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Sequential Application of Insecticides for Resistance Management of African Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Cotton

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Abstract

African bollworm (*Helicoverpa armigera*) (Lepidoptera: Noctuidae) is a major constraint for cotton production and productivity in Ethiopia. The field experiment was conducted during 2017 and 2018 season at Werer Agricultural Research Center aimed to determine the best chemical alternation sequence for insecticide resistance management strategy. It experiment was laid out in a Randomized Complete Block Design (RCBD) with four replications having seven treatments. The assessed parameters were African bollworm population, damaged squares, flowers and bolls at pre and post insecticide application, boll number per plant and seed cotton yield. In this experiment, three round of spray application was made by using insecticides from different chemical class. Using the modified Abbott's formula, the percent efficacy was computed. A highly significant difference ($P<0.0001$) were observed among the treatments for post spray larvae count and damaged squares count in 2017 and 2018 season and non-significance difference among ($P<0.05$) was observed for pre and post spray flower counts in both season of trial. The insecticide rotation chlorantraniliprole, chlorfenapyr, profenofos, and chlorfenapyr, chlorantraniliprole, lufenuron+profenofos gave better control of *H. armigera* larva, damaged squares and flowers and significantly high cotton yield and the lowest seed cotton yield was obtained from unsprayed treatment. The repetitive use of conventional synthetic pyrethroid insecticides might have assisted the increase of resistance in *H. armigera.* The study recommended rotational use of insecticides with different mode of actions. Further studies on monitoring of resistance and evaluation of integrated resistance management methods are recommended.

Keywords: *Helicoverpa armigera*; Resistance management; Insecticide rotation

Introduction

In Ethiopia, cotton is one of the widely cultivated crops both by small and large-scale cotton producers. Production and productivity of cotton in Ethiopia is constrained by a number of factors among which insect pest problem has become the major one. A total of seventy species of insects and mite pest have been known to attack cotton in Ethiopia of which African bollworm (*Helicoverpa armigera*), cotton aphid (*Aphis gossypii*), thrips (*Thrips tabaci*) and Whitefly (*Bemisia tabaci*) are the major. The newly emerged cotton mealybug is also another key pest. African bollworm (*Helicoverpa armigera*) (Lepidoptera: Noctuidae) is a polyphagous insect damaging wide ranges of food, fiber and horticultural crops such as beans, chickpea, peas, sorghum, cotton, tomato, pepper, sunflower, safflower, flax, and Niger seed. Among different pest of cotton, 50%-60% yields were reduced by *H. armigera* each year. Bollworms (*H. armigera, Pectinophora gossypiella, Diparopsis watersi, and Earias spp*) cause 36%-60% yield loss and from that African bollworm, *H. armigera* was the dominant species 60% average yield losses due to bollworms and among the bollworms; *H. armigera* was the most important pest. All parts of the cotton plant are vulnerable to attack by the pests [1].

Management of pests is always being one of the most important tasks in total production cost of cotton in most years. If correct pest control measures are not taken, cotton farms can lose the whole production. Among production control costs, 43% is spent on pesticide purchase and 33% on weeding.

Control of pests with insecticides from a single chemistry group is common in most cotton farms and such a practice for an extended period results in the development of resistance. The *H. armigera* is a multi-resistant insect species; it can express more than one resistance mechanism to a particular insecticide group [2,3]. Application of different insecticides sequentially resulted in significant reduction of larvae as compared with repeated applications of the same insecticide (Designing insecticide resistance management strategy for *H. armigera* is very crucial. The use of pesticide mixtures or mode of action rotation as an important component of an insecticide resistance management approach to delay or mitigate the onset of resistance development in arthropod pest populations. Once resistance is noticed as loss of field efficacy, there are very few practical alternatives other than abandoning the use of the insecticide. Therefore, the present study was proposed to study the effectiveness of selected insecticide application sequences against African bollworm (*Helicoverpa armigera*) on cotton under field condition.

Material and Methods

The experiment was conducted at Werer Agricultural Research Center during 2017 and 2018 season under field conditions.

