

Side-effects of Insecticides on Non-target Organisms: 1- In Egyptian Cotton Fields

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ABSTRACT

Side effects of some groups of recommended insecticides in cotton fields were evaluated on non-target pests and predators through experimental field trials at two Egyptian Governorates for two growing seasons, 2013 and 2014. Direct counts of target and non-target organisms, pre-treatment and at 3 day intervals post treatments for each insecticide/ location were practiced. Reduction rate in insects' population was estimated. As well, a discriminating concentration technique was used for rapid monitoring of insecticidal resistance in field-samples of the pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) and the cotton leaf worm (CLW) *Spodoptera littoralis* (Boisd.) larvae, collected from sprayed cotton fields. Rates of reduction in *P. gossypiella* population (target pest) ranged between 63.2-72.5 and 61.9-74.8% in 2013 and 2014, respectively. Respective rates in non-target pest species; *Aphis gossypii* (Glov.), *Bemisia tabaci* (Genn.) and *Tetranychus urticae* Koch were 35.6-83.3 and 50.3-83.0%, while they attained 79.2-96.5 and 65.2-91.8% in the populations of the predatory species; coccinellids, *Chrysoperla carnea* (Steph.) and spiders. Resistance % in the PBW and CLW collected from sprayed fields was estimated by 19.2- 54.6%.

Key words: Risk Assessment, Insecticides, Side-effects, Non-target organisms, Cotton fields, Egypt.

INTRODUCTION

Pesticides are an integral part of agriculture. They are used by almost all cotton farmers to limit reductions in crop yield and quality from diseases, pests and weeds. Many of the crop production in Egypt are likely to remain dependent on continued pesticide use for the foreseeable future. Destructive insect pests are the most limiting factor of the cotton yield. Bollworms and leaf worms are the key pests and lately aphids and whitefly have become also a problem because of the wide use of pesticides. Although an IPM program is implemented in cotton fields in Egypt, pesticides still the most effective tool for controlling the cotton pests. Side-effects of usage some pesticides result in unfortunate consequences to many non-target organisms, particularly natural enemies and pollinators. Beneficial organisms include various parasitic and/or predacious insects, mites, nematodes, fungi, bacteria, and other microorganisms that feed on or parasitize pest species. The value of these organisms to agriculture and the environment are likely underestimated. Despite many advantages of pesticides, there are some potential hazards or risks when using farm chemicals. These risks may be associated with all chemicals whether they be industrial chemicals, pesticides, household products or even natural chemicals found in the environment. Undesirable side effects of farm chemicals use usually stem from a lack of understanding their risks on the environment, compounded by indiscriminate and overuse of the product.

In Egypt, mostly 70% from the total amount of insecticides, used for pest control in all crops combined, is used in cotton fields. Such applications showed a negative impact of insecticides, as a sharp decline (about 70–80% reduction in the numbers of predatory species populations) occurred in cotton fields post applications (El-Heneidy *et al.*, 1987) as well as in other crops like wheat, as the reduction in numbers of predatory and parasitoid species ranged between 68–72% (El-Heneidy *et al.*, 1991).

Risk assessment of insecticides on beneficial organisms has been carried out under semi-field conditions, following protocols developed by the IOBC (International Organization of Biological Control)-group 'Side-effects of Pesticides on Beneficial Organisms' (Hassan, 1977 and Sterk *et al.*, 1999). The effect of old and new compounds were tested on 1st and 2nd nymphal instars of the predatory species, *Orius laevigatus* (Fieber) (Anthocoridae) and *Macrolophus caliginosus* Wagner (Miridae), adult females of *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae), and pupae and adults of the parasitoid, *Encarsia formosa* Gahan (Aphelinidae). The trials were run using published methods for semi-field trials: *P. persimilis* (Sterk and Van Wetswinkel, 1988), *M. caliginosus* (Van der Linden, 2000), *E. Formosa* (Jaco, 2001) and *O. laevigatus* (Van de Veire *et al.*, 2002). Pesticides were classified into the toxicity categories proposed by the IOBC working group for semi-field trials as: Class 1: harmless (<25% mortality), Class 2: slightly harmful (25-50%), Class 3: moderately harmful (51-75%) and Class 4: harmful (>75%). Selective biological or chemical compounds are needed in such cases (Hassan, 1994).

