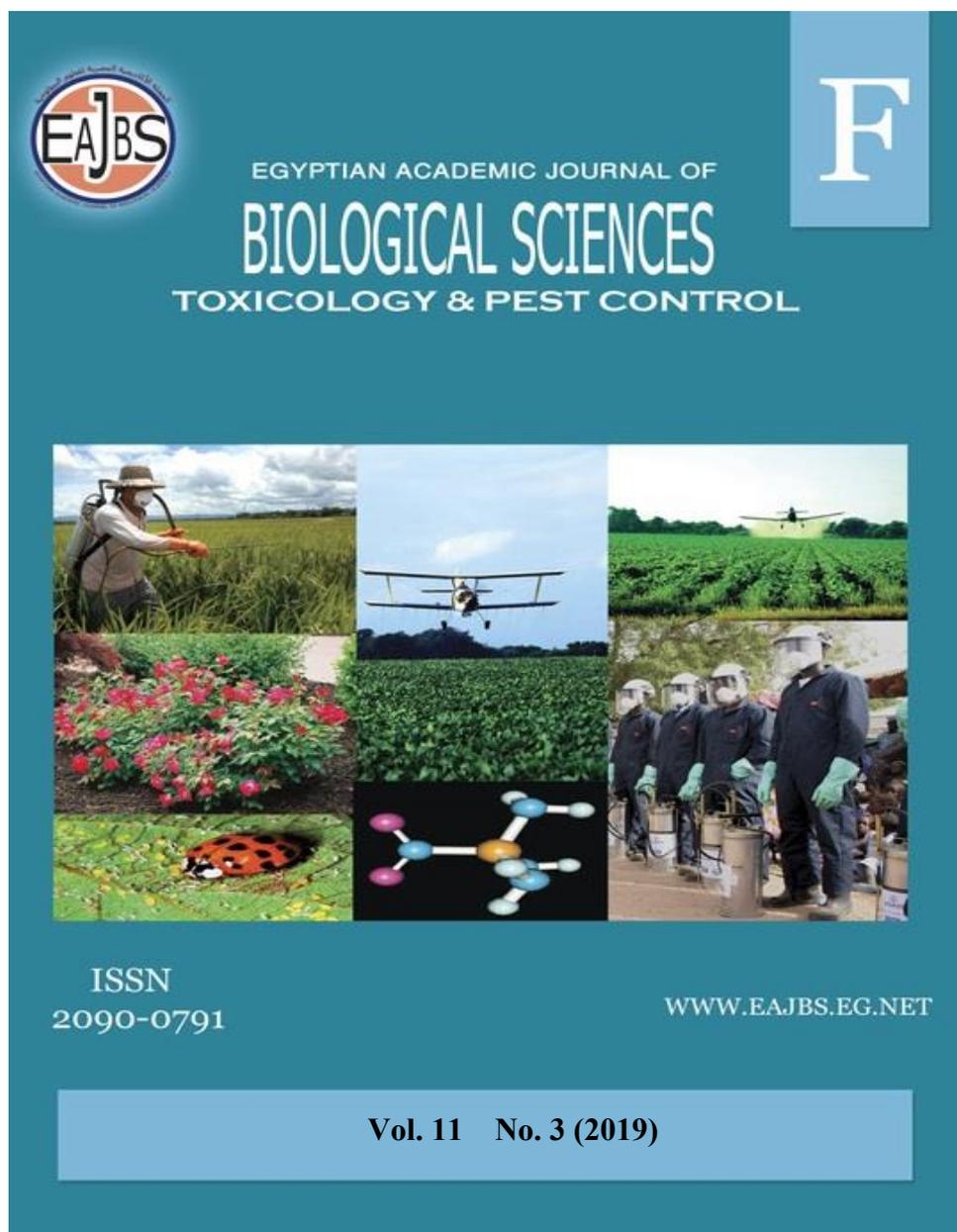


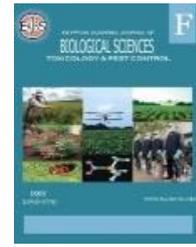
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Evaluation of some Organophosphorous Insecticides against the Tomato Whitefly, *Bemisia tabaci* (GENN.) Collected from some Upper Egypt Governorates with Relation to Esterases Enzyme Activities

