

Evaluating Four Bioassay Techniques for Insecticide Resistance Monitoring in *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT

Laboratory studies were performed on *Helicoverpa armigera* strain collected from Werer Agricultural Research Center cotton fields to investigate resistance to the field rates of profenofos, endosulfan and lambdacyhalothrin using adult vial, topical application, square dip and larval immersion techniques. It was found that LD₅₀ values of endosulfan, profenofos and lambdacyhalothrin in the third instar topical bioassay were 29.84, 4.068 and 0.18 µg/g body weight, respectively. In the square dip test profenofos (Calofos) resulted in 100% kill at two-times lower dose (1.25×10^{-3} g a.i/ml) and 86.67% larval deaths were observed at four-times lower dose. Endosulfan (Ethiosulfan) gave 99.33% only at the field rate (5.25×10^{-3} g a.i/ml). Lambdacyhalothrin (Karate) was observed to yield 99.33% larval death at eight-times lower dose (6.25×10^{-5} g a.i/ml) than the field rate. In larval immersion experiment lambdacyhalothrin and profenofos gave 98.33 and 99.33% kills at eight-time lower dose, respectively, while endosulfan expressed 96.67% efficacy only at the field rate. LC₅₀, and LC₉₀ in adult vial test for endosulfan were found to be 1.333 and 3.099 µg/ml, respectively. However, the observed high mortality (> 25%) in control treatments warranted reevaluation of adult vial technique. Square dip technique was found easy and economical.

Key words : *Helicoverpa armigera*, insecticide resistance, monitoring

INTRODUCTION

The American bollworm, *Helicoverpa armigera* is a serious pest of cotton, *Gossypium spp.* in Ethiopia and is known to attack many field crops including cotton, sorghum, oilseeds, coarse grains, maize, groundnut, tomato, and vegetables in many parts of the world (Mabbett, 1978; Fit, 1989; Deepak and Armes, 1996).

The polyphagous nature of the insect and its serious attack to almost all host plant species

grown for human needs brought it to the number one pest status, and its control is achieved by insecticide application only. Cotton being one of the victims of *H. armigera* attacks bombarded with insecticides more than any crop grown in the world. According to the reports of International Cotton Advisory Committee (ICAC) (1995), cotton is one of the major crops of the world, which consumes up to 20% of global insecticide use. In India alone cotton consumes 50% of the insecticides used annually in the country, even though it

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occupies only 5% of the total cultivated area (Raheja, 1995). In Ethiopia cotton consumes about 65% of the total pesticide purchase (Abate, 1997), while its area coverage is less than 5 percent of the total arable land.

The use of insecticides to control cotton insect pests has been a common practice since the onset of commercial cotton production in Ethiopia. The use of insecticides such as calcium arsenate has started at late 1930's and use of DDT for cotton insect pest control started in the late 1940's (Read, 1950), which continued to early 1970's until endosulfan replaced it. Since then the control of cotton bollworms and chewing-biting insect pests by and large has depended on endosulfan application. The use of a single insecticide for such a long time is anticipated to develop resistance in those areas where the product has been in use. Very recently, reports have been made on control failures of endosulfan (Ethiosulfan 25% ULV) at Dubti Agricultural Development Enterprise, the second largest cotton producing areas of the country. Despite these reports and reduced efficacy of endosulfan, neither resistance monitored nor resistance management strategy has developed.

Resistance monitoring helps to detect changes in sensitivity early and can assist in conserving available major types of insecticides in a resistance management program and helps to avert field control failures (Kanga *et al.*, 1996). As generally accepted rule, changes in gene frequency must be detected at a very early stage for corrective measures to be applied (Muggleton, 1984). Once resistance becomes noticeable as loss of field efficacy, there are very few practical alternatives other than to abandon the use of the insecticide (Leeper and Raffa, 1986). Successful resistance management strategy depends in part on monitoring responses of pests to various insecticides used in production. The current study was initiated with the objectives of determining susceptibility of *H. armigera* to endosulfan (technical grade) and formulated insecticides Ethiosulfan 35% EC,

Calofos 250 EC/ULV and Karate 5% EC using adult vial, topical application, larval immersion and square dip techniques. This paper reports the results of these studies.

