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Research Article

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DOI: https://doi.org/10.21203/rs.3.rs-633192/v1

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Susceptibility of African Bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) to selected pyrethroid Insecticides on Cotton

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Abstract

*Helicoverpa armigera* is a major threat for all cotton production areas in Ethiopia. Pests control with insecticides from a single chemistry group is common practice in most cotton farms, which may help to development of insecticide resistance. The studies aimed to determine the susceptibility of field population of *H. armigera* to pyrethroid insecticides. The experiment was carried out at Werer Agricultural Research Center under the laboratory condition 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. A low level of resistance was detected for all tested locations to alphacypermethrin and a high resistance ratio to lambda-cyhalothrin and deltamethrin for Gewane and Werer populations. Aplhacypermethrin was the most toxic insecticide and its LC<sub>50</sub> was low compared to other tested synthetic pyrethroids. Whereas, deltamethrin was the least toxic insecticide with high LC<sub>50</sub>. The LC<sub>50</sub> value of the 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 the monitoring of resistance are recommended.

**Keywords:** Alphacypermethrin, Bioassay, Deltamethrine, Lambda-cyhalothrin, Resistance
Introduction

African bollworm (*Helicoverpa armigera*) (Lepidoptera: Noctuidae) is a polyphagous insect damaging many crops such as beans, chickpea, peas, sorghum, cotton, tomato, pepper, sunflower, safflower, flax, and Niger seed (Tsedeke, 1982; Waktole, 1996). The pests of cotton cause a 50-60% yield reduction in China (Xiao et al., 2002). In Ethiopia, damage due to bollworms (*H. armigera*, *Pectinophora gossypiella*, *Diparopsis watersi*, and *Earias* spp) inflicts 36-60% yield loss (Tsedeke, 1982; Waktole, 1996); 60% average yield losses (Geremew and Ermias, 2006), of that *H. armigera* is the most important pest.

For decades, cotton farmers have primarily been using chemical pesticides to control pests in Ethiopia. More than six sprays have been done per one cropping season for controlling a different cotton pest; four-round sprays are allotted for control of cotton bollworms and their effect on the environment has not been quantified (EIAR, 2016; Geremew, 2004). Controlling these pests with the available insecticides has become difficult (Geremew, 2004). Pests control method with insecticides from a single chemistry group is common practice in most cotton farms. In the past, development of resistance as in the case of lambda-cyhalothrin for *H. armigera* species at Dubti (Germew, 2004), dimethoate for aphid species at the Middle Awash (IAR, 1990), and carbamate group (carbosulfan, furathiocarb, and pirimicarb) for aphid species resistance to at Arbaminch, Dubti and Werer (Ermias, 2006). Efficacy reduction of endosulfan at Werer area (WARC, 1998) and commercial farms in Ethiopia (Geremew and Surachate, 2005). Currently, the commonly used synthetic pyrethroid insecticides, lambda-cyhalothrine, and deltamethrine have shown efficacy reduction in controlling African bollworm in the Middle Awash area (Personal communication). This might be due to the development of insecticide resistance by African bollworms (*H. armigera*). Because of these, the present study was undertaken to determine the susceptibility of field population *H. armigera* to commonly used synthetic pyrethroid insecticides under laboratory conditions.
Material and methods

The experiment was conducted at Werer Agricultural Research Center (WARC), Amibara District, Gebresu zone of Afar National Regional State during the 2017 cropping seasons 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 40° 09' 811" & N 09° 21' 243"), Gewane (567 m.a.s.l, E 040° 31' 23.0" & N 09° 59' 22.5")) and Upper Awash farms (Merti Jeju (1174 m.a.s.l, E 039° 43' 927" & N 08° 37' 111")). The larvae were reared using cotton squares until pupation. Pupae were collected every morning and transferred to plastic pots (size of 20cm height * 16cm width) embedded with soil. Pairs of male and female emerged adult moths were placed in adult rearing cages. A dissolved sugar was supplied in the rearing cage (size of 30cm height * 27cm width) for adults to feed. The adult diet was prepared from five-gram sugar and 200 ml water (Geremew and Surachate, 2003). 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 close or a detached cotton branch placed inside the cage. The eggs hatch 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 Petri dish 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 (1260m.a.s.l, E 036° 56" & N 06° 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 African bollworm collected from cotton farms which are heavily sprayed for many years.

