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RESEARCH ARTICLE

Residual Effect of profenofos on Cotton Bollworm *Earias insulana* (Boisd.) using two Ground motor sprayer

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Abstract

A field experiment was carried out on spiny bollworm*Eariasinsulana* (Boisd.) larval infestation in cotton field in Qaha district, Qalyoubia Governorate.

Profenofos was sprayed by using Knapsack motor sprayer Cifarilli (20 L./Fed.) and Economy Micron ULVA (15 L./Fed.). The spectrum of droplets range between 142 -165 microns (VMD) with sufficient number ranging from 25- 268 N/cm^2.

The general reduction rates of larval infestation after the treatment with profenofos was 91.15% in case of using Micron ULVA equipment and 91.13% in case of using Knapsack motor Cifarilli sprayer, respectively.
Profenofos residues were extracted from cotton bolls and seeds samples then determined by Gas chromatography (GC) with flame photometry (FPD). The average recoveries ranged between 91.4 % and 96%, and limit of determination was 0.005 mg/ kg. Limit of determination (LOD) were determined on samples at spiking levels 0.01–0.05 mg/ kg from the pesticide standard.

Results indicated that the dissipation the pre harvest interval (PHI) of profenofos residues in cotton seeds was safe in the harvest. Economy Micron ULVA sprayer (15L/Fed.) revealed best reduction in larval infestation and best spray quality with less amount of profenofos residue.

INTRODUCTION

Egypt is known as an exporter of high quality cotton, which has an international reputation in special features that attract niche market consumers. Cotton plays a dominant role in the country's economy by meeting the domestic and export demands, contributing significantly to agriculture, industry, employment and export earnings. The 2006 statistics1 show that Egyptian cotton provided a cash income to roughly one million small farmers. In addition, the cotton industry labor force totals to about 1 million. With the increasing importance of Egyptian cotton at the domestic and international levels, the government pays great importance to and constantly seeks to improve both quantity and quality of cotton. This ensures the competitiveness of Egyptian cotton in the international markets. However, the share of Egyptian cotton exports (ECE) in the world cotton exports has dropped from 4 per cent in 1980 to 1.06 per cent in 2006. The total quantity of ECE has also fallen from 196.8 thousand tones in 2003 to 87.2 thousand tones in 2006. (**Hatab, 2009).**

Eariasinsulana (spiny bollworm) is major lepidopteron pest of many crops and distributed in North Africa and Sub continent (Indo-Pak) **(**Abdul-**Nasr *et al.* 1973),** spiny bollworm is main cotton pest, larvae infesting bolls, damaging cotton squares, flower buds, flowers, seeds and fiber, especially at the late growing stage of cotton plants that cause decreasing in the quality and quantity in the lint and oil of the obtained yield.

Pesticides play an inevitable role in modern agriculture. Rising concern about food safety and environmental impact has led to increasing number of studies on the impact of pesticide residues in agricultural products consumed by humans. With the intensive, indiscriminate and injudicious use of pesticides has resulted in widespread contamination of food and feed. This is related to poor insecticide handling practices and use of more toxic insecticides by farmers (**APHA, 2005**). As a consequence, governments and international organizations have established maximum residue limits (MRLs) (Regulation (EC), No. 396/2005) and pre-harvest intervals (PHI) for fruits and vegetables. It is well known that such pre-harvest intervals defined as the time required reducing the residue levels below the MRL, can be estimated from the residue dissipation curve (**Abd-Alrahman, 2013**).

QuEChERS is a (quick, easy, cheap, effective, rugged and safe) method which has been mainly applied for the extraction of different classes of pesticides. The QuEChERS method is a simple, rapid, and inexpensive procedure requiring little labor and few materials, space, and solvents. This method achieved the status of Official Method of AOAC International **(Lehotay 2007).**

The aim of this research is to evaluate the efficiency of profenofos on E.*insulana*in cotton field with Knapsack motor (Cifarilli) and Economy Micron ULVA sprayer to determined the relationship between spray quality and *E. insulana* larval survival produced by the previous spraying equipment in field and determine the dissipation rate, half-life values (RL_{50}) and pre-harvest interval (PHI).

MATERIAL AND METHODS

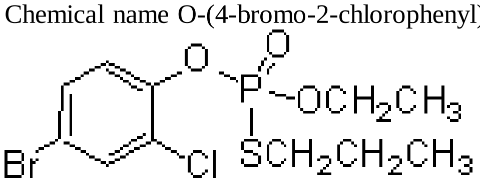
-Insecticide used:

-Organophosphorus:

Common name: Profenofos72%EC.

Trade name**:** Selecron 72%EC.