MATERIALS AND METHODS

Collection and culturing of larvae

H. armigera larvae and eggs were collected from cotton fields in Werer Agricultural Research Center, Ethiopia in July 2002 and June 2003 for population establishment. The larvae were reared on semi-artificial diet prepared from soybean flour (125 g), agar (12.5 g), bread yeast (10 g), ascorbic acid (3 g), sorbic acid (1.5 g), casein salt (3 g), methyl 4-hydroxybenzoate (2.5 g), formalin 40% (2 ml), benomyl 50% WP (1 g), distilled water (750 ml) and 10 ml vitamin stock solution. Diet preparation followed the procedures of Taekle and Jensen (1985). The prepared diet was poured into 12 cc multi-wells with lid or in 45 × 30 × 25mm plastic cups and kept in refrigerator for future use. Since second instar stage, larvae were separated and held singly in cups with fresh diet. All populations were maintained under laboratory conditions (30 ± 3°C, and photoperiod of 10:14 light: dark (L: D)).

Insecticides used

Technical grade endosulfan (95.008%) was obtained from Adamitulu Pesticide Processing and Packing Share Company, Ethiopia, profenofos (88.7%) and lambdacyhalothrin (84.86%) were supplied by Syngenta Bangkok, Thailand. Methomyl (89.6%) was obtained from Aventis Crop Science Bangkok, Thailand.

Formulated insecticides namely Calofos 250 EC/ULV obtained from Cliope, France, Ethiosulfan 35% EC from Adamitulu Pesticide Processing and Packing Share Company, Ethiopia and Karate 5% EC from Almadine (UK) Ltd. No. 2, London, England, were used in square dip and larval immersion studies.

Insecticide preparation

Serial dilutions of technical grade insecticides were prepared in analytical grade acetone. While concentrations of formulated insecticides (Calofos, Ethiosulfan and Karate) were diluted with tap water. Concentrations of formulated insecticides were calculated based on the full-labeled field rate and application volume of 200 liters/ha.

Control insects were treated with water alone in square dip and larval immersion studies, while the insects in third instar topical were treated with acetone. In adult vial tests control insects were treated with acetone and distilled water.

Experiment 1: Adult vial test. The 20-ml glass vials were soaked overnight in soapy water, rinsed with acetone, and air-dried. Then the vials were baked at 100°C for 8 hours to remove oils and other left over from the manufacturing process. Insecticide concentrations were prepared in acetone solutions and anhydrous sodium sulfate was added to technical grade acetone to remove traces of water. Benzoic acid was put in vials to protect biodegradation of the insecticide endosulfan. After putting benzoic acid in vials, required concentrations of endosulfan in 0.5-ml acetone were added. Then, vials were laid on their sides and rolled gently by 2 minutes interval for 12-15 minutes, until dried. The *H. armigera* males, collected from pheromone traps early in the day, brought to the laboratory and fed overnight on a cotton pad soaked in 10% sugar solution were introduced individually into insecticide-treated vials.

Due to lack of standard susceptible strain, six discriminating doses of endosulfan: 2.5, 5, 10, 25, 50 and 100 µg/ml were chosen arbitrarily based on the Australian experience (Australian IPM guideline 1997-98). Doses resulted in 100% death of adults in more than two subsequent tests were excluded from the experiment. Only concentrations that yielded 1-99% of adult deaths were considered.

Experiment 2: Third instar topical application. To determine LD₅₀ and LD₉₀, third instar topical application method was used as recommended by the Entomological Society of America (Anonymous, 1970). The test insecticides were applied to larvae using a Hamilton® repeating dispenser fitted with 50-µl syringe and 1 µl drop was applied to the thoracic dorsum of the third instar larvae 30 - 40 mg weight.

Experiment 3: Square dipping. Square dip studies were conducted from July to August 2002 and 2003, when enough number of squares could be collected from field. Squares collected from unsprayed cotton fields were dipped in test solutions for 10 seconds and transferred onto paper padded trays for air-drying. After 60 minutes of air drying, squares were kept in plastic cups and then larvae were introduced into the cups for feeding on the treated squares.

