

Level of Selected Pyrethroid Insecticides Resistance in African Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) on Cotton

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Abstract

Studies on the level of synthetic pyrethroid insecticides (lambda-cyhalothrin, deltamethrin and alphacypermethrin) resistance in African bollworm, *Helicoverpa armigera* was carried out at Werer agricultural research center. The level of resistance was evaluated under the laboratory conditions on four populations using larva immersion and square dip methods. The selected insecticides were tested in seven dilutions levels. In each dilution 30 larvae of 3rd instars *H. armigera* were treated in three replications along with pure water. Low level of resistance was detected for all tested locations to alphacypermethrin and high resistance ratio to lambda-cyhalothrin and deltamethrin for Gewane and Werer populations. Alphacypermethrin was the most toxic insecticide and its LC₅₀ was low compared to other tested synthetic pyrethroids. Whereas, deltamethrin was the least toxic insecticide with high LC₅₀. The LC₅₀ value of Goffa-Sawla population was significantly different among the populations for Werer, Upper-Awash and Gewane in both bioassay methods. The study concluded that *Helicoverpa armigera* might have resistant to deltamethrin in Werer and Gewane populations. Further studies on monitoring of resistance are recommended.

Keywords: Bioassay; *Helicoverpa armigera*; Pyrethroid; Resistance; Middle Awash

Introduction

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 from 1980-1990 in China. 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. Reported 60% average yield losses due to bollworms and among the bollworms; *H. armigera* was the most important pest [1].

For decades Ethiopian cotton farmers have primarily been using chemical pesticides to control pests. Therefore, the amount of pesticide used on this crop is excessively large (out of six sprays four round sprays or about 66.7% sprays are allotted for control of cotton bollworms) and their effect on environment by this practice have not been quantified. Recently farmers reported that controlling these pests with the available insecticides has become difficult.

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 as in the case of lambda-cyhalothrin for African bollworm species at Dubti, dimethoate for aphid species at the Middle Awash, and carbamate group (carbosulfan, furathiocarb and pirimicarb) for aphid species resistance to at Arbaminch. Efficacy reduction of endosulfan at Werer

agricultural Research Center and commercial farms in Ethiopia. Additionally, the commonly used synthetic pyrethroid insecticides, lambda-cyhalothrin and deltamethrin have shown reduced efficacy in controlling African bollworm in Middle Awash area (Personal communication). This might be due to the development of insecticide resistance by African bollworm. Therefore, the present study was proposed to determine the level of resistance by African bollworm to commonly used synthetic pyrethroid insecticides under laboratory condition [2].

Materials and Methods

The experiment was conducted at Werer agricultural research center, Amibara District, Gebresu zone of Afar national regional state under laboratory conditions.

Laboratory experiments

African bollworm (*Helicoverpa armigera*) larva collection and rearing: The larvae of African bollworm were collected from unsprayed cotton farms in Middle Awash (Werer (734.4 m.a.s.l, E 400 09' 81" and N 090 21' 243"), Gewane (567 m.a.s.l, E 0400 31' 23.0" and N 090 59' 22.5")) and Upper Awash farms (Merti Jeju (1174 m.a.s.l, E 0390 43' 927" and N 080 37' 111")). The larvae were reared using cotton squares until pupation. Pupae were collected every morning and transferred to plastic pots (size of 20 cm height and 16 cm width) embedded with soil. Pairs of male and female emerged adults moths were placed in rearing cages. A dissolved sugar was

supplied in the rearing cage (size of 30 cm height × 27 cm width) for adults to feed. The adult diet was prepared from five gram sugar and 200 ml water. In each adult rearing cage one plastic cup plugged with cotton wool immersed in the sugar solution was kept for the adults to feed. The adults were allowed to lay eggs on cheese cloth or on detached cotton branch placed inside the cage. The eggs hatched after three or four days. The hatched larvae were collected and reared on cotton leaves. Starting the second instar stage, larvae were separated and held singly in petridish with cotton leaves. The experiment was conducted on the third instar larvae. Larvae of ABW were collected from chickpea fields of small-scale farms at Gofa-Sawla area (1260 m.a.s.l, E 0360 56' and N 060 19'), southern Ethiopia, with no insecticide use history in the last six years were brought to Werer agricultural research center and used for comparison with ABW collected from cotton farms which are heavily sprayed for many years [3].