Chemical name O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate



- Field experiment:

Field experiments were carried out on cotton cultivated April 15th during season 2014 at Qaha district, Qalyoubia Governorate, cultivated with Giza 86 cotton variety. The experimental design was randomized complete block with 3 replicates, the whole cultivatedarea(300 m^2) was divided into equally 2 plots, and each plot was treated with profenofos as well as one of the tested equipment; while the remaining plot was left without spraying as control. Cotton seeds were sown at 20 cm distance between hills. Spraying of the tested insecticide took place on cotton plants three times in July, 7th, 21st and 4th August, respectively, with two motor sprayers (Cifarilli, Knapsack-motor sprayer and Economy Micron ULVA sprayer). The experiments were carried out under local meteorological conditions of 32°C average temperatures, 60 % R.H. and 4.6 m/sec. as an average wind velocity during experiment.

To evaluate the effect of profenofos against spiny bollworm, samples of 25 bolls/plot were randomly picked before and week after application. Sampling continued weekly until harvest. The collected bolls were transported to the laboratory, where they were carefully dissected and percent of larval infestation was recorded and the reduction percentages in green boll infestation were determined according to **(Henderson and Tilton 1955).**

-Spraying equipment tested on cotton field:

Two ground application machines were selected to perform the scope of this work, as commonly used equipment in applying pesticides on cotton plants. The tested equipment could be represented according to the technical categorization mentioned in **table (1&2).** Calculations of productivity and rate of performance after **Hindy (1992).**

- Calibration and performance adjustment of the tested equipment:

- **Collection and measurement of Spray deposit**:

Before spraying each cotton field treatments, a sampling line was constructed of five wire holder fixed in diagonal line inside each treatment to collect lost spray between plants; each wire holder top has a fixed water sensitive paper (Novartis Cards) on it. Also, each five cotton plants, the water sensitive paper cards were put at three levels of cotton plant; upper, middle and lower to collect the spray deposit on cotton leaves ,were designed according to the method described by **(Hindy 1989).** All cards were collected and transferred carefully to the laboratory for measuring and calculating the number of droplets/cm² and its volume (VMD) in all treatments.

Table (1): Techno-Operational data of the Economy-Micron - ULVA sprayer used in field during season 2014.

Item	Spining disc ULVA sprayer	Remarks
Type of spraying	Target	Direct spray
Nozzle type	Rotary (spinning disc)	Restrictor
Number of nozzles	1	
Spray tank (L.)	1+10	10 L.spray attached.
Rate of application (L/fed.)	15	
Working speed (Km/h.)	2.4	± 5%
Effective swath width (m.)	1.0	
Flow rate (L/min.)	0.150	Total of the sprayer
Spray height (m.)	0.5	
Productivity * (fed./h.)	0.571	
Rate of performance* (fed./day)	3.04	~ daily hours =8h.

* Number of spraying hours = 8 hours daily.
* Calculations of productivity and rate of performance after **Hindy (1992).**

Table (2): Techno-Operational data of cefarilli ground sprayers applied on cotton field during season (2014).

Item / Type of sprayer	Motorized Knapsack sprayer Cifarilli
Model	Cifarilli
Manufacturing	Italy
Type of atomization	Pneumatic
Nozzle type	Pneumatic
Number of nozzles	One
Pressue (bar)	-
Total Tank capacity (L.)	20.0
Rate of application (L/fed.)	20.0
Working speed (Km/h.)	2.4
Swath width (L/m)	5.0
Flow rate (L/min.)	1.0
Spray height (m.)	0.5
Type of spraying	Drift
Sprayer weight (Kg)	12.2
Productivity (Fed./h.)	2.85
Rate of performance (Fed./day)	12.0
No. of worker's	2

* Number of spraying hours = 8 hours daily.
* Calculations of productivity and rate of performance after **(Hindy 1992).**

- Determination of spray deposit:

Number and size of blue spots (deposited droplets) on water sensitive papers (Novartis cards) were measured with a special scaled monocular lens (Strüben)® with magnification X5.The volume mean diameter (VMD) and number of droplets in one square centimeter (N/cm²) was estimated according to **(Hindy1992).**

- **Determination of profenofos residues in cotton bolls:**

-Sampling:

Samples of cotton bolls with similar ripening stage, size, and shape were located and tagged. Samples about 1.0 kg were taken 1 h and 7 day after the pesticide application in 1st, 2nd and 3rdtreatment and cotton sample in the harvest. During the experiment, a control sample was taken in each sampling time. Immediately after collecting the samples, all the samples were packed in polyethylene bags and transported to the laboratory in an ice box. The samples were homogenized using a food processor (Thermomix Vorwerk). The homogenate of each sample was done where three representative samples of 15 g were taken. Samples were then placed into polyethylene 50-ml centrifuge tube and frozen at -20 °C until the time of analysis.