It is known that the populations of many arthropod species can develop various degree of resistance to the pesticides action. Detoxification of pesticides by metabolism is the common mechanism that has evolved to

protect insects. Early detection of pesticide resistance provides a basis for management of resistant pest and natural enemy populations (Abdel-Baset, 2009). Most of pesticides are subjected to enzymatic reactions after they are entered in the bodies. The most striking effect of organophosphate and carbonate pesticides is their ability to inhibit acetyl cholinesterase in the cholinergic junction of the nervous systems. It had also been clearly demonstrated that several enzymatic systems in resistant organisms such aliphatic esterase, phosphatase and non-specific esterase play an important role in the detoxification of pesticides. For example, population of the American bollworm, *Helicoverpa armigera* (Hb.) had developed various degree of resistance to many insecticides (Panchbhai *et al.*, 2004). Khidr, (1982) determined the levels of resistance in *H. armigera* larvae, collected from tomato fields, to some organophosphorus insecticides. Resistant ratio ranged between 6.96 and 8.43 folds. Young *et al.* (2005) reported that pyrethroids resistance in field population of Australian *H. armigera* is primarily a consequence of the overproduction of esterase isoenzymes which metabolize & sequester pyrethroid insecticide. Aggrawal *et al.* (2006) demonstrated that laboratory tests showed that an insecticide resistance management strategy resulted in a significant decline (1 to 2.9 fold) in resistance of *H. armigera* larvae to Cypermethrin, fenvalerate, Endosulfan, Methomyl, Guinaphos and Chlorpyrifos.

The present study is a contribution for minimizing risk assessments of recommended agricultural insecticides to both common target and their associated non-target organisms in Egyptian cotton fields.

MATERIALS AND METHODS

Working sites

Field studies were implemented at each of 2-4 locations in two Governorates; relatively have different agro-ecosystems, Menoufia representing middle of the Delta (Lower Egypt) and Fayoum representing Middle Egypt in the two successive cotton seasons 2013 and 2014. Working sites included 6 locations: Tala and Quesna (Menoufia Governorate), and Etsa, Sanoures, Fayoum and Abshway (Fayoum Governorate) throughout the study period. Experimental fields (about 2-5 feddan each/ location), received regular agricultural and chemical practices as recommended by the Ministry of Agriculture (MoA), were selected for the evaluation.

Target Insecticides

Risk assessment of insecticides on beneficial organisms, through side-effect trials was carried out under semi-field conditions, following the protocols developed by the IOBC (International Organization of Biological Control)-group 'Side-effects of Pesticides on Beneficial Organisms (Hassan, 1977 and Sterk *et al.*, 1999). Side effects of available different insecticides groups mainly; organophosphorus, carbamates, pyrethroids and bio-insecticides, recommended by the MoA against major pests in Egyptian cotton fields at their recommended doses were targeted in field trials. Insecticides were classified into the toxicity categories proposed by the IOBC (International Organization of Biological Control) working group for semi- and field trials as: Class 1: harmless (<25% mortality), Class 2: slightly harmful (25-50%), Class 3: moderately harmful (51-75%) and Class 4: harmful (>75%) (Hassan, 1977 and 1994).

Methodology

Direct count technique was practiced by experienced specialists to collect field data. The technique varied according to the pest species and growing stage of the crop. A pre-treatment population was estimated one day or on the same day of insecticide's application. Afterwards, inspection's interval was on day 1, 3, 7, and 10. Sample size was 25 leaves x 4 replicates (= 100 leaves/ location/ date). Data were recorded and statistically analyzed. In case of sucking insects, mortality percentages (reduction %) in the population of each of the selected target pest, non-target and predatory species were calculated. For cotton bollworms, reduction % was calculated also by bolls' dissecting and number of larvae in 25 green bolls x 4 replicates (= 100 bolls/ location / date).

Reduction percentages in the populations of non-target organisms were calculated and corrected using Henderson and Tilton (1955) equation:

$$\text{Reduction \%} = (1 - A/B \times C/D) \times 100$$

Where: A = No. of individuals post-treatment, B = No. of individuals pre-treatment, C = No. of individuals in the check pre-treatment and D = No. of individuals in the check post-treatment.

Reduction percentage in the population at day 1 post treatment was calculated as initial kill, while that at the following days post treatment was considered as residual or latent effects.

Monitoring of resistance

Some of the enzymes in different organisms play a vital role in the mechanism of resistance. High levels of enzymes usually increase resistance levels in the organism. The main enzymes in organisms related to

resistance are; Acetylcholinesterase, Glutathione S- transferase, Alkaline phosphatase and α -esterases. Each has its role as its mechanisms are acting different.