Field experiment for resistance management strategy

The field experiment was conducted during the main season of the 2017 and 2018 at Werer Agricultural Research Center. Eight different insecticides belonging to five major insecticide classes were arranged in spray sequences. The selected insecticides have been recommended for the control of African bollworm on cotton by Werer Agricultural Research Center (EIAR) [4]. The insecticides were systematically arranged in six treatments and three spraying sequences; including one untreated check (Table 1). The cotton variety used for the study was Deltpine-90 which was planted on 26th May 2017 and 21st May 2018.

Treatments were arranged in Randomized Complete Block Design (RCBD) with four replications. Individual plots 7 rows of cotton and total size of 63 m^2 (0.9 m × 10 m) and net of plot 5 rows of cotton and area 45 m^2 (0.9 m × 5 m). The distance between ridge to ridge and plant-to-plant was 90 and 20 cm, respectively. The plots were separated from each other by a space of two and four meters between treatments and replications, respectively. All recommended agronomic practices were applied to treatment plots. On each plot examining of ABW was started three weeks after germination and continued until the cotton plants matured [5,6]. Ten plants per plot were selected at random and tagged for the assessment of pest infestation by checking leaves, squares, flowers, and bolls. Then, from tagged plants data on ABW egg and larva, square, flower, and boll damage of *H. armigera* and other sucking insect pests and predators were recorded. On an experimental plot, a total of three rounds of spray were applied using hand-operated knapsack sprayer based on natural infestation when the economic threshold level of 10 larvae per 100 plants.

The evaluated insecticide sprays were prepared according to the companies recommended doses in water application volume of 200 liters/ hectare [6-8]. Low incidence of sucking pest at an early stage of the plant and at cotton maturity, one round of dimethoate 1.5 L/ha and sulfoxaflor 150 ml/ha were applied to control thrips and aphid infestation in both season of trial, respectively. The first round spray application was made on July 6th, 2017 and June 28th with the square and flower formation period of the cotton, and the subsequent two sprays were applied at 15 days intervals. The second round spray application coincided with the pick square and flower formation period and the third round application with the boll formation and boll opening period of cotton plant. Ten plants were tagged in each plots and young shoot leaves, squares, flowers and bolls were examined for data collection. African bollworm egg and larvae, damage squares, flowers, and bolls; non-target and beneficial insects on pre and post spray count of 3, 5, 7 and 10 day were recorded. Data were collected on number of s after treatment. At crop maturity and just before cotton picking healthy numbers of bolls per plant were counted on those ten predetermined plants including on control plots. Finally, seed cotton was harvested and weighed (Table 1).

Treatment	Treatment code	Sequence of treatment		
		1st spray	2nd spray	3rd spray
1	A	chlorantraniliprole 200 SC @150 ml/ha	chlorfenapyr 36 SC@225 ml/ha	profenofos 720 G/L@900 ml/ha
2	B	deltamethrin 2.5EC@600 ml/ha	lufenuron + Profenofos 55EC@650ml/ha	chlorfenapyr 36 SC@225ml/ha
3	C	chlorfenapyr 36 SC@225 ml/ha	chlorantraniliprole 200 SC@150 ml/ha	lufenuron +profenofos 55EC@650 ml/ha
4	D	lufenuron +profenofos 55EC@650 ml/ha	chlorfenapyr 36 SC@225 ml/ha	alphacypermethrin 100 G/L@300 ml/ha
5	E	chlorpyriphos 48% EC@2l/ha	lufenuron +profenofos 55EC@650 ml/ha	lambda-cyhalothrin 5% EC@480 ml/ha
6	F	lambda-cyhalothrin 5% EC@480 ml/ha	lambda-cyhalothrin 5% EC@480 ml/ha	lambda-cyhalothrin 5% EC@480 ml/ha
7	G	Unsprayed	Unsprayed	Unsprayed

Table 1: Insecticide treatments for spray sequence on field experiment.

Statistical analysis

In field experiment, all collected field data were analyzed using PROC GLM (SAS Version 9.0, SAS Institute, 1999). PROC UNIVARIATE was used to test data for normality and homogeneity of variance based on the Shapiro-Wilk statstic. To satisfy the assumptions of ANOVA, the pre and post-spray count mean data were square-root transformed ($\sqrt{x+0.5}$). Seed cotton yield and the transformed data were subjected to Analysis of Variance test. When F-values were significant ($P<0.05$), means were compared by Fisher's Least Significant Difference (LSD) test with SAS software.