- Chemical and reagents:

All organic solvents were of HPLC grade and supplied by Merck, USA. Primary and secondary amines (PSA, 40 ml Bondesil) were purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulphate was of analytical grade, purchased from Merck, USA, and was activated by heating at 250°C for 4 h in the oven before use and kept in desiccators.

- Extraction and Clean up of Pesticides Residues:

Analyses of tested pesticides were carried out in the Central Agricultural Pesticides Laboratory, Agricultural Research Center ARC.

Samples were extracted and cleaned up immediately after sampling using QuEChERS methodology **(Lehotay *et al*., 2010).** About 15 g of the homogenized samples were weighted in a 50-mL centrifuge tube and 10 ml of acetonitrile (1% acetic acid) were added, then the screw cap was closed and the tube vigorously shaken for 1 min using a vortex mixer at a maximum speed. Next 1 g sodium chloride and 4 g anhydrous magnesium sulfate were added. The sample was vortexed for 30 s. The extracts were centrifuged for 5 min at 3800 rpm and 40°C. An aliquot of 4 ml was transferred from the supernatant to new clean 15 ml centrifuge tube and cleaned up by dispersive solid-phase extraction with 100 mg PSA, 20 mg GCB and 600 mg $MgSo_4$. The sample was again vortexed for 1 min and then centrifugation was carried out as mention above. Then, 1ml of the supernatant was taken, mixed with 2ml toluene and evaporated to dryness. The residues were redissolved in 1ml of toluene, filtered through 0.22 um PTFE filter (Millipore, USA) and transferred into a 1.5 ml glass vial for GC analysis.

- **Instrumental determination Gas chromatographic determination:**

Gas chromatography (GC) Hewlett Packard (HP) serial 6890 equipped with flame photometry (FPD) was used. Analysis of the pesticides was performed on capillary column, i.e., HP-5 (5%-Phenylmethylpolysiloxane). The dimensions of column were 30 m length x 0.25 mm inner diameter and coated with 0.25 µm film thickness of the stationary phase. Nitrogen was used as a carrier gas at a flow rate of 1ml/min. The temperatures of injector and interface were 250°C and 300°C, respectively. Temperatures were 220, 320 and 320°C for column, injector and detector, respectively. Gas flow rate of nitrogen was 4 ml min. At these conditions, the retention time (Rt) of profenofos was 2.9 minutes. Recoveries and limit of determination (LOD) were determined on samples at spiking levels 0.01–0.05 mg/ kg from the pesticide standard.

The average recoveries ranged between 91.4 % and 96%, and limit of determination was 0.005 mg/ kg. The results of analyses were corrected for recoveries. Blank samples were fortified with the pesticide and analyzed as a normal sample with each set of samples. The results were recorded on control charts.

Data were subjected to the analysis of variance test (ANOVA) via Randomized Complete Block Design (RCBD) (F. test). The least significant differences (LSD) at the 5% probability level were calculated according to computer program CoStat by (**Steel and Torrie 1981)** to compare the average numbers of inspected pest infestations.

RESULTS AND DISCUSSION

The optimum spectrum of droplets for controlling insects of field crop should be sized between 140 and 200 µm (VMD) with number not less than 30 and 50 droplets/cm^2 distributed homogeneously on the treated target **Himel (1969)**. The following general trends could be extracted from the obtained data and may help in better understanding to the experimental results, in **Table (3)**.

In this work, the minimum size of measured spots was however about 50µm. This is due to the limited capability of the available technique of measurement, which means logically that a lot of invisible fine spots smaller than 50µm should occurred within the measured spots. This might clarify the appearance of certain non-reasonable killing results in some experimental treatments. The range of droplets spectrum (VMD and N/cm^2) deposited on the natural targets by using total recommended dose, insecticides used were 142 & 165µm, and 25 & 268 N/cm^2.

The spray lost on ground, between plants, was the only measured loss, whereas other sources of loss such as by wind (drift), evaporation,... etc, were not subjected to investigation throughout this work.

In the other hand, there were no significant differences between both the distribution percentages of droplet sizes and the droplets number/cm^2 at all targets (cards on cotton plants and cards on ground between cotton plants).

Table (3) Spraying coverage on cotton plants and ground holders produced by certain ground spraying equipment, at season 2014 using total recommended dose rate tested insecticide profenofos against *Eariasinsulana* at Qalubiya Governorate.