Methodology

The four main enzymes; Acetylcholinesterase, Glutathione S- transferase, Alkaline phosphatase and α -esterases were selected to be analyzed as indicators of the level of resistance and detection of biochemical aspects in field strains of the pink bollworm (PBW) *Pectenophora gossypiella* (Saund.) and the cotton leaf worm *Spodoptera littoralis* (Boisd.) as well in the predatory species; *Chrysoperla carnea* (Steph.) and *Orius* spp. compared with laboratory strains. Field samples used in the enzymatic assays represent some of the insecticides recommended in the Egyptian cotton fields against several insect pest species. Laboratory cultures, reared for several generations under laboratory conditions, were used as a standard control. Enzymes' analyses were carried out by the specialists at the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. Response was estimated in the tested species under laboratory conditions by calculating resistance % and relative resistance % using the following equation:

$$\text{Activity ratio} = (\text{Enzymatic activity in field sample}) / (\text{Enzymatic activity in laboratory sample})$$

RESULTS AND DISCUSSION

Side-effect of insecticides

A total of 15 insecticides in 6 field trials, at the 6 locations, 2 Governorates were evaluated under the field conditions in the cotton seasons 2013 and 2014 (Table 1). In general, field data revealed that both target and non-target organisms were influenced by applying the insecticides. Different reduction rates in the populations of target, non-target pests and predators as direct effects of applications were recorded. Reduction percentages varied at different pest and/ or predatory species tested as well for each insecticide used. A total of 18 and 17 trials were practiced in seasons 2013 and 2014, respectively.

Average reduction rates in target pests, mainly; (PBW) *P. gossypiella*, ranged between 65.6-78.9% in both seasons. In non-target pests' populations, that included mostly sucking pests such as; *Aphis gossypii* (Cotton aphid), *Bemesia tabaci* (Cotton whitefly), *Empoasca deipiens* (Leaf hopper), *Tetranychus urticae* (Red spider mite), it ranged between 39.4–83.3 and 51.8-77.8% in seasons 2013 and 2014, respectively. Correspondent reduction rates in the predatory species populations; *Coccinella undecimpunctata* (Lady beetle), *Chrysoperla carnea* (Green lace-wing), *Orius* spp. (Flower bug), *Metasyrphus corella* (Hover fly), *Paederus alfieri* (Rove beetle) and True Spiders ranged between 79.2–96.9 and 59.5-84.6% in seasons 2013 and 2014, respectively. The side effect of insecticidal applications showed higher mortality rates among the natural enemies, particularly the predatory species tested. The effect varied according to the pesticide used. Figure (1) illustrates the effect of 18 insecticides used in the field trials on *C. undecimpunctata* and *C. carnea*. As shown in the figure, mean reduction rate ranged between 50.2–97.2% in case of *C. undecimpunctata*, and was 59.5–93.6% in case of *C. carnea*. Among the insecticides practiced, Actelic was the most toxic one on both predatory species, while Goldben and Confidate were among the least toxic to *C. undecimpunctata* and *C. carnea*, respectively (Fig. 1).

Table (1): List of tested insecticides, rates of application and target pests in Egyptian cotton fields in 2013 and 2014 cotton growing seasons

Trade Name	Common Name	Group	Rate of Application	Target Pests
Actara 25% WG	Thiamethoxan	Neonictinoid	20 gm/ 100l w	<i>Bemesiatabaci</i>
Actelic 50% EC	Pirimiphos-methyl	OP	375 ml/ 100 l w	<i>B. tabaci</i>
Chess	Pymetrozine	Triazine	125 gm/ 100 l w	<i>B. tabaci</i>
Confidate 35% SG	Imidacloprid	Neonicotinoid	25 g/100 l w	<i>B. tabaci</i>
Delcup 6%	Copper sulfate	Inorganic sulfate	250 ml /100 l w	<i>B. tabaci</i> , <i>Thripstabaci</i>
Detergent pH 7	Fatty acid of potassium	Fatty acid of potassium	1 l/ 100 l w	<i>T. tabaci</i>
Dorsil 48% E	Chlorpyrifos	OP	1 l / feddan	<i>Pectinopheragossypiella</i>
Lannate 90% WP	Methonyl	Carbamate	300 gm/ fed	<i>Spodopteralittoralis</i>
Mospilan 20% SP	Acetamiprid	Neonicotinoid	10 g/100 l w	<i>B. tabaci</i> + <i>Aphis gossypii</i>
Nomolt	Teflubenzuron	Benzylurea	50 ml/ 100 l.w	<i>B. tabaci</i>
Pestban 68% EC	Chlorpyrifos	OP	1 l/ 100 l w	<i>P. gossypiella</i>
Pestox 15% SC	Alfa Cypermethrine	Pyrothroids	165 ml / feddan	<i>P. gossypiella</i>
Super actara 25% WG	Thiamethoxan	Neonictinoid	20 gm/ 100 l.w	<i>B. tabaci</i>
Teleton 72% EC	Profenofas	Organophosphate (OP)	750 ml/ feddan	<i>P. gossypiella</i>
Vertimec 1.8% EC	Abamectin	Avermectin	25 ml + 40 ml Z oil /100 l w	<i>Tetranychusurticae</i>

