Ethiop. J. Agric. Sci. 32(4) 56-72 (2022)

# Susceptibility of African Bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) to Different Commercial Pyrethroid Insecticides on Cotton

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## Abstract

*The African bollworm, Helicoverpa armigera (Hubner) is an indigenous species in Africa and has been reported in the destruction of several crops in general and cotton in particular in Ethiopia. Currently, the management of H. armigera is mainly focused on the use of synthetic pyrethroids, which have already led to resistance selection pressure in several field populations of this insect. To know the status of different pyrethroid insecticides monitoring was exercised using populations of H. armigera collected from four different areas of the central and southern rift valley. This study aimed to screen the susceptibility pattern of four field populations of H. armigera to Aplhacypermethrin, lambda-cyhalothrin, and deltamethrin insecticides using third-instar larva immersion and square dip methods. The selected insecticides had been examined in seven concentration levels. For each concentration, thirty-third instar larvae were treated in three replications. An equal number of larvae were treated with tap water as control. The result indicated that Aplhacypermethrin displayed high acute toxicity to H. armigera populations from Werer, Gewane, and Merti Jeju whereas lambda-cyhalothrin and deltamethrin exhibited relatively low toxicity to the populations from Gewane and Werer. The $LC_{50}$ value of the Goffa-Sawla populace became notably exclusive to most of the populations from Werer, Merti Jeju, and Gewane in each bioassay method. The pairwise correlation coefficients of $LC_{50}$ values indicated that the commercial insecticides were not significantly correlated. Therefore, it is concluded that the recorded high levels of deltamethrin resistance in H. armigera from Gewane and Werer may lead to the development of resistance to deltamethrin. Further investigation on the tracking of resistance and its management is needed.*

**Keywords:** Cotton, Helicoverpa armigera, Insecticide, Pyrethroid resistance

## Introduction

Cotton, *Gossypium hirsutum* L. (Malvaceae), is one of the most valuable cash crops in the world grown for its fiber and the oil extracted from the seed (Malinga and Laing, 2022). Cotton production is a major economic component in sub-Saharan Africa, and is a significant contributor to economic growth (Amanet *et al*., 2019). In Ethiopia, cotton is a significant crop produced by both commercial and small-scale farmers in the Awash Valley, Southern Rift Valley, Gambella, Humera, and Metema. But, Ethiopia shares only 5% of the total cotton produced

in Africa (Melesse *et al*., 2019). Its production can be challenging, as the crop is prone to attack by a wide variety of pests, especially African bollworm and sucking pests like aphids and whiteflies, among other factors.

The African bollworm, *Helicoverpa armigera* Hübner is an indigenous species considered to be a major and key insect pest of cotton in Africa (Cherry *et al*., 2003), and ranks as the most important lepidopteran pest on cotton in Ethiopia (Geremew and Ermias, 2006). Unlike most other arthropod pests, *H. armigera* is a polyphagous pest that infests more than 200 crop species worldwide, including cotton, pepper, corn, tomato, lucerne, soybean, sorghum, and tobacco (Cunningham and Zalucki, 2014). In Ethiopia, this pest feeds on a variety of plants, including beans, chickpeas, peas, sorghum, cotton, tomato, pepper, sunflower, safflower, flax, and Niger seed (Tsedeke, 1982; Waktole, 1996). The larva feeds on cotton young leaves, squares, flower buds, flowers, and bolls. Four heliothine species (*H. armigera*, *Pectinophora gossypiella*, *Diparopsis watersi*, and *Earias* spp.) are reported as being of economic importance, but *H. armigera* is the only species of major economic importance that causes 36-60% (Tsedeke, 1982; Waktole, 1996); and about 60% (Geremew and Ermias, 2006) yield losses.

Worldwide, up to 60% of all commercialized insecticides are used in cotton (Yada and Dutta, 2019). Chemical pesticides such as pyrethroids, carbamates, and organophosphates are applied to control Helicoverpa pests (Martin *et al*., 2000). Likewise, cotton producers in Ethiopia have been using chemical insecticides to manipulate pests for decades. To control these cotton pests more than four round sprays were allotted (EIAR, 2016). Geremew (2004) reported that with the use of such monologues chemicals, controlling these pests has proven tough. Excessive and continuous applications of pyrethroid insecticides for *H. armigera* control initiated in the mid-1980s in most countries have led to resistance selection pressure in several field populations of this insect (Brévault *et al*., 2008; Yadouleton *et al*., 2009). With this development of insecticide resistance, the control of *H. armigera* has become critical in many regions worldwide (Chaturvedi, 2007). Recent studies have reported the increased resistance of *H. armigera* to pyrethroids in Pakistan (Ahmad *et al*., 1995), South India (Ramasubramanian and Regupathy, 2004), Spain (Torres *et al*., 2002), and West Africa (Brun *et al*., 2010).