Serial Dilution of Insecticide

The commercial insecticides alphacypermethrin (Fastac 100G/L), lambda-cyhalothrin (Karate 5%EC), and deltamethrin (Decis 2.5% EC) were serially diluted with tap water bioassayed against different strains of African bollworm. 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

The bioassay was conducted using the newly molted F$_1$ generation of 3$^{rd}$ instar larva by using the square dip and larval immersion bioassay procedure recommended by Geremew et al. (2004). The experiments were laid with a 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 3$^{rd}$ 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 Petri dish with insecticide-free cotton a square. The check treatment was treated with pre-water. The mortality rate was assessed 24 hours after placing the larvae by probing the larvae with a fine camel hairbrush. If the larvae respond for probing it was considered alive or dead otherwise.
Figure 2. Adult rearing and hatched larva feeding (A) Adult rearing cage with sugar immersed cotton wool (B) *H. armigera* adult on top and side of the cage (C) Collection of hatched larva from the adult cages (D) Feeding larva with cotton
Experiment 2. Square Dip Method

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

Statistical Analysis

Data from a bioassay were corrected for control mortality using Abbott’s formula (Abbott, 1925):

\[
\text{Percent mortality} = \frac{\text{dead larva}}{\text{total larva treated}} \times 100,
\]

\[
\text{Percent corrected mortality} = \left( \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in the control}} \right) \times 100
\]

The results obtained from the dose-mortality experiments were estimated by probit analysis (Finney, 1971) using the SAS software version 9.0 (SAS Institute, 1999). LC_{50} and LC_{90}, (Lethal Concentrations which kill 50% and 90% test larva), slope, and the 95% Confidence Limit (CL) were determined.

Resistance Ratios were calculated by dividing the LC_{50} values of each field population by the LC_{50} of Gofa-Sawla (susceptible population). The insecticide resistance level was determined using the methods described by Torres-Vila et al. (2002a, b): susceptible (RF=1), low level of resistance (RR=2-10), moderate resistance (RR=11-30), high resistance (RR=31-100), and very high resistance (RR>100).
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 (Table 1). Larval mortality of 100, 100, 100 and 90% mortality was obtained at field rate (5.0 x 10⁻⁴ 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 x 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 (Table 1). In squared dip method the field rate lambda-cyhalothrin (5.0 x 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 (Table 1). The four-times lower dose (1.25 x 10⁻⁴ g. a.i/ml) caused 100% mortality while the eight-times lower dose (2.5 x 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 x 10⁻⁴ g. a.i/ml) and 90% mortality of the Upper-Awash population to field rate dose of Gewane population (Table 1). 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.

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. (Table 2). In the 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 a 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 (Table 2). In the 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 indicate an adequate fit for the model with a normal distribution (Table 2). The Goffa-sawla population had a steep log₁₀ dose probit slope of the line 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 (Table 2).

This study revealed that variation in the level of susceptibility to lambda-cyhalothrin exists in *H. armigera* collected from different locations. Both bioassay methods showed that the tested population had a low level of resistance to lambda-cyhalothrin. The Gewane population has a higher resistance ratio compared to other tested populations. The LC<sub>50</sub> 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 Gewane *H. armigera* population compared to the susceptible Goffa-Sawla population. Both bioassays showed the presence of a low level of resistance to lambda-cyhalothrin in tested locations. Several studies have indicated the development of resistance in *H. armigera* for pyrethroids. A low level of resistance to lambda-cyhalothrin was reported by Karaagac *et al.* (2013) from Turkey and Avilla and González-Zamora (2010) in Spain. Other studies reported moderate to high-level resistance (Hussain *et al.* (2014) and high-level resistance (Duraimurugan & Regupathy, 2005) of *H. armigera* to pyrethroids. This finding contrast with Geremew *et al.* (2004) who found in larva immersion and squared dip methods.
Table 1. Percent of mortality of 3\textsuperscript{rd} instar *H. armigera* larvae in different concentration of lambda-cyhalothrin 5% EC 72 hours after treatment with larva immersion bioassay and 48 hours after treatment in squared dip bioassay (29$\pm$ 2\textdegree C & 48$\pm$4% RH) on Gofa Sawla, Upper Awash, Werer and Gewane populations (N=30).