Experiment 4: Larval immersion. Third instar larvae were immersed into the solutions for 10 seconds and put on tissue padded trays for absorbing excessive liquid from the body. After absorbing process and when the larvae started to move actively then transferred into plastic cups with fresh diet for feeding.

Mortality assessment

After dosage, all test larvae were held individually in cups. In adult vial tests mortalities were assessed after 24 hours of exposure. Males unable to fly short distance (not more than 1m) when tossed in the air were considered susceptible to the insecticide. In third instar topical and larval immersion studies mortality was assessed after 24, 48 and 72 hours while in square dip it was done after 24, 36, and 48 hours of treatment. Mortality was recorded by counting dead or moribund larvae and the larvae that could not respond to pencil tip prodding were considered dead.

Statistical analysis

All treatments were replicated 3 – 4 times

with 10 larvae in each replication. The results obtained from the dose-mortality experiments were estimated by probit analysis as described by (Finney, 1971). Natural mortality was corrected by using Abbott's formula (Abbott, 1925). To calculate the LD₅₀ and LD₉₀ (Lethal Doses) LC₅₀ and LC₉₀, (Lethal Concentrations), slope, and the 95% Confidence Limits (CL) the SAS probit analysis software (SAS Institute, 1985) was used.

RESULTS

Experiment 1: **Adult vial test.**

The adult vial test was conducted on Werer population and for the technical grade insecticide endosulfan only. The study was undertaken from July to September in the years 2002 and 2003 at Werer. Discriminating dose determination started from 100 µg/ml concentration with further dropping-off of concentrations yielded 100% mortality in two subsequent tests. Finally, repeated tests were concentrated on concentrations between 2.5 and 10 µg/ml. The results recorded LC₅₀, and

LC₉₀ to be 1.33 and 3.099 µg/ml, respectively. The recorded level of resistance to endosulfan in the previous studies indicated presence of very low level of resistance to endosulfan. This study also recorded very low LC₅₀ value indicating susceptibility of *H. armigera* moths to endosulfan (Table 1). However, very high control mortality (> 25%) was recorded during this investigation (Table 2). Mortality was high on the control treatment especially in the late hours of bioassay (12:00 to 24:00) (Figure 1). This clearly indicated action of some other mortality factors, which were not related to the insecticides. The high and late mortality factors in the control treatment could possibly be speculated as 1) starvation, 2) physiology and 3) environment (Table 2).

Experiment 2: **Third instar topical application**

Dose mortality response results of the probit analysis for all technical grade insecticides are summarized in Table 3. The results indicated endosulfan to have LD₅₀ and LD₉₀ values of 29.84 and 93.63 µg/g, respectively. The LD₅₀ value for

Table 1 Response of *Helicoverpa armigera* adult moths to various concentrations of technical grade endosulfan at Werer in 2002.

Concentration (µg/ml)	Total No.		Probit	Standard error (SE)	Probability	df
	Tested	Dead				
25	200	200	-	1.11505	0.999	2
10	316	316	-	0.65979	0.998	2
5	316	308	6.622	0.32700	0.979	2
2.5	316	263	5.393	0.14882	0.83	2
Acetone	316	163	-	-	-	-
Water	196	76	-	-	-	-
LC ₅₀		1.33383 (0.95259 – 1.62355)				
LC ₉₀		3.09904 (2.81674 – 3.43056)				
LC ₉₉		6.16192 (5.14779 – 8.36036)				
Slope ± SE		1.520 ± 0.219				
χ ²		0.4911 (df = 2; P = 0.7823, thus P> 0.1000)				

µg/ml = microgram per milliliter; df = degree of freedom; LC₅₀ = median lethal concentration; LC₉₀ = lethal concentration which killed 90% of test larvae; χ² = Chi square

