)^{b}	(41.2)^{d}	
T_{10} Cotton (NSKE treated)	20.6	24.8	16.3	33.7	17.7	40.8	24.3	37.2	21.0	42.5	10.5	45.0	11.8^{b}
	(4.5)^{c}	(29.8)^{f}	(4.0)^{d}	(35.5)^{f}	(24.8)^{c}	(39.6)^{d}	(29.5)^{c}	(37.5)^{c}	(27.2)^{c}	(40.6)^{d}	(18.8)^{b}	(41.2)^{cd}	
T_{11} Cotton (*T. chilonis* released)	23.8	13.1	21.3	13.4	25.9	13.4	34.2	11.6	28.9	20.8	17.7	7.3	7.5^{de}
	(4.9)^{d}	(21.2)^{g}	(4.6)^{fg}	(21.4)^{I}	(30.5)^{f}	(21.4)^{I}	(35.7)^{f}	(19.9)^{h}	(32.5)^{f}	(27.1)^{h}	(24.8)^{cd}	(15.6)^{g}	
T_{12} Cotton sole crop	27.4	-	24.6	-	29.9	-	38.7	-	36.5	-	19.1	-	6.3^{e}
(untreated check)	(5.2)^{f}		(5.0)^{h}		(33.1)^{g}		(38.4)^{g}		(37.1)^{g}		(25.8)^{cd}		

1 = Mean No. of eggs per 10 plants 2 = % reduction over untreated check 3 = Mean No. of larvae per 10 plants 4 = % reduction over untreated check
5 = % damage on fruiting bodies 6 = % reduction over untreated check 7 = % boll damage (Open boll basis) 8 = % reduction over untreated check
9 = % locule damage 10 = % reduction over untreated check 11 = % inter locule damage 12 = % reduction over untreated check
13 = Kapas Yield (q ha^{-1})
# Figures in parentheses are square root transformed values * Figures in parentheses are arcsine transformed values
Means in a column followed by same letter(s) are not significantly different (p = 0.05) by DMRT

Table 2: Effect of trap crops and restricted application of NSKE on cotton on the preference of cotton bollworms (Vaigaidam, Summer 2003)

			H. armigera			
			Eggs		Larvae	
Cropping system	NSKE 5% spray on cotton	Crops	P	PR	P	PR
Cotton + okra	Cotton untreated with NSKE	Cotton	24.16	-	21.90	-
		Okra	32.66	1:1.35	24.44	1:1.12
	Cotton treated with NSKE	Cotton	13.83	-	13.27	-
		Okra	41.88	1:3.02	32.55	1:2.45
Cotton + pigeonpea	Cotton untreated with NSKE	Cotton	26.16	-	19.00	-
		Pigeonpea	36.80	1:1.40	24.18	1:1.27
	Cotton treated with NSKE	Cotton	15.00	-	11.63	-
		Pigeonpea	36.50	1:2.43	30.72	1:2.64
Cotton sole crop	Cotton untreated with NSKE	Cotton	27.40	-	24.63	-
	Cotton treated with NSKE	Cotton	20.58	-	16.27	-

P = Mean population per ten plants PR = Preference Ratio

from 10.40 to 12.0 and 14.50 to 15.50%, respectively. The percent parasitization of eggs of *H. armigera* on cotton sole crop ranged from 14.80 to 16.40% in the, respective *T. chilonis* released plots (Table 3). The movement of the parasitoid on okra fruits was inhibited by long trichome and wasps were trapped by sticky trichome exudates. It leads to the lower per cent parasitization on okra fruits compared to cotton and pigeonpea.

Effect of trap crops, neem and *T. chilonis* against Resistance Frequency (RF) of *H. armigera* to synthetic pyrethroids: The extent of resistance before spraying was 94.0, 92.5, 88.2, 90.2 and 85.3% to cypermethrin, fenvalerate, deltamethrin, lambda-cyhalothrin and beta-cyfluthrin, respectively. The resistance of field survived population at the end of the season was 90.4, 89.1, 89.5, 92.1 and 84.8% to cypermethrin, fenvalerate, deltamethrin, lambda-cyhalothrin and beta-cyfluthrin, respectively (Table 4).

Trap cropping is based on the pest's host plant preference and accomplished by providing a plant variety or species that is preferred over the main crop. A pest moving into an area is likely to be attacked to the preferred trap plants and be diverted away from the main crop[8]. The diverse biological effects of neem are repellency, phagodeterrence, growth inhibition, abnormal development[9] and oviposition suppression[10].

Table 3: Mean per cent parasitisation of *H. armigera* eggs by *T. chilonis* on trap crops and cotton (Vaigaidam, Summer 2003)

Treatments	Trap crop/ cotton	Percent of parasitisation on trap crops/cotton
T_1 Cotton (NSKE treated)+ okra (*T. chilonis* released)	Okra	12.0 (20.2)[bc]
T_2 Cotton (NSKE treated)+ okra (*T. chilonis* unreleased)	Okra	1.2 (5.9)[e]
T_3 Cotton (NSKE untreated)+ okra (*T. chilonis* released)	Okra	10.4 (18.8)[c]
T_4 Cotton (NSKE untreated)+ okra (*T. chilonis* unreleased)	Okra	3.7 (11.0)[d]
T_5 Cotton (NSKE treated)+ pigeonpea (*T. chilonis* released)	Pigeonpea	14.5 (22.3)[ab]
T_6 Cotton (NSKE treated)+ pigeonpea (*T. chilonis* unreleased)	Pigeonpea	2.5 (9.0)[d]
T_7 Cotton (NSKE untreated)+ pigeonpea (*T. chilonis* released)	Pigeonpea	15.5 (23.1)[a]
T_8 Cotton (NSKE untreated)+ pigeonpea (*T. chilonis* unreleased)	Pigeonpea	1.2 (6.0)[e]
T_9 Cotton (both NSKE and *T. chilonis* treated)	Cotton	14.8 (22.6)[ab]
T_{10} Cotton (NSKE treated)	Cotton	2.5 (9.0)[d]
T_{11} on (*T. chilonis* released)	Cotton	16.4 (23.8)[a]
T_{12} Cotton sole crop (untreated check)	Cotton	3.8 (11.3)[d]