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ABSTRACT

The tomato whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is a worldwide pest of many crops. Organophosphorous (OP) insecticides have been used to control this insect for many years and are still being used despite the development of resistance. In this work' evaluation of the biological activity of three organophosphorous insecticides, profenofos 72% E.C. (Selecron); chlorpyrifos 48% E.C.(Dursban) and fenitrothion 50% E.C. (Sumithion) on the felid strain of tomato whitefly was carried out on tomato plants. Whitefly adults were collected from four different Egyptian Governorates throughout three successive years (2016-2018). The LC₅₀ value of each compound in each Governorate was used to determine the biochemical responses of this insect pest. The colorimetric test of esterase activity is a useful tool to detect the level of resistance against this group of insecticides. The obtained results showed that profenofos insecticide was more toxic on the tested insects followed by chlorpyrifos, while fenitrothion showed a low toxic effect on the tested insects in all years. The activities of α and β esterases have fluctuated up and down in some Governorates.

INTRODUCTION

Tomato whitefly *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is a small pest with a great agricultural importance worldwide (Bellows *et al.*, 1994). The insects not only feed on leaves resulting in delayed growth and even death of the plants (Liu *et al.*, 2007), but also deposit honeydew on leaves that often lead to sooty mold and reduction in photosynthesis (Ghanim *et al.*, 1998). Additionally, the insect is also known to transmit tomato yellow leaf curl virus (TYLCV) (Abdel-Salam, 1991).

In Egypt, tomato is considered to be one of the most important vegetable crops. *B. tabaci* is a destructive pest on tomato plants (Shaheen, 1997). The marked capacity of whiteflies to develop resistance to insecticides must, therefore, be viewed with grave concern and as serious threat to the sustainability of current control programs. Resistance already extends to all chemical groups (Dittrich *et al.*, 1990). Insecticides resistance has become a major obstacle to successful chemical control with conventional insecticides. The evaluation of resistance to insecticides is governed by a complex of events and

factors; mainly, intense and repeated applications of insecticides which are often from the same chemical group or which employ the same mode of action. Chlorpyrifos has been used to control tobacco whitefly and other insect pests for many years (Pasteur and Sinègre, 1978; Milio *et al.*, 1987; Rust and Reiersen, 1991; Archer, 1994; Guides *et al.*, 1996; Liu *et al.*, 2005; Curtis and Pasteur, 2009) and is still used in many countries.

In the early days, studies of whitefly insecticide resistance mechanisms were mainly at biochemical and toxicological levels (Hansen and Hodgson, 1971; Gunning *et al.*, 1992; Byrne *et al.*, 1995; Gunning *et al.*, 1996; Valles and Woodson, 2002). Esterase enzymes play an important role in conferring or contributing to insecticide resistance in insects (Guillemaud *et al.*, 1997; Campbell *et al.*, 1998; Field and Devonshire, 1998; Claudianos *et al.*, 1999). Esterases are also used as bioindicators to measure the toxic potency of pesticide residues usually applied in agriculture. Esterase isozyme appears to be playing a critical role in offering resistance to insecticides (Karunaratne *et al.*, 1999).

Organophosphorus acts as an inhibitor of certain esterases. When organisms are treated with insecticides, continuous nerve impulse transmission due to inhibition of acetylcholine esterase causes them to be shaky and have writhing movements. This may result in sudden death of an organism. The low esterase activity may be used as a marker for resistant individuals in populations of *B. tabaci* (Wool and Greenberg, 1990).

In the present investigation the state of adult resistance to some organophosphorus (OP) insecticides was evaluated and estimate the non-specific esterase enzymes activity (α -E & β -E) of different field strains of tomato whitefly in six different Egyptian Governorates. The study was extended over three years.

MATERIALS AND METHODS

The toxicological studies were carried out on tomato plants for three successive years (2016, 2017 and 2018). The bioassay method was done according to Dittrich *et al.*, (1985 & 1990). The tomato whitefly adults of both sexes were exposed to leaf discs dipped in aqueous media of the formulated tested insecticides' profenofos 72% E.C. (Selecron); chlorpyrifos 48% E.C. (Dursban) and fenitrothion 50% E.C. (Sumithion) for ten seconds and then laid flat on a thin layer of 1% agar in plastic Petri dishes. About 30-40 collected adults from six different Governorates were placed in each Petri dish. Mortality counts were made after 24 hours. The average mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971). Resistance indexes (RI) are calculated by dividing each LC_{50} values of each strain on that of the strain with the lower LC_{50} value for each insecticide.