Equipment	Economy Micron ULVA		Cifarilli Knapsack-motor-sprayer	
Application rate L./fed.	15		20	
Insecticide	profenofos			
Upper level	N/cm^2	VMD	N/cm^2	VMD
	172^a	157^a	268^a	156^b
Middle level	170^a	165^b	252^b	142^c
Lower level	126^b	162^c	240^c	154^b
Mean	156	161	253	151
Ground	25^c	159^a	86^d	160^a
% N/Cm^2 on ground (spray lost)	5	-	10	-

Numbers followed by the same letter(s) in each column are not significantly different at P ≤ 0.05 level.

Data in **Table (3)** showed that, there was a significant differences between both the distribution percentages of droplet sizes (LSD=2.38 for equipment, 2.58 for levels and for the droplets number/cm^2 (LSD=3.60 for equipment, 3.51 for levels).

A satisfactory coverage was obtained on cotton plants, the droplet spectrum was obtained in field experiment was agreed with the optimum droplet sizes which mentioned by **(Himel and moore 1969).** The best obtained result was 15 L/Fed. As spray volume, 157 µm and 156 droplets/cm², these results agreed with **(Himel 1969)** in the optimum droplet size to control cotton leaf worm in the cotton fields by ground equipment. The data showed that Economy Micron ULVA sprayer (15 L./fed.) is the best equipment to control Spiny bollworm on cotton plants. Also, the lowest spray volume and the lowest percentage of lost spraying between plants; these results were agreed with **(Hindy *et al.*, 1997)** and **(2011)** mentioned that, there was a positive relationship between rate of application and spray lost on ground.

Data presented in **Table (4),** showed that application of profenofos with Micron ULVA sprayer caused significant reduction in percentages of infested cotton bolls caused by the spiny bollworm *E. insulana*. The reduction percentages in the green bolls caused by *E. insulana* using Micron ULVA sprayer increased gradually during July, August and September by 80.17, 92.96 and 93.89%, respectively.

As shown in **Table (4),** General reduction percentages in the green bolls infestation caused by *E. insulana* larvae in the whole season associated to the treatments with profenofos was 91.15%.

The reduction percentage of infested cotton bolls caused by the spiny bollworm *E. insulana* after profenofos application using motor sprayer Cifarilli illustrated in **Table (4)**. Earliest incidence of the spiny bollworm larvae of the experimental trails was on June, 30th. Number of spiny bollworm larvae was mostly lower in profenofos treatment than the control. Reduction means in the infestation percentages of bollworm larvae of plots treated with profenofos were 79.01, 92.70 and 93.67% during July, August and September and 91.13% in the whole season.

In a similar study **Salem (2002)** recorded that the chemical insecticide (Herculis) was the most effective in reducing the infestation and larval content in green cotton bolls. Also, **(Al-Shannaf 2010)** found that all the tested sprays (Profenofos, S-fenvalerate and Chlorpyrifos methyl caused highly decreasing in cotton bollworms larvae compared with untreated area.

Also, (Abdalla 1991) stated that the effects of chemical control programs on the rate of infestation of cotton bolls by the *E. insulana*in Egypt. The obtained results revealed that three or four sprays through the season caused a satisfactory decrease of infestation and loss of yield. **(Simwat and Dhawan 1992)** assessed the efficacies of conventional insecticides were the most compounds potent against cotton bollworms. **(Abdel Megeed 2008)** found that foliar treatment of EastenaAminofert with spinosad and chloropyirifos reduced levels of spiny bollworm infestation.

Data in **Table (4)** represent the amounts of profenofos in cotton bolls and seeds with two tested equipment under field conditions, after different intervals. It is obvious that, the amounts of profenofos initially after 1st, 2nd and 3rd treatments with Micron ULVA sprayer detected in cotton bolls were 35.7, 40.1 and 38.65 mg/kg, respectively. While in the case of motor sprayer Cifarilli the amounts of profenofos initially after 1st, 2nd and 3rd treatments were 54.98, 56.74 and 48.5 mg/kg, respectively.

Table (4): Residues of profenofos (mg/kg) in /on cotton bolls and reduction percentages of spiny bollworm *E. insulana*larvae by different spraying techniques under field conditions.