In Ethiopia, few studies reported resistance of *H. armigera* to lambda-cyhalothrin in the Dubti area (Germew, 2004) and endosulfan in the Werer area (WARC, 1998; Geremew and Surachate, 2005). In contrast, Lambda-cyhalothrin and Deltamethrin, the two commonly used synthetic pyrethroid pesticides, have shown efficacy in suppressing African bollworms in the Middle Awash area (Personal communication). Unfortunately, information about *H. armigera* resistance to different insecticides in different locations of Awash valley representing major cotton cropping systems is limited. Even the technical literature which has reported on control failures of *H. armigera* was too preliminary. Thus it was

essential to understand the status of resistance to selected pyrethroid insecticides which are still in use in different locations to know their contribution to controlling failures. Therefore, the study was conducted to determine the sensitivity of different field-collected *H. armigera* populations to commonly used synthetic pyrethroid pesticides in laboratory settings.

# Material and Methods

The experiment was conducted at Werer Agricultural Research Center (WARC) which is located in Amibara District, Gebresu zone of Afar National Regional State in Ethiopia during the 2017 cotton production season under laboratory conditions. The test insect, *H. armigera*, larvae were collected from different locations of Awash valley and Gofa-Sawla (Fig. 1), representing different cropping patterns and agro-ecosystems which are dominating with host crops of *H. armigera*.