Results and Discussion

First spray

The larval population, damaged squares, and flower damage in all the treatments were not significantly different before application for all treatments in both season. In 2017 season the post spray mean larva count revealed, a highly significant difference ($P<0.01$) among treatments. The highest larvae count was observed from control treatment (0.18/plant) and the lowest larvae mean from chlorfenapyr insecticides (0.04/plant) treated plots. In 2018 there was a significance difference ($P<0.05$) among treatment was observed.

A post spray mean damage squares count for 1st round spray application showed a highly significant difference ($P<0.0001$) and significant different ($P<0.05$) among the different treatments were oberevd in 2017 and 2018 season (2 and 3). In 2017 season, lower square damaged were recorded for chlorantraniliprole and chlorfenapyr, which was statistically significant from treatment deltamethrin and control but par with the rest treatments. Control plots had the highest number of damaged square numbers both season of trail. Among treatments, the highest numbers of damage flowers (0.06/plant) were recorded with control in both season of trial, while the lowest (0.01/plant) were with lufenuron+profenofos in 2017 and (0.01/plant) from lamdacyhalothrin. Among tested insecticides the maximum efficacy percent was obtained from chlorfenapyr (81.90%)

and highest percent mortality from chlorantraniliprole (80.43%). Whereas, deltamethrin was the least effective in both season of trial.

Second spray

The post-spray mean larva count and square damage of 2017 season showed significantly varied ($P<0.0001$) among the different insecticides. Both season of trail Chlorfenapyr showed the highest efficacy (85.61% and 80.85%) and the lowest from lambda-cyhalotrine (55.69% and 53.12%). In 2018 season the post-spray mean larvae count, damaged squares, flowers and bolls revealed significant variation ($P<0.05$) were obtained among different insecticides treated.

In 2017 trial the highest damage of flowers of (0.17/plant) were recorded with lufenuron+profenofos and the lowest (0.07/plant) and number of damaged bolls (0.06/plant) for with chlorfenapyr treatments. Control plots had 0.24/plant number of damaged flowers and 0.19 damaged bolls. The highest number of damage bolls (0.18/plant) were recorded for lambda-cyhalothrin. In 2018 season maximum mean damage squares, flowers and bolls count were recorded in control treatment, while minimum mean damaged squares, flowers and bolls count were recorded on test-plots treated by with Chlorfenapyr.

Third spray

The lowest larvae counts were from profenofos (0.03/plant and 0.056/plant) and the highest from unsprayed treatments (0.18/plant and 0.388/plant), in 2017 and 2018 season, respectively. The number of square damage did not differ among the different treatments. Conversely, there were fewer fruit bodies damages in plants treated with insecticides, presumably because of the high mortality of young larvae. The highest efficacy was obtained from chlorfenapyr (83.10%) and Profenofos (84.27%); and the lowest from lambda-cyhalothrin (57.09% and 59.68%) in 2017 and 2017 season, respectively. There was significantly difference ($P<0.01$) in number damaged bolls among treatments in both seasons of trial and combined analysis.

Yield and yield component

There was significant difference ($P<0.01$) in the number of boll number/plant among the insecticide treatments in both season of trial. The highest number of boll (16.53/plant) in 2017 and 20.85/plant in 2018 and seed cotton yield (3.84 ton/ha) in 2017 and (3.17 tonn/ha) in 2018 were obtained from the treatment with rotation of chlorantraniliprole, Chlorfenapyr, profenofos and the lowes numbers of boll (10.38/plant) in 2017 and (9.0/plant) in 2018 season and seed cotton yield 2.56 ton/ha and 1.91 ton/ha from the control treatment. The rotation of chlorantraniliprole, cholefenapyrr gave cotton yield advantage of of 0.72 ton/ha and 0.75 ton/ha in 2017 and 2018 season than the convectional three times lambdacyhalothrin treatment. The cotton yield of the rotation deltamethrine (Table 2).