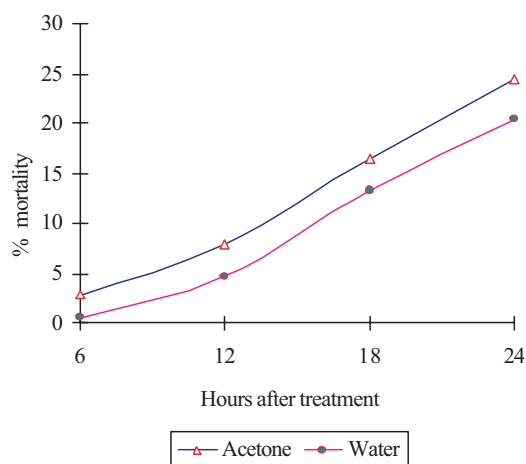


Figure 1 Percent mortality of male *Helicoverpa armigera* moths in acetone and water 6, 12, 18 and 24 hours after treatment.

profenofos was 4.07, while LD₉₀ was 11.24 µg/g larval body weight. Lambdacyhalothrin gave LD₅₀ value of 0.18 and LD₉₀ value of 1.09 µg/g larval body weight. Recorded slope values for endosulfan, profenofos and lambdacyhalothrin were 2.58 ± 0.60 , 1.26 ± 0.15 and 1.62 ± 0.25 , respectively. Among the three insecticides tested, the highest LD₅₀ and LD₉₀ values of endosulfan were recorded followed by profenofos and lambdacyhalothrin.

Experiment 3: Square dipping

Square dip studies were conducted from July to August 2002 and 2003, when enough number of squares could be collected from the field. Results of the three formulations tested on the third instar larvae under laboratory conditions

Table 2 Percent mortality of male *Helicoverpa armigera* moths in different concentrations of endosulfan 6, 12, 18 and 24 hours after treatment.

Concentration (µg/ml)	Total tested	No. dead per ____ hours				Total dead	Percent mortality	
		6	12	18	24		Observed	Corrected
25	200	196	4	-	-	200	100	100
10	316	273	37	6	-	316	100	100
5	316	205	71	27	5	305	97.5	94.8
2.5	316	88	69	53	53	263	83.23	65.3
Acetone	316	9	25	52	77	163	51.58	-
Water	196	1	9	26	40	76	38.78	-

µg/ml = microgram per milliliter

Table 3 LD₅₀, LD₉₀, confidence limit, slope and chi square ratio of endosulfan, profenofos and lambdacyhalothrin in topical application.

Insecticide	LD ₅₀ (µg/g)	95% CL	LD ₉₀ (µg/g)	95% CL	Slope ± SE	χ ²
Endosulfan	29.84	14.05 – 42.53	93.63	69.14 – 154.3	2.58 ± 0.60	3.26
Profenofos	4.07	3.33 ± 4.93	11.24	8.76 – 16.06	1.26 ± 0.15	2.86
Lambdacyhalothrin	0.18	0.1 – 0.25	1.09	0.76 – 1.90	1.62 ± 0.25	1.20

LC₅₀ = median lethal concentration; LC₉₀ = lethal concentration which killed 90% of test larvae; CL = confidence limit; χ² = Chi square; µg/g = microgram per gram body weight of larva

are presented in Table 4. Log-dose-probit line parameters estimated for the three insecticide formulations and this LC_{50} and LC_{90} are presented in Table 5. Calofos resulted in 100% kill at two-times lower dose than the field rate (1.25×10^{-3} g a.i/ml) and 86.67% larval deaths were observed at three-times lower dose than the field rate

(Table 4). Ethiosulfan recorded 99.33% only at the field rate (5.25×10^{-3} g a.i/ml) and subsequently, diluting decreased efficacy very fast and resulted in 71.7, 53.5, 32.0 and 14.4% larval deaths at the second, third, fourth and fifth concentrations of the field rate, respectively (Table 4). Karate gave 99.33% larval death at four-times lower dose (6.25

Table 4 Percent mortality of third instar larvae of *Helicoverpa armigera* to feeding on squares treated with different concentrations of Calofos 250 EC/ULV, Ethiosulfan 35% EC and Karate 5% EC under laboratory conditions ($30 \pm 3^\circ\text{C}$) at Werer.