Times	Tested motor sprayer	Residues Cotton bolls	
1st spray	Econom Micron ULVA	mg/kg*	35.7^{c}
		%loss	-
	Cifarilli Knapsack	mg/kg*	54.98^{b}
		%loss	--
7days	Economy Micron ULVA	mg/kg*	3.56^{d}
		%loss	90.02
	Cifarilli Knapsack	mg/kg*	4.86^{d}
		%loss	91.16
2nd spray	Economy Micron ULVA	mg/kg*	40.1^{a}
		%loss	-
	Cifarilli Knapsack	mg/kg*	56.74^{a}
		%loss	-
7days	Economy Micron ULVA	mg/kg*	2.67^{d}
		%loss	-
	Cifarilli Knapsack	mg/kg*	4.9^{d}
		%loss	-
3rd spray	Economy Micron ULVA	mg/kg*	38.65^{b}
		%loss	93.34
	Cifarilli Knapsack	mg/kg*	48.5^{c}
		%loss	91.36
7days	Economy Micron ULVA	mg/kg*	3.2^{d}
		%loss	91.72
	Cifarilli Knapsack	mg/kg*	3.97^{e}
		%loss	91.81
Harvest	Economy Micron ULVA	mg/kg*	$*0.11^{e}$
		%loss	99.77
	Cifarilli Knapsack	mg/kg*	$*0.31^{f}$
		%loss	99.36
LSD		0.45	
Fvalue		0.0000***	
MRL*	Economy Micron ULVA	2	
	Cifarilli Knapsack	2	

Inspection months	Tested motor sprayer	Mean	% Reduction
July	Econom Micron ULVA	4.8^{a}	80.17^{c}
	Cifarilli Knapsack	5.08^{a}	79.01^{d}
	Untreated	24.2^{c}	-
August	Econom Micron ULVA	4.75^{a}	92.96^{a}
	Cifarilli Knapsack	4.93^{b}	92.70^{b}
	Untreated	67.5^{b}	-
September	Econom Micron ULVA	4.5^{a}	93.89^{a}
	Cifarilli Knapsack	4.67^{b}	93.67^{a}
	Untreated	73.67^{a}	-
General Mean/ reduction	Econom Micron ULVA	4.68^{a}	91.15^{b}
	Cifarilli Knapsack	4.89^{a}	91.13^{c}
	Untreated	55.12^{c}	-

Numbers followed by the same letter(s) in each column are not significantly different at $P \leq 0.05$ level.

- *=Average of 3 replicates
- **= Blow detection limit(0.005mg. kg^{-1})
- ***Residues of profenofos in cotton seeds
- MRL=2 mg. kg^{-1} **CODEX 2014**

Pesticide dissipated rapidly after each application. The concentrations of profenofos in cotton bolls 7 days after1st, 2nd and 3rd treatments with Micron ULVA sprayer were 3.56, 2.67 and3.2 mg/kg, respectively. While in the case of motor sprayer Cifarilli the amounts of profenofos in cotton bolls 7 days after 1st, 2nd and 3rd treatments were 4.86, 4.9 and 3.97 mg/kg, respectively.

The concentrations of profenofos in cotton seeds with Micron ULVA sprayer and motor sprayer Cifarilli on the harvest were 0.11 and 0.31 mg/kg, respectively.

The dissipation/degradation of various pesticide compounds after their application depends on various factors, including plant species, chemical formulation, and mode of application (**Womac *et al*. 1994**), climatic conditions, physical phenomena (mainly volatilization), and photo degradation, in which sunlight plays a prominent role (**Papadopoulos *et al*. 1995**). Therefore, dissipation studies for a given crop under the open field conditions of each growing area are necessary to test if the pesticide residue levels soon after the preharvest interval (PHI) are below the maximum residue limit (MRL). PHI, which is defined as the period between the last pesticide application and harvesting the crop, after which the pesticide residue level is expected to be below the established maximum residue level (MRL), is one of the important pesticide registration requirements in Egypt.

CODEX MRL for profenofos in cotton seeds was 2 mg/kg. It could be concluded that the pre harvest interval (PHI) of profenofos in cotton seeds. It was safe at harvest under using two tested equipment under field conditions, these results harmonize with other investigators working on residues of profenofos in cotton bolls **(Zhao Wen Ying 2009).**

(**ZhaoWenYing 2009**), In 2006 and 2007, after spraying 500 g/l profenofos modifiable concentration on the cotton in Jinan and Changsha trial site, the residues of profenofos in the samples of cotton seeds, cotton leaves and soil were studied . The dynamic residue results indicated that the initial deposits in cotton leaves were 83.0 - 170 mg/kg. The final residue test results showed that the maximum value of final residue of profenofos in cotton seeds was 0.125 mg/kg, which was under the MRL2 mg/kg constituted by CODEX whatever the high or low dosage. The later was high because of the shorter interval to harvest.

CONCLUSION

The significant differences between the two ground motor sprayers consequently differences in residue results and thus differences in reduction percentages of spiny bollworm *E. insulana*.

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