Serial dilution of insecticide

The commercial insecticides alphacypermethrin (Fastac 100 G/L), lambda-cyhalothrin (Karate 5% EC) and deltamethrin (Decis 2.5% EC) were serially diluted with tap water bioassayed against different strains of ABW. Concentrations of formulated insecticides were calculated based on the market available full-labeled field rate and application volume of 200 liters/ha.

Laboratory bioassay methodology

Bioassay was conducted using the newly molted F1 generation of 3rd instar larva by using the square dip and larval immersion bioassay procedure recommended. The experiments were laid with Completely Randomized Design (CRD) with three replications. For each replicate of a serial dilution ten larvae were used.

Experiment 1: Larval immersion method

Thirty larvae were used in each treatment and each treatment was replicated three times. For each treatment ten 3rd instar larvae per replication were used. The larvae were dipped into individual dilutions for ten seconds and placed on tissue paper padded trays for absorbing excessive liquid from the body. Larvae were transferred into glass petridish with insecticide free cotton square. The check treatment treated with pre water. The mortality rate was assessed 24 hours after placing the larvae by probing the larvae with fine camel hair brush. If the larvae respond for probing it was considered alive or dead otherwise [4].

Experiment 2: Square dip method

Medium size cotton squares which weigh 700 mg-1000 mg were collected from the unsprayed cotton field and dipped into individual dilutions of insecticides for ten seconds and transferred onto paper padded tray for air-drying. After 60 minutes of drying, single dipped squares were kept in glass petridishes and a single 3rd instar larva was introduced for feeding on the treated squares. The check treatment treated with pre water. The mortality rate was assessed 24 hours after placing the larvae by probing the larvae with fine camel hair brush. If the larvae respond for probing it was considered alive or dead otherwise.

Data collected

The dose-mortality larvae were recorded after 24, 48 and 72 hours of treatment for larval immersion bioassay while after 24, 36, and 48 hours of treatment for square dip method bioassay. Larvae were regarded as dead if they are not able to move when probed with a blunt probe or brush. Results were expressed as percentage mortality. The daily minimum and maximum temperature and RH of the laboratory were recorded [5].

Statistical analysis

Data from a bioassay were corrected for control mortality using Abbott's formula

$$\text{Percent mortality} = \frac{\text{dead larva}}{\text{total larva treated}} * 100,$$

Percent corrected mortality

$$= \left(\frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \right) * 100$$

The results obtained from the dose-mortality experiments were estimated by probit analysis using the SAS software version 9.0. LC₅₀ and LC₉₀, (Lethal Concentrations which kill 50% and 90% test larva), slope and the 95% Confidence Limit (CL) was determined.

Resistance Ratios were calculated by dividing the LC₅₀ values of each field population by the LC₅₀ of Gofa-Sawla (susceptible population). The insecticide resistance level was determined using the methods described by susceptible (RR=1), low level of resistance (RR=2-10), moderate resistance (RR=11-30), high resistance (RR=31-100) and very high resistance (RR>100) [6].

Results and Discussion

Larva immersion and square dip method

Lambda-cyhalothrin: *H. armigera* larva mortality ranged from 3.3 to 100 percent when exposed to different concentrations (2, 1, 0.5, 0.25, 0.12, 0.0625 and 0.03125 µl/ml) of lambda-cyhalothrin. Larval mortality of 100, 100, 100 and 90% mortality was obtained at field rate (5.0×10^{-4} g g. a.i/ml) of the insecticide for Goffa-Sawla, Upper-Awash, Werer and Gewane population, respectively. Four times lower dose from the recommended rate (1.25×10^{-4} g. a.i/ml) resulted in 100% mortality on Gofa-Sawla population, which was higher than field rate dose-mortality (90%) of Gewane and two times lower dose rate mortality percent (96%) upper-Awash and (86.7%) of Werer population. In square dip method the field rate lambda-cyhalothrin (5.0×10^{-4} g. a.i/ml) resulted in 100%, 100%, 96.7% and 93.3% mortality on Goffa-Sawla, Upper-Awash, Werer and Gewane populations, respectively. The four-times lower dose (1.25×10^{-4} g.a.i/ml) caused 100% mortality while the eight-times lower dose (2.5×10^{-5} a.i/ml) 93.3% mortality on the Goffa-Sawla population while only 83.3% mortality was recorded for Werer to two times-lower doses (2.5×10^{-4} g. a.i/ml) and 90% mortality of the Upper-Awash population to field rate dose of Gewane population. Both bioassay method showed the Gewane population was less susceptible to lambda-cyhalothrin in larva immersion method and larva susceptibility were in the decline sequence of Goffa-Sawla Upper-Awash>Werer>Gewane population [7].