Calofos 250 EC/ULV concentration (g a.i/ml)	% mortality	Ethiosulfan 35% EC concentration (g a.i/ml)	% mortality	Karate 5% EC concentration (g a.i/ml)	% mortality
2.5×10^{-3}	100	5.25×10^{-3}	99.33	5.0×10^{-4}	100
1.25×10^{-3}	100	2.625×10^{-3}	71.7	2.5×10^{-4}	100
6.25×10^{-4}	86.67	1.3125×10^{-3}	53.5	1.25×10^{-4}	100
3.125×10^{-4}	76	6.5625×10^{-4}	32.0	6.25×10^{-5}	99.33
1.563×10^{-4}	56	3.28125×10^{-4}	14.4	3.125×10^{-5}	93.33
7.81×10^{-5}	29.33	1.64×10^{-4}	5.7	1.563×10^{-5}	72.67
Control	0	Control	0.67	Control	1.33

g a.i/ml = gram active ingredient per milliliter

Table 5 LD_{50} , LD_{90} , 95% confidence limit and regression coefficients of Calofos 250 EC/ULV, Ethiosulfan 35% EC, and Karate 5% EC in square feeding studies under laboratory condition ($30 \pm 3^\circ\text{C}$) at Werer.

Parameter	Calofos 35% EC/ULV (profenofos)	Insecticides ethiosulfan 35% EC (endosulfan)	Karate 5% EC (lambdacyhalothrin)
n	1650	1050	1650
LC_{50}	1.2×10^{-4} g a.i/ml	1.17×10^{-3} g a.i/ml	8.0×10^{-5} g a.i/ml
95% CL	$9.0 \times 10^{-5} - 1.6 \times 10^{-4}$	$9.1 \times 10^{-4} - 1.51 \times 10^{-3}$	$5.0 \times 10^{-5} - 1.0 \times 10^{-4}$
LC_{90}	6.6×10^{-4} g a.i/ml	4.17×10^{-3} g a.i/ml	2.7×10^{-4} g a.i/ml
95% CL	$4.6 \times 10^{-4} - 1.08 \times 10^{-3}$	$2.97 - 7.15 \times 10^{-3}$	$2.0 \times 10^{-4} - 4.3 \times 10^{-4}$
Slope $\pm$ SE	1.74359 ± 0.16438	2.3272 ± 0.325308	2.29465 ± 0.37808
χ^2	5.029	2.95362	1.151
df	8	4	7

n = total number of larvae tested; LC_{50} = median lethal concentration; LC_{90} = lethal concentration which killed 90% of test larvae; CL = confidence limit; χ^2 = Chi square; df = degree of freedom

$\times 10^{-5}$ g a.i./ml) than the field rate (5.0×10^{-4} g a.i./ml), while the fifth concentration resulted in 93.33% kill (Table 4). Karate caused significantly higher mortality of third instar larvae than those of Calofos and Ethiosulfan. Insecticide efficacy magnitude was in the sequence of Ethiosulfan < Calofos < Karate.

Generally, results indicated a low response of *H. armigera* to the full-labeled field rate of endosulfan. On the contrary, effective control of the cotton bollworm larvae was achieved by very low concentrations of the EC formulations of lambdacyhalothrin and profenofos. The endosulfan-based formulation (Ethiosulfan 35% EC) showed very low efficacy as compared to pyrethroid and organophosphate insecticides, Karate and Calofos respectively.

Experiment 4: Larval immersion

Larval immersion studies were conducted from July to September 2002 and 2003 at Werer. Results of the three formulations tested on the third instar larvae and LC_{50} and LC_{90} values obtained in these tests for the insecticide formulations used in the study are presented in Tables 6 and 7, respectively.