Treatments code	Healthy boll/ plant		Seed cotton yield (ton/ha)		Healthy boll/ plant	Seed cotton yield (ton/ha)
A	16.53a		3.84a		20.85a	3.17a
B	12.85b		3.35bc		12.68b	2.48b
C	14.08b		3.68ab		15.53ba	3.08a
D	14.15b		3.51abc		14.05b	2.42b
E	12.63b		3.39bc		10.5b	2.31bc
F	12.40bc		3.12c		10.13b	2.26bc
G	10.38c		2.56d		9.0b	1.91c
LSD(0.05)	2.05		0.44		6.78	5.08
CV (%)	10.39		8.87		15.37	13.57

Note: Means followed by the same letter (s) within a column are not significantly different from each other at 5% levelof significance, Least significance Difference (LSD), CV=Coefficient of Variability, NS=None

Table 2: Mean value of number of healthy bolls per plant at harvest and seed cotton yield in field experiment, werer 2017.

Discussion

The present study indicated application of chlorantraniliprole insecticide in the 1st spray gave better control for *H. armigera* larvae and reduced damage to fruiting bodies in both season of trial found application of chlorantraniliprole 20 SC@40 g a.i. ha^{-1} effectively controlled *H. armigera* pest on cotton. Chlorantraniliprole insecticide activates ryanodine receptors via stimulation of the release of calcium stores from the sarcoplasmic reticulum of muscle cells (i.e., for chewing insect pests) causing impaired regulation, paralysis and ultimately death of sensitive species. This might be the reason for high mortality percent with this insecticide. In the current study chlorfenapyr 36 SC, effectively controled *H. armigera* larvae and reduced damage to cotton fruiting bodies when applied at the manufacturers' recommendation in both season of trial. A number of authors reported spraying chlorfenapyr insecticide was effectual in *controlling H. armigera.* Chlorfenapyreffectively controlled *H. armigera* on a soybean in Brazil with 90.9% efficacy. Indicated that chlorfenapyr has good efficacy against *H. armigera* because of its knockdown chemical nature. Insecticide lambda-cyhalothrin and deltamethrin, which had a mode of action of sodium canal modulator, had low efficacy spraying of deltamethrin and lambda-cyhalothrin insecticides were least effective in controlling H. armigera pest. The IGR+OP insecticide was also good in reducing *H. armigera* larvae and damage to cotton squares, flower and boll. Lufenuron insecticide has Chitin Synthesis Inhibitors (CSI) that act on the incorporation of N-acetyl glucosamine monomer into chitin in the integument, resulting in the formation of abnormal new cuticle and death of the insect (Profenophos insecticide toxicity can occur in two ways: inhibition of acetylcholine esterase, and cytotoxic effects on immune cells. A repetitive spraying of synthetic insecticide in three successive sprays induced variable effectiveness against African bollworm larvae and damaged cotton fruiting bodies. The result showed that the lowest population of H. armigera larvae and highest efficacy was when insecticides with different mode of actions were rotated, i.e., chl orantraniliprole (diamide), chlorfenapyr (pyrole), profenofos (organop hosphate). The lowest efficacy was recorded for the conventional insecticides lambda-cyhalothrin (pyrethroid) applied in the three sequences. Additionally, study was noticed that IGR+OP insecticides in sequence with new and conventional insecticides gave good reduction of the African bollworms larvae and reduced damage cotton fruiting bodies. Conventional insecticides like deltamethrin and lambdacyhalotrine, when applied in spray sequence, gave low larvae mortality against *H. armigera* and damage to fruiting bodies of cotton while the new insecticide chlorfenapyr proved to have high efficacy against *H armigera* larva. The study confirmed in both seasons of trial that inclusion of deltamethrin in to the rotation reduced the efficacy. Use of insecticides with the same mode of action continuously has been one of the causes for resistance development in insect pests.

Conclusion

Deltamethrin might have reduced its efficacy for controlling *Heliothis armigera* to; thus, there is a need to replace it with new insecticides with different mode of actions. The study showed that chlorfenapyr is a good substitute for providing good control against *H. armigera* on cotton. Use of insecticides in rotations gave better control of *H. armigera* larvae, had lower damages of squares and bolls, and gave higher yield than the conventional way of applying lambda-cyhalotrhin repeatedly. It is better knowing of use insecticide with a different mode of action, they belong to different chemical groups have no positive cross-resistance with each other.

Synthetic pyrethroids should not be applied alone repeatedly for many years to control ABW in cotton. Application of insecticide with a different mode of action in rotations is important to reduce the development of insecticide resistance in *H. armigera.* Future studies are needed in design insecticide resistance management strategy.

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