| Concentration (µl/ml) | Gofa Sawla | Upper Awash | Werer | Gewane | Concentration (µl/ml) | Gofa Sawla | Upper Awash | Werer | Gewane |
|-----------------------|------------|-------------|-------|--------|-----------------------|------------|-------------|-------|--------|
| 2                     | 100        | 100         | 100   | 90.0   | 2                     | 100        | 100         | 96.7  | 93.3   |
| 1                     | 100        | 96.7        | 86.7  | 70.0   | 1                     | 100        | 90.0        | 83.3  | 73.3   |
| 0.5                   | 100        | 83.3        | 70.0  | 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       | 40.0        | 23.3  | 13.3   | 0.12                  | 73.3       | 36.7        | 23.3  | 16.7   |
| 0.0625                | 50.0       | 23.3        | 10.0  | 6.7    | 0.0625                | 56.7       | 20.0        | 10.0  | 3.3    |
| 0.03125               | 16.7       | 10.0        | 10.0  | 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 2.** Comparative toxicity of lambda-cyhalothrin 5% EC to *H. armigera* populations in larva immersion and squared dip study.

### Larva immersion

| Location       | N  | $\text{LC}_{50} \mu l/ml$ (lower-upper) | $\text{LC}_{90} \mu l/ml$ (lower-upper) | The fit of probit analysis | $\chi^2$ (df) | P     | RR   |
|----------------|----|----------------------------------------|----------------------------------------|----------------------------|----------------|-------|------|
| Gofa-Sawla     | 180| 0.074 (0.057-0.094)                     | 0.260 (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)                     | 2.870 (1.578-8.204)                   | 1.69±0.256                 | 0.622 (4)      | 0.9606| 6.73 |

### Squared dip

| Location       | N  | $\text{LC}_{50} \mu l/ml$ (lower-upper) | $\text{LC}_{90} \mu l/ml$ (lower-upper) | The fit of probit analysis | $\chi^2$ (df) | P     | RR   |
|----------------|----|----------------------------------------|----------------------------------------|----------------------------|----------------|-------|------|
| Gofa-Sawla     | 180| 0.060 (0.044-0.075)                     | 0.193 (0.144-0.306)                   | 2.52±0.384                 | 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 |