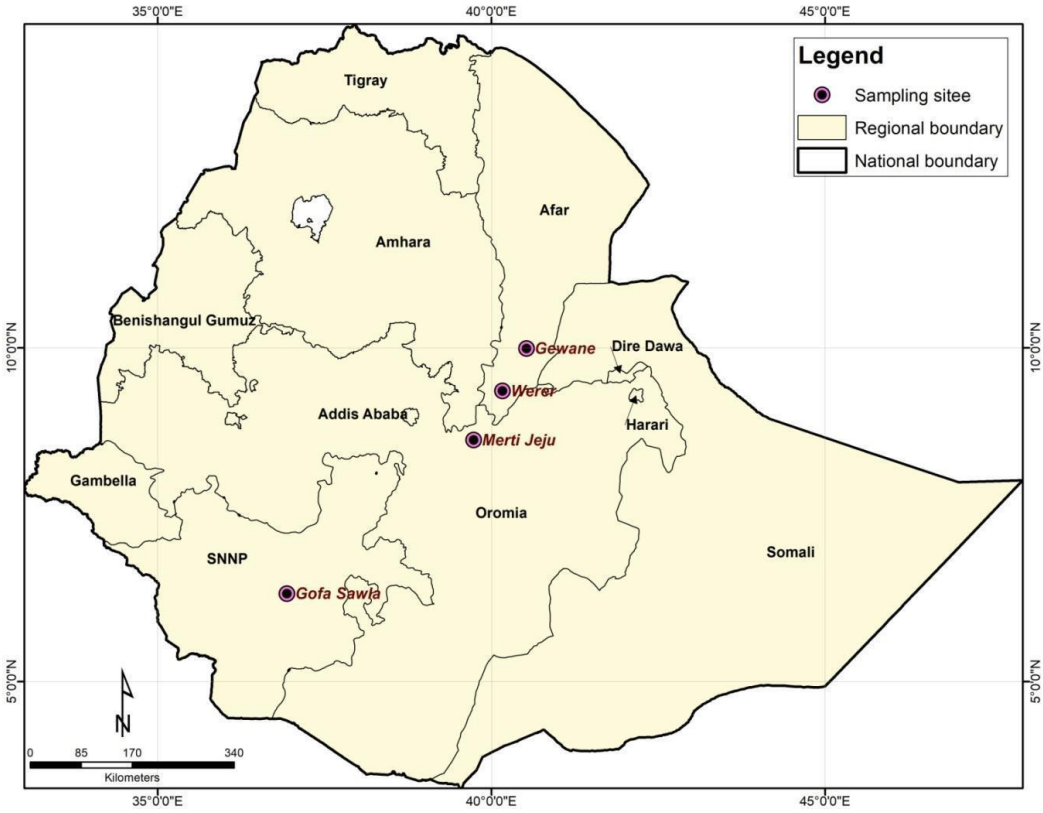


**Figure 1**. Map of Ethiopia showing selected sampling location of *H. armigera* larvae collection.

## Collection and rearing of *H. armigera*

Field populations of *Helicoverpa armigera* were collected from unsprayed cotton farms in Middle Awash (Werer &, Gewane) and Upper Awash farms (Merti Jeju) with substantial pyrethroid pesticide use history which led to suspect the

development of pyrethroid resistance. Besides, Larvae of *H. armigera* were also collected from chickpea small-scale farms at Gofa-Sawla, Southern Ethiopia, with no pesticide use history for the last six years, for the sake of comparison with those populations collected from cotton farms with a history of heavy pesticide use for several years. Detailed descriptions of four sample larvae collection areas are stated in Table 1.

Larvae collected in the field were introduced into plastic vials (4cm diameter; 5cm height) with a cover punched with holes to allow ventilation for the breathing of larvae. Each vial was filled with host plant leaves for feeding larvae throughout their transportation from the field to the laboratory. The field-collected larvae of *H. armigera* were brought to the entomology laboratory at Werer Agricultural Research Center and reared on natural hosts of cotton fruiting bodies till pupation at 29 $\pm$ 2 $^0$C, with a relative humidity of 48 $\pm$4% and 12:12 h Light: Dark photoperiod. The culture was maintained location-wise separately. Then pupae were collected each morning and transferred to plastic pots (15 pupae/pots) with a size of 20cm height * 16cm width embedded with soil and placed in adult cages. Pairs of emerged male and female adult moths (1:1) were transferred into separate adult rearing cages (30cm height *27cm width). The adults were provided with a sugar solution and allowed for mating. The adult diet was prepared from five-gram sugar and two-hundred cubic centimeters of water (Geremew and Surachate, 2003). The adults were allowed to lay eggs on cheese close and a detached cotton branch was placed within the cage. The eggs hatch after 3 or four days. After hatching first instar larvae were reared in groups in large Petri plates provided with a natural diet i.e. cotton leaves. While larvae reached second instar status they were reared individually in a large petri dish with its natural diet of cotton leaves. Every morning the cotton leaves were changed and the petri-dish was cleaned throughout the rearing period. A pictorial representation of the rearing process is illustrated in Figure 2. The experiment was conducted using third-instar larvae.

Table 1. Geo-reference data of surveyed localities for *H. armigera* sampling

| Collection Site | Agro Ecosystem | Host Plant | Altitude (m.a.s.l) | Latitude (E) | Longitude (N) |
|---|---|---|---|---|---|
| Werer | Middle Awash | Cotton | 734.4 | 40$^0$ 09' 811" | 09$^0$ 21' 243" |
| Gewane | Middle Awash | Cotton | 567 | 040$^0$ 31' 23" | 09$^0$ 59' 22.5" |
| Merti Jeju | Upper Awash | Cotton | 1174 | 039$^0$ 43' 93" | 08$^0$ 37' 111" |
| Gofa-Sawla | Southern Ethiopia | Chickpea | 1260 | 036$^0$ 56' | 06$^0$ 19' |


**Figure 2.** Adult rearing and hatched larva feeding process (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

## The serial concentration of test insecticides

All test insecticides namely alphacypermethrin **(**Fastac 100G/L**),** lambda-cyhalothrin **(**Karate 5%EC**),** and deltamethrin (Decis 2.5% EC) were obtained as commercial formulations available on the market in 2017. The required concentrations of test insecticides lambda-cyhalothrin (2, 1, 0.5, 0.25, 0.12, 0.0625 µL/mL and control); deltamethrin (3, 1.5, 0.75, 0.375, 0.1875, 0.046875 µL/mL and control) and alphacypermethrin (1.5, 0.75, 0.375, 0.1875, 0.046875, 0.0234375 µL/mL and control) were prepared from the formulated products by serially diluted the required quantities in tap water after accurate weightiness of insecticides

## Laboratory Bioassay Method for Susceptibility Study

Bioassays were conducted using the fresh molted $F_1$ generation of third instar larvae of *H. armigera* by the victimization of the cotton square dip and larval

immersion bioassay procedure suggested by Geremew *et al*. (2004). The experiments were arranged in a completely randomized design (CRD) with 3 replications. For every replicate of a serial concentration and control, 10 larvae were used.