The different concentrations of lambda-cyhalothrin 5% EC resulted in variable levels of, mortality when tested against *H. armigera* larvae which originated from different locations. In larva immersion method the Goffa-sawla population had a comparatively low value of LC₅₀ (0.074 µl/ml) and LC₉₀ (0.260 µl/ml) with steep log dose probit slope of the mortality regression line of 2.36. Whereas, high LC₅₀ (0.498 µl/ml) and LC₉₀ (2.870 µl/ml) values were obtained for the Gewane population with slope values of 1.69. In squared dip method P-values in the goodness-of-fit table of Werer (0.9967), Upper-Awash (0.9985), Goffa-Sawla (0.9134) and Gewane (0.9389) for the Pearson chi-square indicates an adequate fit for the model with normal distribution. The Goffa-sawla population had steep log₁₀ dose probit slope of line the 2.52. Whereas, Werer (1.84), Upper-Awash (1.80) and Gewane (1.78) slope values. Both bioassay method indicates the Goffa Sawla population is much more sensitive to lambda-cyhalothrin compared to other population. Goffa-Sawla population was significantly different (P<0.05) from Werer, Upper-Awash and Gewane populations without any overlap of 95% CL [8].

This study revealed that variation in the level of susceptibility to lambda-cyhalothrin exists in collected from different locations. Both bioassay methods showed that the tested population had low level of resistance to lambda-cyhalothrin. The Gewane population has higher resistance ratio compared to other tested populations. The LC50 of Gewane population in larva immersion and square dip technique recorded 0.498 and 0.447 values, respectively. The RF for the respective larval immersion and square dip study showed 6.73 and 7.45 times more resistance of the population compared to the susceptible Goffa-Sawla population. Both bioassays showed the presence of low level of resistance to lambda-cyhalothrin in tested locations. Several studies have indicated the development of resistance in for pyrethroids. Low level of resistance to lambda-cyhalothrin was reported from Turkey and Avilla and in Spain. Other studies reported moderate to high level resistance and high level resistance of to pyrethroids. The study contrast with who found in larva immersion and squared dip methods (Tables 1 and 2) [9].

Larva immersion					Squared dip				
Concentration (µl/ml)	Percent mortality				Concentration (µl/l)	Percent mortality			
	Goffa Sawla	Uppr Awah	Werer	Gewae		Goffa Szawla	Upper Awash	Werer	Gewane
2	100	100	100	90	2	100	100	96.7	93.3
1	100	96.7	86.7	70	1	100	90	83.3	73.3
0.5	100	83.3	70	53.3	0.5	100	76.7	63.3	53.3
0.25	83.3	63.3	46.7	26.7	0.25	93.3	56.7	46.7	33.3
0.12	66.7	40	23.3	13.3	0.12	73.3	36.7	23.3	16.7
0.0625	50	23.3	10	6.7	0.0625	56.7	20	10	3.3
0.03125	16.7	10	10	3.3	0.03125	23.3	6.7	3.3	3.3
Control	6.7	0	6.7	3.3	Control	3.3	3.3	6.7	6.7

Table 1. Percent of mortality of 3rd instar *H. armigera* larvae in different concentration of lambda-cyhalotrin 5% EC 72 hours after treatment with larva immersion bioassay.

Larva immersion									
Location	N	LC ₅₀ µl/ml	95% CL (lower-upper)	LC ₉₀ µl/ml	95% CL (lower-upper)	Fit of probit analysis			RR
						Slope ± SE	χ ² (df)	P	
Goffa-Sawla	180	0.074	(0.057-0.094)	0.26	(0.192-0.415)	2.36 ± 0.333	2.778 (4)	0.5957	—
Upper Awash	180	0.153	(0.118-0.199)*	0.693	(0.476-1.226)	1.96 ± 0.250	0.512 (4)	0.9723	2.07
Werer	180	0.264	(0.199-0.361)*	1.419	(0.886-3.022)	1.75 ± 0.236	2.15 (4)	0.7089	3.57
Gewane	180	0.498	(0.364-0.763)	2.87	(1.578-8.204)	1.69 ± 0.256	0.622 (4)	0.9606	6.73
Squared dip									
Location	N	LC ₅₀ µl/ml	95% CL (lower-upper)	LC ₉₀ µl/ml	95%CL (lower-upper)	Fit of probit analysis			RR
						Slope ± SE	X ² (df)	P	
Goffa-Sawla	180	0.06	(0.044-0.075)	0.193	(0.144-0.306)	2.5 ± 20.384	0.976 (4)	0.9134	—
Upper Awash	180	0.194	(0.147-0.258)*	1.007	(0.657-1.969)	1.80 ± 0.237	0.113 (4)	0.9985	3.25
Werer	180	0.302	(0.230-0.41)*	1.505	(0.949-3.162)	1.84 ± 0.249	0.168 (4)	0.9967	5.03
Gewane	180	0.447	(0.334-0.651)*	2.338	(1.364-5.869)	1.78 ± 0.261	0.797 (4)	0.9389	7.45