*N= total number of larva used for probit analysis, $\text{LC}_{50}$ = median lethal concentration, $\text{LC}_{90}$= the lethal concentration which killed 90% of the test *H. armigera* population, 95% CL= the lower and the higher confidence limits at which the $\text{LC}_{50}$ and $\text{LC}_{90}$ value can fall at 95% probability, SE= standard Error, $\chi^2$ =Chi-square, RR (Resistance Ratio) = $\text{LC}_{50}$ of the field population / $\text{LC}_{50}$ of Gofa-Sawla population, superscript denoted astric*=the collected *H. armigera* populations were not significantly different (P<0.05) among each other in their susceptibility to lambda-cyhalothrin insecticide.*
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 x 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 x 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 (Table 3). The field-collected *H. armigera* larva from Goffa-Sawla experienced 100% mortality at two times lower dose (1.5 x 10^{-4} g. a.i/ml) of deltamethrin which was higher than the field rate mortality of Werer, Upper-Awash, and Gewane populations, respectively (Tables 3). Werer population showed the 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 indicates an adequate fit for the model with the normal distribution (Table 4). 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 the Goffa-Sawla population with no overlapping 95% CL (Table 4). The LC_{50} of Gewane population both in larva immersion (0.900) and square dip method (1.171) was lower than the Werer population of the respective LC_{50} values of 1.257 and 1.435 (Table 4). In the 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 (Table 4). Similarly, the square dip method also showed that the Werer and Gewane populations are 9.25 and 7.55 more resistant to the susceptible Goffa-Sawla population (Table 4). These indicate that there is a high resistance development in *H. armigera* for deltamethrin at Werer and Gewane. According to the resistance grouping of Torres-Vila *et al.* (2002a, b) *H. armigera* in Middle Awash, Ethiopia showed a low level of resistance to deltamethrin. Deltamethrin has been used to control *H. armigera* and sucking pests in cotton for a long time. Recently, due to lack of the ultra-low volume (ULV) formulation, the emulsify concentrate (EC) formulation of deltamethrin has been applied like ULV by mixing with a small volume of water to save time and labor (Personal communication). Such misuse of an insecticide against *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 reported by Faheem *et al.* (2013) and Hussain *et al.*, (2014) in Pakistan
Table 3. Percent of mortality of 3rd instar *H. armigera* larvae in different concentrations of deltamethrin 2.5% EC 72 hours after treatment with larva immersion bioassay and 48 hours after treatment in squared dip bioassay (29 ± 2°C & 48 ± 4% RH) on Gofa-Sawla, Upper Awash, Werer and Gewane population (N= 30).

| Concentration (μl/ml) | Goфа Sawla | Upper Awash | Werer | Gewane | Concentration (μl/ml) | Goфа Sawla | Upper Awash | Werer | Gewane |
|-----------------------|------------|-------------|-------|--------|-----------------------|------------|-------------|-------|--------|
| 3                     | 100        | 93.3        | 80.0  | 86.7   | 100                   | 90.0       | 80.0        | 83.3  |
| 1.5                   | 100        | 76.7        | 56.7  | 66.7   | 100                   | 76.7       | 50.0        | 60.0  |
| 0.75                  | 93.3       | 50.0        | 30.0  | 43.3   | 96.7                  | 56.7       | 23.3        | 33.3  |
| 0.375                 | 76.7       | 26.7        | 13.3  | 20.0   | 76.7                  | 40.0       | 3.3         | 6.7   |
| 0.1875                | 53.3       | 13.3        | 3.3   | 6.7    | 56.7                  | 20.0       | 3.3         | 3.3   |
| 0.09375               | 30.0       | 3.3         | 0     | 0      | 26.7                  | 6.7        | 0           | 0     |
| 0.046875              | 13.3       | 0           | 0     | 0      | 6.7                   | 0          | 0           | 0     |
| Control               | 3.3        | 6.7         | 6.7   | 6.7    | Control               | 6.7        | 6.7         | 0     | 6.7    |
Table 4. Comparative toxicity of deltamethrin 2.5% EC to *H. armigera* populations in larva immersion and squared dip study.

### Larva immersion

| Location      | N   | LC$_{50}$ µl/ml (lower-upper) | LC$_{90}$ µl/ml (lower-upper) | The fit of probit analysis | RR |
|---------------|-----|-------------------------------|-------------------------------|---------------------------|----|
| Gofa-Sawla    | 150 | 0.143 (0.104- 0.246)          | 0.572 (0.430- 0.966)          | 2.59 ± 0.563              | 0.915 | _ |
| Upper Awash   | 150 | 0.690 (0.533 - 0.890)$^*$     | 2.690 (1.863 - 4.894)         | 2.17 ± 0.313              | 0.933 | 4.83 |
| Werer         | 150 | 1.257 (0.980 - 1.690)$^*$     | 4.814 (3.146 - 9.990)         | 2.20 ± 0.331              | 0.998 | 8.79 |
| Gewane        | 150 | 0.922 (0.717 -1.207)$^*$     | 3.633 (2.446 - 7.017)         | 2.15 ± 0.314              | 0.977 | 6.45 |