Figures in parentheses are square root transformed values

Means in a column followed by same letter(s) are not significantly different ($p = 0.05$) by DMRT

Table 4: Effect of push-pull strategy with conjunctive use of trap crops, neem and *T. chilonis* against Resistance Frequency (RF) of *H. armigera* to synthetic pyrethroids (Vaigaidam, Summer 2003)

Synthetic pyrethroids	DD dose ($\mu g\ \mu L^{-1}$)	% resistance of F_1 field population before first spray % resistance±SE	% resistance of F_1 field survived population after last spray % resistance±SE
Cypermethrin	0.1000	94.0±3.4	90.4±4.1
Fenvalerate	0.2000	92.5±3.7	89.1±4.6
Deltamethrin	0.0125	88.2±4.6	89.5±4.6
Lambda-cyhalothrin	0.0250	90.2±4.2	92.1±3.5
Beta-cyfluthrin	0.2000	85.3±5.1	84.8±5.1

SE- Pooled binomial standard error (±)

Biological control in IRM/IPM system is a subject of considerable current interest because of a perceived urgency to develop and adopt safe and efficient methods for managing agricultural pests[11]. The inability of *Trichogramma* to disperse in larger areas are the major constraints in cotton ecosystem[5]. However the above difficulties could be overcome through a concept called push-pull strategy[12] , in which host-masking agents, repellents, antifeedants or oviposition deterrents are deployed to push colonizing insects away from the harvestable crop and also to attract predators or parasitoids into the area. At the same time, the pests are aggregated on a sacrificial or trap crop so that a selective control agents, eg. *Trichogramma* sp. can be used directly to reduce the pest population.

Field trial was conducted on push-pull strategy with conjunctive use of three components such as trap crops, neem spray on cotton alone along with restricted release of *Trichogramma* on trap crops against *H. armigera*. Application of NSKE on cotton in conjunction with restricted release of *Trichogramma* on trap crops under trap cropping system in cotton was most effective in controlling *H. armigera* and increasing the yield.

In the present study, okra acted as a good trap for *H. armigera* and the efficiency of the trap crop was improved by applying NSKE on cotton. Similar observation on noctuid's ovipositional preference to okra than cotton in Punjab was reported by Singh *et al.*[13], suggested intercropping of okra in cotton for *H. armigera* as an useful cultural practices. Similar diversion of *A. devastans, B. tabaci, A. gossypii* and semiloopers from cotton to okra[14] was observed when the Non-edible Oil (NEO) formulations were applied on the main crop.

The percent parasitism on okra, pigeonpea and cotton observed in the present study is very low compared to 37.5% on okra[15], 55% on pigeonpea[16] and 32 to 96% on cotton[17,18]. This might be due to the variation in the variety of the crop in relation to trichome characters and environmental conditions of the experimental area.

Trichogramma eggs parasitism is much influenced by host plants and rarely parasitise eggs on pigeonpea[19-21]. Romeies *et al.*[20] recorded 2.2% parasitisation on pigeonpea. However, Duffield[22] reported high levels of egg parasitisation on short duration pigeonpea, when it was inter cropped with sorghum due to the movement of parasitoids from sorghum to pigeonpea. This is in confirmation with the present study, where the spraying of NSKE forced the parasitoid on the trap crops and increased the percent parasitisation.

The efficiency of *T. chilonis* on pigeonpea is dependent on the plant surface structure on which the host eggs were found[16]. Romeies *et al.* [23] observed the percent egg parasitisation of 3.6, 0.3 and 40.7 on calyxes, pods and leaves of pigeonpea, respectively. However, in the present study, lower level of parasitism in pigeonpea was observed though eggs were observed on leaves and petals.

In the conjunctive use of trap crops, NSKE on cotton and *T. chilonis* on trap crops, the resistance of the field collected population of *H. armigera* to the pyrethroids showed lesser percent survival compared to the survival of the field collected population before spraying. Botanicals may be used to increase the susceptibility of the target pest. The exposure to a stressor might influence the susceptibility of the host to an active pathogen. The biologically active compounds from the plant products penetrate the gut wall which allows the easy penetration of the pathogen into the haemocoel[24].

The non-chemical methods used in the present study in cotton provides scope for relaxation in selection pressure of *H. armigera* to certain extent. Use of botanical pesticides and trap crop are the best choice for the cotton IPM. Different modes of actions like antifeedant, antiovipositional, IGR, cidal, antisymbiont and reduction in reproduction make the insects more vulnerable and prevent the development of resistance and resurgence. Plant products are an excellent choice for preservation of beneficial organisms. This is important in light of today's expanding IPM programme. Use of these plant derivatives and biocontrol agents as a component of IRM will go a long way in the management of insect pests.

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