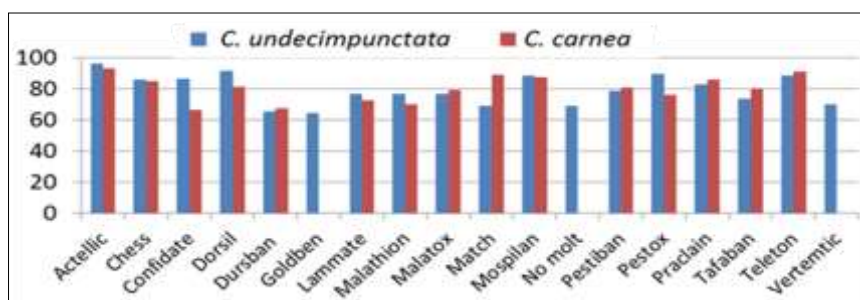


Fig. (1): Mean percentages' reduction in the populations of the predatory species; *C. undecimpunctata* and *C. carnea*, as side effects on non-target organisms resulted from chemical control of pests, using different groups of insecticides, in Egyptian cotton field seasons 2013 and 2014.

According to the IOBC categories, the tested insecticides could be classified to: Actellic, Chess, and Mospilan as class 4 (harmful >75%), Dorsil, Nomolt, Pesteban, Pestox, Teleton and Vertimic as class 3 (moderately harmful 51-75%), and Match as class 2 (slightly harmful 25-50%) in case of the non-target sucking insect pests. None was classified as class 1 harmless (<25% mortality). Dorsil and Pesteban, which are classified as class 3 (moderately harmful 51-75%) on sucking insects showed less toxicity on the spider mite *T. urticae*, as they were classified as class 2 (slightly harmful 25-50%). None was classified as class 1 harmless (<25% mortality). As well, the tested insecticides could be classified according to their toxicity to the predatory species to: Actellic, Chess, Dorsil, Mospilan, Pesteban, Pestox and Teleton as class 4 (harmful >75%) and Lannate, Nomolt and Vertimic as class 3 (moderately harmful 51-75%).

In conclusion, the tested insecticides showed different toxicity classifications toward non-target pests and predatory species. According to the IOBC classification, in case of the non-target pests; 6, 8 and 2 insecticides were classified as class 4, 3 and 2, respectively. In case of the predatory species, they were 9, 4 and 3, respectively. Predatory species were highly susceptible to be affected by insecticidal applications compared even with non-target pests. Working on the side effect on non-target pests, Dar *et al.* (2015) evaluated the toxicity of selecron and trebon against the spiny bollworm, *Earias insulana* and recorded that reduction percentages in the pest ranged between 79.01 and 94.57 %.

In general, Maloney *et al.* (2003) reported that spiders are more sensitive than many pests to some pesticides, such as the synthetic pyrethroids, (cypermethrin and deltamethrin); the organophosphates, (dimethoate and malathion) and the carbamate, (carbaryl). A decrease in spider populations as a result of pesticide use can result in an outbreak of pest populations. Mandour (2009) studied toxicity of spinosad to immature stages of *C. carnea* and its effect on the reproduction and survival of adult stages after direct spray and ingestion treatments. Spinosad was harmless to *C. carnea* eggs and pupae, irrespective of concentrations or method of treatments.