The biochemical assay was done on the adult homogenates of different insect strains collected from different Governorates. After centrifugation the supernatant was used directly for enzyme assay. Alpha- and Beta- esterases activities (α -E & β -E) were determined according to the method of Van Asperen (1962) using alpha- and beta-naphthyl acetate (α - and β -NA) as substrates. The percentage activity is calculated by dividing the activity of each strain on the activity of the strain that has the lower LC_{50} for each insecticide.

RESULTS AND DISCUSSION

Toxicological Study:

The toxicological study was carried out on adults of tomato whitefly during three successive cultivated years (2016, 2017 and 2018). Table 1 illustrates the toxicities of

each tested OP insecticide (ppm/mg b.wt.) on each strain. Table 2 represents the resistance index (RI) of each insecticide related to the strain that has the lower LC₅₀ value for each insecticide. During the first year (2016) as shown in Table (2), Giza strain and Menia strain showed a high resistance index (47.50, 31.30) respectively for chlorpyrifos insecticide compared to the other strains, while Bani-Swief- strain showed moderate tolerance (RI = 7.2). On the other hand, Faium showed high susceptibility to the chlorpyrifos insecticide. Regarding to fenitrothion insecticide, Giza strain and Faium strain showed high resistance index (44.60, 15.1) respectively. On the other hand, Bani-Swief and Menia strain show high sensitivity to fenitrothion insecticide. While the insecticide profenofos exhibited low levels of toxicity to all tested strains expect Giza strain showed high resistance index (103.2). Form this result suggested that, Giza strain showed high resistance to all insecticide.

Table 1: Toxicity (ppm/mg b.wt.) of some OP insecticides against different field strains of tomato whitefly during three successive years

Strains	First year (2016)			Second year (2017)			Third year (2018)		
	Chlorp.	Fenit.	Profen.	Chlorp.	Fenit.	Profen.	Chlorp.	Fenit.	Profen.
Giza	15.2	316.4	16	229.7	745.3	68.1	19.9	188.1	1.45
Faium	0.3	107.0	0.155	970.9	837.3	79.3	104.7	40.3	11.17
Bani-Swief	2.3	7.1	0.37	132	103.5	356	216.0	318.47	174.24
Menia	10.0	13.0	0.167	439.3	28.5	90	81.3	87.2	126.04

Chlorp. = chlorpyrifos, Fenit. = fenitrothion, profen. = profenofos

Table 2: Resistance index (RI) of the tested OP insecticides of different tested field strains of whiteflies during three successive years

Strains	First year (2016)			Second year (2017)			Third year (2018)		
	Chlorp.	Fenit.	Profen.	Chlorp.	Fenit.	Profen.	Chlorp.	Fenit.	Profen.
Giza	47.5	44.6	103.2	1.7	26.2	1.0	1.0	4.7	1.0
Faium	1.0	15.1	1.0	7.4	29.4	1.2	5.3	1.0	7.7
Bani-Swief	7.2	1.0	2.4	1.0	3.6	5.2	10.9	7.9	120.2
Menia	31.3	1.8	1.1	3.3	1.0	1.3	4.1	2.2	86.9

Chlorp. = chlorpyrifos, Fenit. = fenitrothion, profen. = profenofos

During the second year (2017), all tested insecticides showed high resistance on Giza and Faium governorates for chlorpyrifos insecticide (26.2, 29.4) respectively. The average of tested governorates in the second years clearly reveals that the profenofos insecticide was more toxic on the tested insects, while chlorpyrifos and fenitrothion insecticide showed low toxic effect on the tested insects. Profenofos show low resistance for all governorates expect Bani-Swief shows high resistance for all treated insecticide.

During the third year (2018), Bani-Swief shows high resistance for all treated insecticide was more toxic on the tested insects except Menia strain show high resistance. Chlorpyrifos show moderate resistance, while fenitrothion insecticide showed low toxic effect on the tested insects. The result suggested that the profenofos insecticide was more toxic on the tested insects, Giza governorate more resistance in the first year, Faium governorate more resistance in the second year and Bani-Swief show high resistance during the third year. All tested OP compounds become less effective on whiteflies during the second and third years. The variation in the sensitivity of whiteflies may be

due to the variation in the cultivated crops in these areas, lead to building up resistance against them.