### Experiment I. Larval Immersion Experiment

Doses were applied in order of increasing concentration, and the same syringe was used to apply all doses of the same insecticide. For every treatment, 10 third instar larvae per replication were used. The larvae were dipped into individual dilution for 10 seconds and placed on tissue soft trays for gripping excessive liquid from the body. Larvae were transferred into a glass petri dish with an insecticide-free cotton square. The control larvae were treated with tap water. Observation of mortality started 24 h after treatment.

### Experiment II. Square Dip Experiment

Medium-size cotton squares that weigh 700-1000 milligrams were collected from the unsprayed cotton field and dipped into the individual concentration of insecticide for 10 seconds and transferred onto a paper soft receptacle for air-drying. After 30 min of drying, these cotton squares were placed into glass petri dishes, and 10-third instar larvae used per replication were used for feeding on the treated. The control larvae were allowed to feed on tap water-dipped and dried cotton squares. Observation of mortality started 24 h after treatment.

### Data Collected

The dose-mortality larvae were recorded after 24, 48, and 72 hours of treatment for larval immersion bioassay whereas after 24, 36, and 48 hours of treatment for square dip bioassay. Larvae were thought to be dead if they are ineffectual to maneuver once probed with a blunt probe or brush. Results were expressed as percentage mortality. The resistance ratio (RR) was determined as the ratio of the lethal dose for 50% ($LD_{50}$) of each field population to the $LD_{50}$ of the Gofa-Sawla susceptible population (Torres-Vila *et al.*, 2002a, b). The calculated RR was used to categorize the tested population into different pesticide resistance groups in which RR=1 was considered as susceptible, 2-10 as low level of resistance, 11-30 as moderate resistance, 31-100 as high resistance, and above 100 as extreme resistance. The daily minimum and maximum temperature and RH of the laboratory during the study period were recorded (Table 2).

Table 2. Mean monthly temperature and relative humidity of the laboratory during the study period at Werer (2017)

| Month | Temperature ($^0$C) | | Relative humidity (%) |
|---|---|---|---|
| | Minimum | Maximum | |
| Month 1 | 27.6 | 33.9 | 57.0 |
| Month 2 | 27.3 | 31.2 | 53.5 |
| Month 3 | 26.6 | 29.5 | 45.2 |
| Month 4 | 26.4 | 29.5 | 40.7 |
| Month 5 | 25.4 | 29.0 | 40.1 |
| Mean | 26.6 | 30.5 | 48.1 |

### Statistical Analysis

Mortality in the control was always <10%. Therefore, data from all bioassays were corrected for control mortality using Abbott's formula (Abbott, 1925):

Percent corrected mortality=(% mortality in treatment-% morality in control 100-% mortality in control)*100

The statistical analyses of data obtained from the dose-mortality experiments were performed by probit analysis (Finney, 1971) with SAS software version 9 (SAS Institute, 1999). The mortality data were arcsine transformed prior to analysis to stabilize variances. $LC_{50}$ and $LC_{90}$ (Lethal Concentrations that kill 50 and 90% larva, respectively), slope, and 95% Confidence Limit (CL) were also determined by probit analysis. The least significant difference (LSD) test was used to separate means at a 5% probability level. $LD_{50s}$ and $LD_{90s}$ of two different populations were considered significantly different when their 95% confidence intervals did not overlap. Cross-resistance among the insecticides was determined through pair-wise correlation coefficients of log $LC_{50}$ values of the common populations for each insecticide

## Results and Discussion

The current study assessed the susceptibility of three pyrethroids insecticides that were available on the market, and farmers have been applying them indiscriminately to manage an *H. armigera* pest. The results of this study showed the response of *H. armigera* pest to these insecticides across the sampled locations

### Susceptibility of Helicoverpa armigera to Lambda-cyhalothrin

In larva immersion techniques, at recommended rate larval mortality was 90-100% mortality while in square dip techniques was 93.3-100% mortality in four *H. armigera* field populations (Table 3). For lambda-cyhalothrin insecticides, the four-times decrease dose ($1.25 \times 10^{-4}$ g. a.i/mL) precipitated 100% mortality (Table 3).