Susceptibility of cotton bollworm larvae to the three insecticide formulations was determined. Out of the tested compounds, Karate gave 98.33% kill at four-times lower dose (6.25×10^{-5} g a.i./ml) than the field rate (5.0×10^{-4} g a.i./ml) (Table 6). Similarly, Calofos yielded 99.33% kill at four-times lower dose (3.125×10^{-4} g a.i./ml) (Table 6), while Ethiosulfan gave 96.67% at the field rate (5.25×10^{-3} g a.i./ml). The subsequent dilutions of Ethiosulfan decreased efficacy very fast and resulted in 53.33, 20.00, 6.67 and 3.33% larval death at the second, third, fourth and fifth concentrations lower than the field rate, respectively (Table 6). As in the previous tests, the magnitude of efficacy was in the order of Karate > Calofos > Ethiosulfan. Therefore, effective control of the bollworm larvae was achieved by very low concentrations of the EC formulations of Karate and Calofos. The endosulfan-based formulation (Ethiosulfan 35% EC) showed very low efficacy as compared to lambdacyhalothrin (pyrethroid) and profenofos (organophosphate), respectively.

DISCUSSION

Susceptibility of *H. armigera* male moths

Table 6 Percent mortality of third instar larvae of *Helicoverpa armigera* immersed in different concentrations of Calofos 250 EC/ULV, Ethiosulfan 35% EC and Karate 5% EC under laboratory condition ($30 \pm 3^\circ\text{C}$) at Werer.

Calofos 250 EC/ULV concentration (g a.i./ml)	% mortality	Ethiosulfan 35% EC concentration (g a.i./ml)	% mortality	Karate 5% EC concentration (g a.i./ml)	% mortality
2.5×10^{-3}	100	5.25×10^{-3}	96.67	5.0×10^{-4}	100
1.25×10^{-3}	100	2.625×10^{-3}	53.33	2.5×10^{-4}	100
6.25×10^{-4}	100	1.3125×10^{-3}	20	1.25×10^{-4}	100
3.125×10^{-4}	99.33	6.5625×10^{-4}	6.67	6.25×10^{-5}	98.33
1.563×10^{-4}	89.33	3.28125×10^{-4}	3.33	3.125×10^{-5}	88.33
7.81×10^{-5}	66	1.64×10^{-4}	0	1.563×10^{-5}	73.33
Control	0	Control	0	Control	1.11

Table 7 LC₅₀, LC₉₀, 95% confidence limits and regression coefficients of Calofos, Ethiosulfan, and Karate in larval immersion studies at Werer.

Parameters	Calofos 35% EC/ULV (profenofos)	Insecticides ethiosulfan 35% EC (endosulfan)	Karate 5% EC (lambdacyhalothrin)
n	1650	1260	1980
LC ₅₀	4.0×10^{-5} g a.i/ml	2.11×10^{-3} g a.i/ml	1.0×10^{-5} g a.i/ml
95% CL	$3.0 - 6.0 \times 10^{-5}$	$1.73 - 2.61 \times 10^{-3}$	$0.0 - 1.0 \times 10^{-5}$
LC ₉₀	1.8×10^{-4} g a.i/ml	5.49×10^{-3} g a.i/ml	3.0×10^{-5} g a.i/ml
95% CL	$1.3 \times 10^{-4} - 2.8 \times 10^{-4}$	$4.12 - 8.66 \times 10^{-4}$	$2.0 - 5.0 \times 10^{-5}$
Slope $\pm$ SE	2.09427 ± 0.21685	3.082 ± 0.425	1.93395 ± 0.35550
χ^2	3.930	6.757591	1.238
df	8	4	7

n = total number of larvae tested; LC₅₀ = median lethal concentration; LC₉₀ = lethal concentration which killed 90% of test larvae; CL = confidence limit; χ^2 = Chi square; df = degree of freedom

obtained from pheromone trap catch was studied to technical grade endosulfan response. The low LC₅₀ (1.334 µg/ml) and LC₉₀ (3.099 µg/ml) values revealed susceptibility of the insect to endosulfan. However, the observed high percentage mortality on the control treatment put the usefulness of the result and the adult vial technique under question marks. Apart from exposure to a particular insecticide concentration, death of moths could occur due to various factors, which could not be always measured or quantified.