### Squared dip

| Location      | N   | LC$_{50}$ µl/ml (lower-upper) | LC$_{90}$ µl/ml (lower-upper) | The fit of probit analysis | RR |
|---------------|-----|-------------------------------|-------------------------------|---------------------------|----|
| Gofa-Sawla    | 150 | 0.155 (0.097 - 0.234)          | 0.515 (0.391 - 0.870)         | 2.74 ± 0.626              | 0.829 | _ |
| Upper Awash   | 150 | 0.563 (0.400 - 0.758)          | 3.111 (1.970 - 7.063)         | 1.727 ± 0.287             | 0.9913 | 3.63 |
| Werer         | 150 | 1.435 (1.137- 1.899)$^*$     | 4.712 (3.199- 9.103)          | 2.48 ± 0.371              | 0.639 | 9.25 |
| Gewane        | 150 | 1.171 (0.935-1.504)$^*$     | 3.751 (2.643- 6.632)          | 2.53 ± 0.359              | 0.834 | 7.55 |

$N=$ total number of larva used for probit analysis, $LC_{50}=$ median lethal concentration, $LC_{90}=$ the lethal concentration which killed 90% of the test *H. armigera* population, 95%CL= the lower and the higher confidence limits at which the LC$_{50}$ and LC$_{90}$ value can fall at 95% probability, SE= standard Error, $\chi^2=$Chi-square, RR (Resistance Ratio) = LC$_{50}$ of the field population / LC$_{50}$ of Goffa-Sawla population, superscript denoted astric*=the collected *H. armigera* populations were not significantly different (P<0.05) among each other in their susceptibility to deltamethrin insecticide.
Alphacypermethrin

*Helicoverpa armigera* larva from different locations had varied mortality when exposed to different concentrations of alphacypermethrin (Table 5). Alphacypermethrin caused 100% larva mortality at field rate (1.0 x 10^-3 g. a.i/ml) on Werer, Upper-Awash, and Gewane populations in both bioassay methods (Table 5). Except for Werer the two times-lower concentration alphacypermethrin resulted in 100% mortality. The four-time lower concentration (2.5 x 10^-4 a.i/ml) resulted in 100% mortality only for the Goffa-Sawla population (5). Subsequent dilutions of the insecticide resulted in lower percent mortality of larva to alphacypermethrin (Tables 5). 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 LC$_{50}$ 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 (Table 6). In this study, the probit analysis indicated showed resistance ratio in the range of 1.86-1.93 in the larval immersion method (Table 6) and 1.76-1.94 in the square dip method (Table 6). As a result, the level of resistance to alphacypermethrin was comparatively lower compared with other compounds of the pyrethroids group (lambda-cyhalothrin and deltamethrin) tested, which indicates 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 a 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 (BASF Chemical Company, 2014). Therefore, alphacypermethrin could be used for the resistance management program as one of the insecticides in the alternation scheme.
Table 5. Percent of mortality of 3\textsuperscript{rd} instar *H. armigera* larvae in different concentrations of alphacypermethrin 100G/L 72 hours after treatment with larva immersion bioassay and 48 hours in squared dip bioassay (29 ± 2\textdegree C 48±4% RH) on Gofa Sawla, Upper Awash, Werer and Gewane populations (N=30).

| 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    |
| 0.375                 | 100               | 90.0     | 83.3  | 83.3    | 0.375                 | 100               | 80.0    | 83.3  | 76.7    |
| 0.1875                | 90.0              | 73.3     | 73.3  | 63.3    | 0.1875                | 86.7              | 76.7    | 66.7  | 56.7    |
| 0.09375               | 76.7              | 60.0     | 53.3  | 46.7    | 0.09375               | 70.0              | 60.0    | 53.3  | 43.3    |
| 0.046875              | 56.7              | 43.3     | 40.0  | 30.0    | 0.046875              | 53.3              | 36.7    | 36.7  | 26.7    |
| 0.0234375             | 26.7              | 16.7     | 16.7  | 10.0    | 0.0234375             | 23.3              | 16.7    | 16.7  | 10.0    |
| Control               | 0                 | 0        | 6.7   | 6.7     | Control               | 10.0              | 0       | 3.3   | 6.7     |
Table 6. Comparative toxicity of alphacypermethrin 100G/L to *H. armigera* populations in larva immersion and squared dip study.