The Goffa-sawla populace had a relatively low value of $LC_{50}$ and $LC_{90}$ in both methods. Whereas, high $LC_{50}$ (0.498µL/mL) and $LC_{90}$ (2.870 µL/L) values had been acquired for the Gewane populace with showed low levels of resistance (RR = 6.73 -7.45-fold) difference compared to Goffa-sawla populations (Table 4). The Goffa Sawla population was significantly more sensitive to lambda-cyhalothrin than the Werer, Merti Jeju, and Gewane populations without any overlap of 95% CL (Table 4).

A determined resistance ratio of *H armigera* has shown that the Gewane population is resistant to the insecticide lambda-cyhalothrin to a low degree, compared to other populations studied. This indicates that lambda-cyhalothrin insecticide is less efficient in controlling the pest in Gewane regions. This might be the insecticide that was used for a long time in Middle Awash for controlling chewing and sucking pests. This suggests an enormous amount of insecticides is required for *H. armigera* pest management. Different scientists found the susceptibility pattern to different insecticide groups. Honnkaerappa and Udikeri (2022) reported the field-collected population *H. armigera* peak 14.18-fold resistance to lambda-cyhalothrin. Likewise, many studies reported *H armigera* has developed a low level of resistance (Avilla and González-Zamora, 2010; Karaagac *et al*., 2013) and high-level resistance (Duraimurugan & Regupathy, 2005; Hussain *et al*., 2014) to lambda-cyhalothrin. Geremew *et al*. (2004) also discovered similar results of high-level resistance in larva immersion and squared dip procedures in populations from these areas to Endosulfan insecticide. Additionally, a low level of resistance was reported for FAW (Gichere *et al.*, 2022); moderate resistance of *Spodoptera littura* (Sahd *et al*., 2012) to lambda-cyhalothrin. The increase in the use of pyrethroids in Hunan Province results in rising resistance to pyrethroids is consistent (Huang *et al*., 2006; Xie *et al.*, 2010).

Table 3. Percentage mortality of different *H. armigera* larvae populations (N=30) in different concentrations of lambda-cyhalothrin

| **Larva immersion** | | | | | **Squared dip** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (µL/mL) | Percent mortality | | | | Concentration (µL/mL) | Percent mortality | | | |
| | Gofa Sawla | Merti Jeju | Werer | Gewane | | Gofa Sawla | Merti Jeju | 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 4. Comparative toxicity of lambda-cyhalothrin 5% EC to different *H. armigera* populations

**Larva immersion**

| Location | N | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slope ± SE | $\chi^2$ (df) | P | |
| Gofa-Sawla | 180 | 0.074 | (0.057 -0.094) | 0.260 | (0.192- 0.415) | 2.36± 0.333 | 2.778 (4) | 0.5957 | _ |
| Merti Jeju | 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 | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slope ± SE | $\chi^2$ (df) | P | |
| Gofa-Sawla | 180 | 0.060 | (0.044 -0.075) | 0.193 | (0.144 - 0.306) | 2.52±0.384 | 0.976 (4) | 0.9134 | _ |
| Merti Jeju | 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 larvae 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 lambda-cyhalothrin insecticide.*

## Susceptibility of *H. armiger* to Deltamethrin

*Helicoverpa armigera* populations exposed to different concentrations of deltamethrin 2.5% EC experienced varying levels of mortality at different locations. Larvae from Goffa-Sawla exhibited 100% mortality at two times lower doses (1.5 x 10-4 g. a.i/mL) (Table 5). $LC_{50}$ values suggest that Werer, Merti Jeju, and Gewane populations have been no longer extensively distinctive amongst every difference ($P< 0.05$) from the Goffa-Sawla population with no overlapping 95% CL (Table 5).

The $LC_{50}$ values of deltamethrin were 0.690–1.257 µL/mL and 0.563-1.435 µL/mL for populations collected in the Werer, Merti Jeju, and Gewane with resistance ratio (RR= 3.63-9.25-fold) when compared to Goffa-sawla populations (Table 6). In general, the resistance of *H. armigera* to deltamethrin was lowest in the population collected from Merti Jeju, while the highest resistance was obtained in a population collected from Gewane (Table 6).