Note: N: Total number of larva used for probit analysis; LC₅₀: Median lethal concentration; LC₉₀: The lethal concentration which killed 90% of the test *H. armigera* population; 95% CL: The lower and the higher confidence limits at which the LC₅₀ and LC₉₀ value can fall at 95% probability; SE: Standard Error; X²: Chi-square; and the RR (Resistance Ratio)=LC₅₀ of the field population/LC₅₀ of Goffa-Sawla population; superscript denoted asterisk*: The collected *H. armigera* populations were not significantly different (P<0.05) among each other in their susceptibility to lambda-cyhalothrin insecticide.

Table 2. Comparative toxicity of lambda-cyhalotrin 5% EC to *H. armigera* populations in larva immersion and squared dip study.

Deltamethrin

Helicoverpa armigera populations of Werer, Upper-Awash, Goffa-Sawla and Gewane populations exposed to different concentrations of deltamethrin 2.5% EC experienced a varying level of mortality. At field rate (3×10^{-4} g. a.i/ml) deltamethrin gave 100%, 93.3%, 86.7% and 80.0% mortality 72 hours after larvae were immersed for Goffa-Sawla, Upper-Awash, Gewane and Werer populations, respectively. In squared dip method at field rate (3.0×10^{-4} g. a.i/ml) deltamethrin gave 100%, 90%, 83.3% and 80.0% larval mortality after 48 hours in square dip method of Goffa-Sawla, Upper-Awash, Gewane and Werer population, respectively. The field collected *H. armigera* larva from Goffa-Sawla experienced 100% mortality at two times lower dose (1.5×10^{-4} g. a.i/ml) of deltamethrin which was higher than the field rate mortality of Werer, Upper-Awash and Gewane populations, population showed the were respectively. lowest susceptibility to deltamethrin in both bioassay methods. The P-values in the goodness-of-fit table of Werer, Upper-Awash, Goffa-Sawla and Gewane for the Pearson chi-square indicate an adequate fit for the model with the normal distribution. The LC_{50} values indicate that Werer, Upper-Awash, and Gewane populations were not-significantly different among each other but differ ($P < 0.05$) from Goffa-Sawla population with no overlapping 95% CL.

The LC_{50} of Gewane population both in larva immersion (0.900) and square dip method (1.171) were lower than the Werer population of the respective LC_{50} values of 1.257 and 1.435. In larva immersion method the probit analysis showed that the Werer population is 8.79 times and Gewane populations 6.45 times more resistant to the susceptible Goffa-Sawla population. Similarly, the square dip method also showed that the Werer and Gewane population are 9.25 and 7.55 more resistant to the susceptible Goffa-Sawla population. These indicate that there is a high resistance development in *H. armigera* for delametrinat Werer and Gewane.

According to resistance grouping of *H. armigera* in Middle Awash, Ethiopi showed the resistance to deltamethrin. low level of Deltamethrin have been used to control *H. armigera* and sucking pests in cotton for long time. Recently, due to lack of the Ultra-Low Volume (ULV) formulation, the Emulsifiable Concentrate (EC) formulation of deltamethrin have been applied like ULV by mixing with small volume of water to save time and labor (personal communication). Such misuse of an insecticide against a *H. armigera*, may result in the selection of resistant forms of the pest population. Development of low to high level resistance in different strains of *H. armigera* for deltamethrin (Tables 3 and 4).