As reviewed by Schowalter (2000), age, nutrition, temperature, humidity, handling and caging greatly affected the mortality of insects exposed to insecticides. This was similar to the results of the study where most insects in the control treatment died during 12–24 hours, while in insecticide treated vials death occurred in the first 12 hours. For example in California, Varela *et al.* (1993) observed high control mortality (exceeding 20%) in adult vial tests on codling moth. Similarly in Texas, Kanga *et al.* (1995) recorded > 20% mortality in adult tobacco budworm and discarded the data from analysis.

Brewer *et al.* (1990) omitted ten bioassay results from analysis because of high control mortality (> 15%) in California. Daly (1992) recorded < 6% control mortality for ≤ 5 days old moths and 6–18% in older ones in Australia. Generally, Daly and Fitt (1990) and Glenn *et al.* (1994) reported decline of the expression of resistance in adults with ages. Thus, from the results obtained it was possible to speculate starvation as major cause of aggravated late period deaths of moths in the control treatment. Gause (1934) indicated hunger, food abundance and other physiological and behavioral factors as major constituents to stabilization of insects for feeding. Therefore, the disturbed moths were less responsive to feeding, which probably resulted in high mortality in the latter hours of bioassay.

The second point of argument could be the cumulative physiological stress from pheromone trapping to vial test. The long and repeatedly fight to escape captivity caused the insects to lose energy, damage to external body parts and unstabilization which predisposed the insect for weakness and finally to death.

Thirdly, the environmental factors such as humidity and temperature in the vial were different from those in the field condition and acted on more time on the control insects as they survived longer. Studies of Gause (1934) pointed out the importance of environmental heterogeneity as an important factor in population interactions. According to the overall result, the data on adult resistance monitoring provided some indication to base future monitoring works. However, the low level of lethal dose recorded in the study did not match to the field application rate. In addition, the adults were not the primary interest in *H. armigera* management and thus it was difficult to relate results from adult bioassay to the most dangerous stage, the larvae. Besides, the age of male moths obtained from pheromone traps could not be determined before testing. The results from elsewhere indicated female *H. armigera* adults to be more resistant than males. Therefore, the usefulness of this technique to determine resistance level in the field populations is less sounding and needs further refinement. Above all, the inability of the test method to partition causes of adult deaths especially in the latter period of bioassay was found to be the major limiting factor, which needed further validation in other situations where the use of adult vial test was contemplated in resistance monitoring program.

Results of topical application test demonstrated that *H. armigera* at Werer developed low level of resistance to endosulfan. The steep ldp line slope (2.58), coupled with relatively high LD₅₀, (29.84) are indicators for presence of low-level resistance in Werer strain. Similar results were recorded in Thailand (Ahmad and McCaffery, 1988), India (McCaffery *et al.*, 1989; Armes *et al.*, 1992), and Australia (Forrester *et al.*, 1993), Cote d'Ivoire (Martin *et al.*, 2002). Ahmad *et al.* (1995) also reported moderate level of resistance to endosulfan in Pakistan.

The recorded LD₅₀ value 4.07 µg/g larval body weight for profenofos was generally found

very low. Therefore, it was difficult to delineate the field population into resistant or susceptible strains due to lack of standard susceptible strain. However, the low LD₅₀ value recorded in the study was comparable with the LD₅₀ values of susceptible strain from Cote d'Ivoire (Martin *et al.*, 2002). The West African susceptible strain (BK77) recorded 6.84 (Martin *et al.* 2002), while that of Werer strain was 4.07 µg active ingredient per gram of larvae. In general, the low LD₅₀ value indicated susceptibility of the field population to profenofos. This finding was in full agreement with results that of Ahmad *et al.* (1995). Similarly, low level of resistance to profenofos was reported in Australia (Gunning and Easton, 1993; McCaffery, 1989) and Pakistan (Ahmad *et al.*, 1995).