The present study suggests that deltamethrin can not provide effective control for this pest in all tested locations. This implies that insecticide control is in the field in question. Deltamethrin is registered in Ethiopia for controlling *H. armigera* and other pests and has long been used to control *H. armigera* and sucking pests either in single or combination in many cotton farms. But, because of the misapplication of a pesticide against *H. armigera* may result in the selection of resistant variants of the pest population. Many researchers reported the resistance level of this pest in many countries. Honnkaerappa and Udikeri (2022) reported about 24.95-fold resistance *H. armigera* to deltamethrin. Likewise, Tossou *et al.* (2019 found high resistance levels of *H. armigera* to deltamethrin on cowpea tomato and cotton farms. Similarly, Faheem, *et al*. (2013) and Hussain *et al*. (2014) stated low-to-high-level for variant *H. armigera* populations; the lowest level of resistance for the population *Spodoptera litura* to deltamethrin insecticide (Tong *et al.,* 2013). Sene *et al* (2020) mentioned deltamethrin had low and moderate susceptibility to the *H. armigera* population in tomato and cotton farms respectively.

Table 5. Percentage mortality of different *H. armigera* larvae populations (N=30) in different concentrations of deltamethrin 2.5% EC

| Larva immersion | | | | | Squared dip | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (µL/mL) | Percent mortality | | | | Concentration (µL/mL) | Percent mortality | | | |
| | Gofa Sawla | Merti Jeju | Werer | Gewane | | Gofa Sawla | Merti Jeju | Werer | Gewane |
| 3 | 100 | 93.3 | 80.0 | 86.7 | 3 | 100 | 90.0 | 80.0 | 83.3 |
| 1.5 | 100 | 76.7 | 56.7 | 66.7 | 1.5 | 100 | 76.7 | 50.0 | 60,0 |
| 0.75 | 93.3 | 50.0 | 30.0 | 43.3 | 0.75 | 96.7 | 56.7 | 23.3 | 33.3 |
| 0.375 | 76.7 | 26.7 | 13.3 | 20.0 | 0.375 | 76.7 | 40.0 | 3.3 | 6.7 |
| 0.1875 | 53.3 | 13.3 | 3.3 | 6.7 | 0.1875 | 56.7 | 20.0 | 3.3 | 3.3 |
| 0.09375 | 30.0 | 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 6. Comparative toxicity of deltamethrin 2.5% EC to different *H. armigera* populations

**Larva immersion**

| Location | N | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **Slope ± SE** | **$\chi^2$ (df)** | **P** | |
| Gofa-Sawla | 150 | 0.143 | (0.104- 0.246) | 0.572 | (0.430- 0.966) | 2.59 ± 0.563 | 0.517 (3) | 0.915 | _ |
| Merti Jeju | 150 | 0.690 | (0.533 - 0.890)* | 2.690 | (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**

| Location | N | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slope ± SE | $\chi^2$ (df) | P | |
| Gofa-Sawla | 150 | 0.155 | (0.097 - 0.234) | 0.515 | (0.391 - 0.870) | 2.74 ± 0.626 | 0.884 (3) | 0.829 | _ |
| Merti Jeju | 150 | 0.563 | (0.400 - 0.758) | 3.111 | (1.970 - 7.063) | 1.727 ± 0.287 | 0.104 (3) | 0.9913 | 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 |

*N= total number of larvae 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*

## Susceptibility of *H. armigera* to Alphacypermethrin

Alphacypermethrin induced 100% *H. armigera* larvae mortality at the indicated rate (1.0 x $10^{-3}$g.i/mL) on Werer, Merti Jeju, and Gewane populations. Subsequent insecticide dilutions resulted in reduced percent mortality of larvae (Table 7). Goffa-Sawla population was significantly different (P <0.05) from Werer, Merti Jeju, and Gewane populations with non-overlapping 95% CL (Table 8). There was only a 1.6-fold difference in tested populations on the basis of $LC_{50}$. These showed that the pest has responded to narrow variability in different geographical locations; there have been no control failures, given the reduction in the use of insecticides in sampled areas. Alphacypermethrin is a new insecticide that was registered for controlling this pest (BASF Chemical Company, 2014). A similar

result reported by Ishtiaq *et al.* (2012) mentioned that the reduced use of insecticides in controlling pests results in a low level of resistance. The result contradicts, Ahmad *et al.*(1998) reported there was a chronological increase of *H. armigera* insecticide resistance to alpha-cypermethrin due to subjection to continuous high selection pressure.