Concentration (µl/ml)	Larva immersion				Concentration (µl/ml)	Squared dip			
	Percent mortality					Percent mortality			
	Gofa Sawla	Upper Awash	Werer	Gewan-e		Gofa Sawla	Upper Awash	Werer	Gewan-e
3	100	93.3	80	86.7	3	100	90	80	83.3
1.5	100	76.7	56.7	66.7	1.5	100	76.7	50	60.0
0.75	93.3	50	30	43.3	0.75	96.7	56.7	23.3	33.3
0.375	76.7	26.7	13.3	20	0.375	76.7	40	3.3	6.7
0.1875	53.3	13.3	3.3	6.7	0.1875	56.7	20	3.3	3.3
0.09375	30	3.3	0	0	0.09375	26.7	6.7	0	0
0.046875	13.3	0	0	0	0.046875	6.7	0	0	0
Control	3.3	6.7	6.7	6.7	Control	6.7	6.7	0	6.7

Table 3. Percent of mortality of 3rd instar *H. armigera* larvae in different concentration of deltamethrin 2.5% EC 72 hours after treatment with larva immersion bioassay.

Larva immersion									
		95% CL		95% CL		Fit of probit analysis			
Location	N	LC_{50} µl/ml	(lower-upper)	LC_{90} µl/ml	(lower-upper)	Slope + SE	χ^2 (df)	P	RR
Gofa-Sawla	150	0.143	(0.104-0.246)	0.572	(0.430-0.966)	2.59+0.563	0.517 (3)	0.915	—
Upper Awash	150	0.69	(0.533-0.890)*	2.69	(1.863-4.894)	2.17+0.313	0.433 (3)	0.933	4.83

Werer	150	1.257	(0.980-1.690)*	4.814	(3.146-9.990)	2.20+0.331	0.044 (3)	0.998	8.79
Gewane	150	0.922	(0.717-1.207)*	3.633	(2.446-7.017)	2.15+0.314	0.203 (3)	0.977	6.45
Squared dip									
			95% CL		95% CL	Fit of probit analysis			RR
Location	N	LC ₅₀ µl/ml	(lower-upper)	LC ₉₀ µl/ml	(lower-upper)	Slope ± SE	X ² (df)	P	
Goffa-Sawla	150	0.155	(0.097-0.234)	0.515	(0.391-0.870)	2.74 ± 0.626	0.884 (3)	0.829	–
Upper Awash	150	0.563	(0.400-0.758)	3.111	(1.970-7.063)	1.727 ± 0.287	0.104 (3)	0.991	3.63
Werer	150	1.435	(1.137-1.899)*	4.712	(3.199-9.103)	2.48 ± 0.371	1.689 (3)	0.639	9.25
Gewane	150	1.171	(0.935-1.504)*	3.751	(2.643-6.632)	2.53 ± 0.359	0.865 (3)	0.834	7.55

Note: N: Total number of larva used for probit analysis; LC₅₀: Median lethal concentration; LC₉₀: The lethal concentration which killed 90% of the test *H. armigera* population; 95% CL: The lower and the higher confidence limits at which the LC₅₀ and LC₉₀ value can fall at 95% probability; SE: Standard Error; X²: Chi-square; and the RR (Resistance Ratio)=LC₅₀ of the field population/LC₅₀ of Goffa-Sawla population; superscript denoted asterisk*: The collected *H. armigera* populations were not significantly different (P<0.05) among each other in their susceptibility to deltamethrin insecticide.

Table 4. Comparative toxicity of deltamethrin 2.5% EC to *H. armigera* populations in larva immersion and squared dip study.

Alphacypermethrin

Helicoverpa armigera larva from different locations had varied mortality when exposed to different concentrations of alphacypermethrin. Alphacypermethrin caused 100% larva mortality at field rate (1.0×10^{-3} g. a.i/ml) on Werer, Upper-Awash and Gewane populations in both bioassay method. Except for Werer the two times-lower concentration alphacypermethrin resulted in 100% mortality. The four-times lower concentration (2.5×10^{-4} a.i/ml) resulted in 100% mortality only for Goffa-Sawla population. Subsequent dilutions of the insecticide resulted in lower percent mortality of larva to alphacypermethrin. Both bioassay methods showed, effective control of bollworm larva was achieved by alphacypermethrin compared with other insecticides tested. According to the current study the order of importance of pyrethroids used to combat *H. armigera* damage on cotton was alphacypermethrin >lambda-cyhalotrin>deltamethrin.

Based on LC₅₀ values, and the probit analysis Goffa-Sawla population was significantly different (P<0.05) from Werer, Upper-Awash and Gewane population with non-overlapping 95% CL.