On the other hand, Nadeem *et al.* (2011) designed a study to ascertain the comparative survival of natural population of predator, *C. carnea* in cotton under field conditions, conducted in 2007. The results showed that count of eggs on unsprayed plants was in increasing trend from 2.8 to 25 per plant, larvae from 1.3 to 6.3, while pupae recorded as 0.4 to 3.4 per plant from July to October. Few eggs, pupae and larvae of *C. carnea* were observed in insecticide treatment, which showed the survival of naturally resistant strains of this predator. Cabral *et al.* (2011) evaluated the effects of primicarb and pymetrozine on the voracity of the 4th instar larvae and adults of *C. undecimpunctata* under distinct scenarios of exposure to chemicals. When the insecticides were sprayed on the pray/plant system, the predator's voracity was significantly increased.

Estimation of resistance

As the main enzymes in organisms related to resistance are; Acetylcholinesterase, Glutathione S-transferase, alkaline phosphatase and α -esterases and each has its role as its mechanisms are acting different, the four enzymes were analyzed in the two insect pests; the PBW and the CLW field and laboratory strains as target pests and *C. carnea* and *Orius* spp. as non-target organisms). Results indicated in table (2) showed that biochemical aspects were mostly much higher in the field strains (ranged between 2.99 and 4.21) which means that insecticidal applications induced resistance in such target pests' strains. As well a relative resistance was also recorded in non-target organisms (predators) but with much less levels (ranged between 1.09 and 1.35 in *C. carnea* and 1.04 and 1.41 in *Orius* spp. Hence, enzymes activity plays an important role as a mechanism factor for resistance in organisms.

Table (2): Enzymatic activity in pink bollworm and cotton leaf worm and predatory species

Enzymes/ Species	Acetylcholinesterase		Glutathione S- transferase		Alkaline phosphatase		α-esterases	
	Activity	Activity ratio	Activity	Activity ratio	Activity	Activity ratio	Activity	Activity ratio
<i>P. gossypiella</i>								
Laboratory	2084	1.00	2501	1.00	11.3	1.00	29.7	1.00
Field	8191	4.21	9991.3	3.99	39.7	3.51	88.7	2.99
<i>S. littoralis</i>								
Laboratory	2140.3	1.00	2451.3	1.00	26.3	1.00	23.3	1.00
Field	6985.3	3.27	9805.3	4.00	87.7	3.33	98.0	4.21
<i>Chrysoperla carnea</i>								
Laboratory	1498	1.00	1566.7	1.00	4.7	1.00	24.4	1.00
Field	1903	1.27	1716.3	1.09	5.0	1.06	33.0	1.35
<i>Orius spp.</i>								
Laboratory	2106.7	1.00	2255.0	1.00	2.6	1.00	11.6	1.0
Field 1	2885.7	1.37	3072.7	1.36	3.3	1.27	16.3	1.41
Field 2	2656.7	1.26	3019.3	1.34	3.1	1.19	15.3	1.32
Field 3	2226.0	1.06	2348.0	1.04	2.7	1.04	14.3	1.23

Development of resistance countermeasures and a successful resistance management strategy depends on the nature, frequency and evolution of resistance mechanisms in field population of the organisms (Abdel-Baset, 2009). Most of pesticides are subjected to enzymatic reactions after they are entered in the bodies. The most striking effect of organophosphate and carbonate pesticides is their ability to inhibit acetyl cholinesterase in the cholinergic junction of the nervous systems. Young *et al.* (2005) reported that pyrethroids resistance in field population of Australian *H. armigera* is primarily a consequence of the overproduction of esterase isoenzymes which metabolize & sequester pyrethroid insecticide. Aggrawal *et al.* (2006) demonstrated that laboratory tests showed that an insecticide resistance management strategy resulted in a significant decline (1 to 2.9 fold) in resistance of *H. armigera* larvae to Cypermethrin, fenvalerate, Endosulfan, Methomyl, Guinaphos and Chlorpyrifos. Saleman (2014) mentioned that profenofos resistance was found in field strains of *P. gossypiella* collected at the late season of 2013 from four governorates in Egypt. Resistance was high in regions where profenofos was frequently used. Profenofos resistance was the highest in Gharbia strain as compared with the other field strains; whereas the lowest resistance percentage was noticed in Kafr El-Sheikh strain. Colorimetric assay showed a significant positive correlation between increased resistance to profenofos in strain of *P. gossypiella* tested and the hydrolytic enzymes activity, Acetylcholinesterase, alkaline & acid phosphatases and α- and β- nonspecific esterases as well total protein content.

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