Table 7. Percentage mortality of different *H. armigera* larvae populations (N=30) in different concentrations of alphacypermethrin 100G/L.

| **Larva immersion** | | | | | **Squared dip** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration (µL/mL) | Percent mortality | | | | Concentration (µL/mL) | Percent mortality | | | |
| | Gofa Sawla | Merti Jeju | Werer | Gewane | | Gofa Sawla | Merti Jeju | 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 8. Comparative toxicity of alphacypermethrin 100G/L to different *H. armigera* populations

| Location | N | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Slope ± SE | $\chi^2$ (df) | P | |
| **Larva immersion** | | | | | | | | | |
| Gofa-Sawla | 180 | 0.043 | (0.031 - 0.055) | 0.157 | (0.114- 0.265) | 2.28 ± 0.366 | 0.992 (3) | 0.803 | _ |
| Merti Jeju | 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 | $LC_{50}$ µL/mL | 95% CL (lower-upper) | $LC_{90}$ µL/mL | 95%CL (lower-upper) | The fit of probit analysis | | | RR |
| | | | | | | Slope ± SE | $\chi^2$ (df) | P | |
| Gofa-Sawla | 180 | 0.049 | (0.036 - 0.063) | 0.186 | (0.134 - 0.320) | 2.21 ± 0.347 | 1.666 (3) | 0.664 | _ |
| Merti Jeju | 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 larvae 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 alphacypermethrin insecticide*

### Cross Resistance Pattern

Any insecticide's effectiveness could be hampered by the possible issue of cross-resistance. According to the current study, the three pyrethroid insecticides log $LC_{50}$ were compared pair-wise correlation across common populations, and the results revealed non-significant ($P < 0.05$) positive correlations present among the insecticides (Table 9). Thus each insecticide could have a cross-resistance to each other. This resistance development might be due to the over-dependence of farmers on a similar group of insecticides in the study areas. Scholars at different times reported that insecticides could have a cross-resistance to chemicals belonging to the same group and from different groups (Honnkaerappa and Udikeri, 2018, 2022; Ishtiaq *et al*., 2012; Ramasubramanian and Regupathy, 2004; Saddiq, *et al*., 2015; Sene *et al*., 2020; Tong *et al*., 2013).

Table 9. Pairwise correlation coefficient comparisons of log $LC_{50}$ values *Helicoverpa armigera* for different insecticides

| Larva immersion | | | |
|---|---|---|---|
| Insecticides* | Lambda-cyhalothrin | Deltamethrin | Alphacypermethrin |
| Lambda-cyhalothrin | 1.00 | | |
| Deltamethrin | $0.63^{ns}$ | 1.00 | |
| Alphacypermethrin | $0.80^{ns}$ | $0.93^{ns}$ | 1.00 |
| Square dip method | | | |
| Lambda-cyhalothrin | 1.00 | | |
| Deltamethrin | $0.80^{ns}$ | 1.00 | |
| Alphacypermethrin | $0.90^{ns}$ | $0.86^{ns}$ | 1.00 |

Superscript ns correlation is non-significant at the 0.05 level

## Conclusion

Results from this study revealed high levels of pyrethroid resistance in several populations of *H. armigera* in Werer and Gewane locations. An increasing resistance pattern was observed as we moved from the Gofa-Sawla to the Gewane areas. A resistance contrast was also recorded between populations of *H. armigera* from cotton and chickpea fields. The current bioassay test showed low efficacy and the development of a low level of resistance in the *H. armigera* population to lambda-cyhalothrin in Werer and Gewane locations. The efficacy of deltamethrin also turned into a reasonable decrease with very high percentages of survival at dose-response and resistance levels in the Gewane and Werer *H. armigera* populations in comparison to lambda-cyhalothrin. This indicated that *H. armigera* may have developed resistance to deltamethrin and is exceedingly unlikely to be successful in eliminating this pest. Alphacypermethrin insecticide may be used for the resistance management program but careful selection of insecticide is crucial due to the presence of cross-resistance. For the development of more tailored, cost-effective, and sustainable IPM strategies against this highly polyphagous pest, further investigation is needed to assess the insecticide resistance profiles of *H. armigera* to other insecticides.

# Acknowledgments

The authors acknowledge the Ethiopian Institute of Agricultural Research for providing research facilities and financial support for the study.

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