Biochemical Study:

The data resulted from the biochemical assay represented changes in the activity of both esterases enzymes in different tomato whitefly strains collected from four different Egyptian Governorates during three successive years in relation to the toxicities of some OP insecticides. The percentage activity is calculated by dividing the activity of each strain on the activity of the strain that has the lower LC₅₀ for each insecticide.

Changes in Esterases Activity:

The data in Table 3 revealed that the highly resistant strain to chlorpyrifos, (Giza) showed low level of activity of both esterases during the first year compared with the sensitive one (Faium). While in the second year there are differences in esterases activities between the sensitive and resistant strains. On the other hand, the high resistant strain (Bani-Swief) to chlorpyrifos characterized by a high level of α -esterase activity during the third year compared to more sensitive one (Giza).

Table 3. Esterases activity in the different tomato whitefly strains collected from four different Governorates during three successive years in relation to chlorpyrifos insecticide toxicity

Strains	First year (2016)			Second year (2017)			Third year (2018)		
	LC ₅₀	%Activity		LC ₅₀	%Activity		LC ₅₀	%Activity	
	Chlorp.	α -est.	β -est.	Chlorp.	α -est.	β -est.	Chlorp.	α -est.	β -est.
Giza	15.20	60.60	69.90	229.70	66.10	53.10	19.87	100.00	100.00
Faium	0.30	100.0	100.00	970.90	122.60	95.00	104.73	188.20	254.30
Bani-Swief	2.30	89.10	63.30	132.00	100.00	100.00	216.00	62.40	95.10
Menia	10.00	130.20	100.20	439.30	41.70	46.60	81.27	167.30	142.40

% Enzyme activity was calculated by dividing the activity value of each strain on that of the base strain having the lower LC₅₀ value. Chlorp. = chlorpyrifos

Table 4. Esterases activity in the different tomato whitefly strains collected from four different Governorates during three successive years in relation to fenitrothion insecticide toxicity

Strains	First year (2014)			Second year (2015)			Third year (2016)		
	LC ₅₀	%Activity		LC ₅₀	%Activity		LC ₅₀	%Activity	
	Fenit.	α -est.	β -est.	Fenit.	α -est.	β -est.	Fenit.	α -est.	β -est.
Giza	316.4	68.0	110.4	745.3	158.4	114.0	188.1	53.1	39.3
Faiume	107.0	112.2	158.1	837.3	293.9	203.8	40.3	100.0	100.0
Bani-Swief	7.1	100.0	100.0	103.5	239.8	214.6	318.47	33.2	37.4
Menia	13.0	146.1	158.4	28.5	100.0	100.0	87.2	88.9	56.0

Fenit. = fenitrothion,

The relations between fenitrothion insecticide toxicity and the changes in esterases enzymes activities in different tomato whitefly strains collected from different Egyptian Governorates were represented in Table(4). *From the present results it is evident there is no relation between changes in esterase activities and the toxicity of the compound, however, the high resistant strain (Giza) showed low level of α esterases activities during first year , on the other hand the highly resistant strain (Faium) showed high level of α and β esterases activities during second year, while in the third year, Bani-Swief showed

low level of α and β esterases compared with the sensitive one (Faium). On the other hand, the highly resistant strain (Bani-Swief) to fenitrothion characterized by a low level of α and β esterase activities during the third year compared to the sensitive ones.

In regard to the data of the relation between the toxicity of profenofos insecticide and the changes in esterases enzyme activities in different tomato whitefly strains collected from different Egyptian Governorates were represented in Table (5). There was distinct relation between changes in esterase activities and the insecticide toxicity. However, the high resistant strain (Giza) showed low levels of esterases activities during the first year. On the other hand, the high resistant strain (Bani-Swief) showed high levels of esterases activities during the second year, and low levels of esterases during the third year in the high resistant strain (Bani-Swief).

From the present results, it is obvious that profenofos insecticide was more toxic on the tested insects followed by chlorpyrifos insecticide, while fenitrothion insecticide showed a low toxic effect on the tested insects in all years, but in the third year. The variation of resistance in whiteflies may come back to the variation in the cultivated crops in different governorates, lead to building up resistance against them.