### Larva immersion

| Location   | N  | $L_{C_{50}}$ μl/ml | 95% CL (lower-upper) | $L_{C_{90}}$ μl/ml | 95% CL (lower-upper) | The fit of probit analysis | RR |
|------------|----|--------------------|----------------------|--------------------|----------------------|---------------------------|----|
| Gofa-Sawla | 180| 0.043              | (0.031 - 0.055)      | 0.157              | (0.114- 0.265)       | 2.28 ± 0.366              | 0.992 (3) 0.803     |
| Upper Awash| 180| 0.070              | (0.051- 0.091) *     | 0.335              | (0.232 - 0.591)      | 1.88 ± 0.261              | 2.039 (4) 0.729      | 1.62 |
| Werer      | 180| 0.080              | (0.057 - 0.107)*     | 0.471              | (0.310 - 0.922)      | 1.66 ± 0.236              | 0.978 (4) 0.913      | 1.86 |
| Gewane     | 180| 0.083              | (0.078 - 0.133)*     | 0.459              | (0.318 - 0.806)      | 1.97 ± 0.256              | 2.62 (4) 0.620       | 1.93 |

*Squared dip*

| Location   | N  | $L_{C_{50}}$ μl/ml | 95% CL (lower-upper) | $L_{C_{90}}$ μl/ml | 95% CL (lower-upper) | The fit of probit analysis | RR |
|------------|----|--------------------|----------------------|--------------------|----------------------|---------------------------|----|
| Gofa-Sawla | 180| 0.049              | (0.036 - 0.063)      | 0.186              | (0.134 - 0.320)      | 2.21 ± 0.347              | 1.666 (3) 0.664     |
| Upper Awash| 180| 0.079              | (0.055 -0.107) *     | 0.528              | (0.338- 1.096)       | 1.55± 0.228               | 1.648 (4) 0.8001    | 1.62 |
| Werer      | 180| 0.086              | (0.062 -0.115)*      | 0.516              | (0.336-1.029)        | 1.65 ± 0.234              | 0.977 (4) 0.9133    | 1.76 |
| Gewane     | 180| 0.095              | (0.100- 0.185)*      | 0.852              | (0.527- 1.871)       | 1.61± 0.226               | 0.743 (4) 0.9459    | 1.94 |

N= total number of larva used for probit analysis, $L_{C_{50}}$ = median lethal concentration, $L_{C_{90}}$= the lethal concentration which killed 90% of the test *H. armigera* population, 95%CL= the lower and the higher confidence limits at which the $L_{C_{50}}$ and $L_{C_{90}}$ value can fall at 95% probability, SE= standard Error, $\chi^2$=Chi-square, RR (Resistance Ratio) = $L_{C_{50}}$ of the field population/$L_{C_{50}}$ of Goffa-Sawla population, superscript denoted asteric* = the collected *H. armigera* populations were not significantly different (P <0.05) among each other in their susceptibility to alphacypermethrin insecticide.
Conclusions

The current study confirmed a reduction in efficacy and the development of a low level of resistance in the *H. armigera* population to lambda-cyhalothrin at Werer and Gewane tested locations. The efficacy of deltamethrin was moderately reduced and had a 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 a different mode of action. These insecticides were used for a long time to control cotton bollworm and sucking pest. Alphacypermethrin insecticide could be used for the resistance management program as one of the insecticides in the alternation scheme. The study included a limited number of insecticides out of the commercially registered cotton *H. armigera* control in Ethiopia. Future studies are needed to monitor the level of insecticide resistance.

Acknowledgments

The authors acknowledge the Ethiopian Institute of Agricultural Research for providing research facilities and financial supports for the study. The authors would also highly thankful to Mr. Ermias Shonga and the Entomology section staff of Werer Agricultural Research Center, especially Mr. Workishet Taye, is highly appreciated for their unreserved technical, material, and moral contributions.

Conflict of interest statement

The authors declare no conflict of interest.

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