The lowest LD₅₀ value (0.18 µg/g) larval body weight recorded in lambdacyhalothrin bioassay was well comparable with similar results obtained by different investigators elsewhere. Martin *et al.* (2000) recorded LD₅₀ values between (0.046–0.069) for deltamethrin on the susceptible (BK77) strain in Cote d'Ivoire. Ahmad (1994), Ahmad and McCaffery (1988) reported that the susceptible Thai strain, recorded LD₅₀ values ranging from 0.009 - 0.031 for cypermethrin and 0.015 µg/g for fenvalerate. Based on the results of this study therefore, it was possible to consider the Werer strain susceptible. As results of this monitoring work indicated, the field collected *H. armigera* larvae were found susceptible to lambdacyhalothrin. However, it is very essential to monitor continuously or exert selection pressure for many generations to be sure of the pyrethroid susceptibility or resistance, as the insect is known to migrate long distances as observed in Australia and West Africa (Daly and Gregg, 1985).

The square dip test closely approximated the effects of field application of insecticides as the young larvae were found on or in fruiting structures. Thus, most mortality probably was resulted from exposure to residues as young larvae

moving to different plant parts. The results of Calofos recorded to have 100% kill at two-time lower concentration and 86.67% larval deaths were observed at four-times lower concentration. Ethiosulfan gave 99.33% kill only at the field rate with sharp decline in subsequent dilutions. Karate gave 99.33% larval death at eight-time lower concentration than the fields rate while the fifth concentration resulted in 93.33% kill. Therefore, the high percentage kill obtained from Calofos and Karate treatments indicated their efficacy in the cotton bollworm control. Ethiosulfan showed very low efficacy as compared to Karate and Calofos. Thus, results of the study expressed reduced efficacy of Ethiosulfan in controlling *H. armigera*.

Larval immersion studies were undertaken under laboratory condition on Calofos, Ethiosulfan and Karate. It was found that out of tested products Karate gave 98.33% kill at eight-time lower concentration. Similarly, Calofos yielded 99.33% kill at eight-time lower concentration, while Ethiosulfan recorded 96.67% only at the field rate. This study again showed comparative effectiveness of Karate and Calofos in controlling bollworm larvae. The eight-time low-percentage kill observed by endosulfan-based formulation, Ethiosulfan 35% EC also reconfirmed the reduced efficacy of endosulfan to *H. armigera* control.

Although, Ethiosulfan 35% EC provided similar results in both tests evaluated (square dip and larval immersion) at full-labeled field rate, achieving this level under field conditions is less probable. The reasons were as follows: firstly the full coverage of the larval surface with insecticide droplets might be difficult due to the possibility of shading of leaves and squares, or boring of the larvae into the bolls or squares. Secondly, as the larvae bored into the bolls, the chance of feeding to treated surface alone was minimized. Thus, the results obtained from endosulfan treatment in both methods were unsatisfactory as compared to Karate and Calofos. The reports from lower Awash

Agricultural Development Enterprise showed failures in *H. armigera* control by Ethiosulfan 25% ULV during past few years. The field experience also indicated a decline of Ethiosulfan in residual efficacy. Since these failures were correlated with bioassay results obtained in square dip and larval immersion studies it was possible to conclude that *H. armigera* developed resistance to endosulfan. Thus, until IPM is developed and implemented, replacing chemical should be screened and the use of endosulfan restricted to only one application per season and the alternative control methods must be studied. Results of Calofos showed slight variation in the two tests. However, its efficacy was far above endosulfan. Karate yielded very similar results in all tests and revealed its efficacy against *H. armigera* in eight-times lower concentration than the field rate.

CONCLUSION

It was demonstrated that endosulfan resistance was present in Werer strain of *H. armigera* in the years 2002 and 2003. Generally, adult vial test technique should be improved to the status that treated adults get enough food and aeration during the test period. The result showed the control mortality to be < 15% between 6–12 hours. Thus, after this much exposure, the insects could be transferred into rearing cage for feeding. In comparing the relative utility of four test methods, it was found that the use of inexpensive and rapid assay, such as square dip and larval immersion methods could be used successfully in resistance monitoring as they yielded almost similar results. However, square dip technique is more recommendable due to similarity of the field sprays, availability of squares in cotton growing seasons, and reduced cost of larval maintenance after treatment. Square dip can also be used as a rapid bioassay where it is desirable to quickly determine the cause of control failure and in developing countries where facilities and equipment may be

limited.

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