Table 5. Esterases activity in the different tomato whitefly strains collected from four different Governorates during three successive years in relation to profenofos insecticide toxicity

Strains	First year (2016)			Second year (2017)			Third year (2018)		
	LC ₅₀	%Activity		LC ₅₀	%Activity		LC ₅₀	%Activity	
	Profen.	α -est.	β -est.	Profen.	α -est.	β -est.	Profen.	α -est.	β -est.
Giza	16	60.6	109.4	68.1	100.0	100.0	1.45	100.0	100.0
Faiume	0.155	100.0	100.0	79.3	185.6	178.7	11.17	188.2	254.3
Bani-Swief	0.37	89.1	99.1	356	151.4	188.2	174.24	62.4	95.1
Menia	0.167	130.2	156.9	90	63.1	87.7	126.04	167.3	142.4

profen. = profenofos

New chemistry, such as the insect growth regulators, provides additional options in integrated control management programs and may delay or reduce resistance to conventional chemistries (Ellsworth *et al.*, 1996; Palumbo *et al.*, 2001). Using Insecticide mixtures to avoid the development of insecticide resistance has been advocated by several researchers (Ishaaya *et al.*, 1985; Ascher *et al.*, 1986; Mushtaq, 2004). Esterase activity is useful as an indicator of the frequency of organophosphorous resistant strains. The low esterase activity may be used as a marker for resistant individuals in the populations of *B. tabaci* (Wool and Greenberg, 1990). The effect of chlorpyrifos insecticide on the activity of α and β esterases enzymes in *B. tabaci* in the third year shows a decrease in α - β esterase activity in the high resistant strain (Bani-Swief) that agree with Wool and Greenberg (1990). The effect of fenitrothion insecticide on the activity of α and β esterases enzyme activities in third year shows a decrease in α -esterases activity in high resistant strain (Bani-Swief). The effect of profenofos insecticide on the activity of α and β esterases enzyme activities in third-year show decrease in α -esterases activity in the high resistant strains (Bani-Swief) and these binding agree with those of Wool and Greenberg (1990). Esterase enzymes play an important role in conferring or contributing to insecticide resistance in insects (Claudianos *et al.*, 1999).

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ARABIC SUMMARY

تقييم بعض المبيدات الفوسفورية على ذبابة الطماطم البيضاء المجمعّة من بعض محافظات الوجه القبلي وعلاقتها
بأنشطة أنزيمات الإستيريزات

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ذبابة الطماطم البيضاء آفة واسعة الانتشار وتصيب العديد من المحاصيل وقد تم استخدام المبيدات الفوسفورية للسيطرة على هذه الحشرة لسنوات عديدة ، وما زالت قيد الاستخدام على الرغم من ظهور صفة المقاومة ضدها، في هذا العمل تم تقييم الفاعلية لثلاث مبيدات من المبيدات الفوسفورية المختلفة كمرکبات قابلة للاستحلاب وهي بروفينوفوس 72% (سليكرون) ، كلوربيريفوس 48% (دروسبان) وفينيتروثيون 50% (سوميثيون) على السلالة الحقلية لذبابة الطماطم البيضاء على نباتات الطماطم التي تم جمعها من أربع محافظات مصرية مختلفه طوال ثلاث سنوات متتالية (2016-2018). وتم استخدام قيم الجرعات النصفية المميّة للمركبات المختبرة في تقدير الإستجابات البيوكيميائية على هذا النوع من الذباب الأبيض. يعتبر الاختبار اللوني لإنزيمات الإستيريزات أداة مفيدة للكشف عن مقاومة الحشره للمبيدات الفوسفورية. وأظهرت النتائج للمركبات المختبرة أن مبيد البروفينوفوس أكثر سمية على الحشرات المختبرة يليه الكلوربيريفوس ، بينما أظهر مبيد الفينيتروثيون تأثير منخفض للسمية على الحشرات المختبرة في جميع السنوات وجد ارتفاع في مستوى المقاومة للذبابة البيضاء بالنسبة لجميع المبيدات المختبرة ، ولقد أظهرت النتائج حدوث تقلبات في أنشطة أنزيمي ألفا و بيتا إستيريزات لأعلى ولأسفل في بعض المحافظات.