In this study the probit analysis indicated showed resistance ratio in the range of 1.86-1.93 in larval immersion method and 1.76-1.94 in square dip method. As a result, level of resistance to alphacypermethrin was comparatively lower compared with other compounds of pyrethroids group (lambda-cyhalothrin and deltamethrin) tested, which indicate that there is no resistance to the insecticide in all populations tested. The toxicity of alphacypermethrin was high compared to lambda-cyhalothrin and deltamethrin.

Alphacypermethrin insecticide is used for control of cotton bollworm in Middle Awash,. Because of its broad spectrum mode of action, typically it is applied one time during peak squaring and flowering period. That could be the reason for high level of *H. armigera* mortality compared to other insecticides evaluated in this study. Alphacypermethrin is a newer insecticide in the study areas and has not been widely used compared to the other tested insecticides. Alpha-cypermethrin, a third generation pyrethroid is now one of the top-selling insecticides globally. Therefore, alphacypermethrin could be used for resistance management program as one of the insecticides in the alternation scheme (Tables 5 and 6) [10].

Concentration (µl/ml)	Larva immersion				Concentration (µl/ml)	Squared dip			
	Percent mortality					Percent mortality			
	Goffa Sawla	Upper Awash	Werer	Gewane		Goffa Sawla	Upper Awash	Werer	Gewane
1.5	100	100	100	100	1.5	100	100	100	100

0.75	100	100	96.7	100	0.75	100	93.3	96.7	90
0.375	100	90	83.3	83.3	0.375	100	80	83.3	76.7
0.1875	90	73.3	73.3	63.3	0.1875	86.7	76.7	66.7	56.7
0.09375	76.7	60	53.3	46.7	0.09375	70	60.0	53.3	43.3
0.046875	56.7	43.3	40	30	0.046875	53.3	36.7	36.7	26.7
0.0234375	26.7	16.7	16.7	10	0.0234375	23.3	16.7	16.7	10.0
Control	0	0	6.7	6.7	Control	10	0	3.3	6.7

Table 5. Percent of mortality of 3rd instar *H. armigera* larvae in different concentration of alphacypermethrin 100 G/L 72 hours after treatment with larva immersion bioassay.

Concentration (µl/ml)	Percent mortality				Concentration (µl/ml)	Percent mortality			
	Gofa Sawla	Upper Awash	Werer	Gewane		Gofa Sawla	Upper Awash	Werer	Gewane
1.5	100	100	100	100	1.5	100	100	100	100
0.75	100	100	96.7	100	0.75	100	93.3	96.7	90
0.375	100	90	83.3	83.3	0.375	100	80	83.3	76.7
0.1875	90	73.3	73.3	63.3	0.1875	86.7	76.7	66.7	56.7
0.09375	76.7	60	53.3	46.7	0.09375	70	60.0	53.3	43.3
0.046875	56.7	43.3	40	30	0.046875	53.3	36.7	36.7	26.7
0.0234375	26.7	16.7	16.7	10	0.0234375	23.3	16.7	16.7	10.0
Control	0	0	6.7	6.7	Control	10	0	3.3	6.7

Note: N: Total number of larva used for probit analysis; LC₅₀: Median lethal concentration; LC₉₀: The lethal concentration which killed 90% of the test *H. armigera* population; 95% CL: The lower and the higher confidence limits at which the LC₅₀ and LC₉₀ value can fall at 95% probability; SE: Standard Error; X²: Chi-square; and the RR (Resistance Ratio)=LC₅₀ of the field population/LC₅₀ of Gofa-Sawla population; superscript denoted asterisk*: The collected *H. armigera* populations were not significantly different (P<0.05) among each other in their susceptibility to alphacypermethrin insecticide.

Table 6. Comparative toxicity of alphacypermethrin 100 G/L to *H. armigera* populations in larva immersion and squared dip study.

Conclusion

The current study confirmed reduction in efficacy and the development of low level of resistance in *H. armigera* population to lambda-cyhalothrin at Werer and Gewane tested locations.

The efficacy of deltamethrin was moderately reduced and had higher resistance ratio compared to lambda-cyhalothrin in Werer and Gewane locations. *Helicoverpa armigera* might have resistant to deltamethrin; thus, there is a need to replace it with new insecticides with different mode of actions.

These insecticides were used for a long time to control to control cotton bollworm and sucking pest. Alphacypermethrin insecticide could be used for resistance management program as one of the insecticides in the alternation scheme.

The study included limited number of insecticides out of the commercially registered for cotton *H. armigera* control in Ethiopia. Future studies are needed to monitor the level of insecticide resistance.

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