Assessment of Certain Neonicotinoid Compounds on *Tetranychus cucurbitacearum* (Sayed) and Predatory Mite, *Phytoseiulus macropilis* (Banks)

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ABSTRACT

Generally, the two tested neonicotinoides, acetamiprid and thiamethoxam were more toxic against both adults and one day old eggs of *T. cucurbitacearum* using spraying method than dipping technique method under laboratory conditions. Moreover, positive relationship was established between the number of treated prey (devoured females of *T. cucurbitacearum*) consumed per predatory mite, *Phytoseiulus macropilis* (Banks) (15.28±0.34, 11.17±0.89 and 28.22±0.70) and number of deposited eggs per predator (3.00 ±0.27, 2.33±0.35 and 6.33±0.06 eggs) for acetamiprid, thiamethoxam and control, respectively. Field experiments were carried out with the two neonicotinoides against moving stages of *T. cucurbitacearum* during 2014 season at Zagazig district, Egypt. Data revealed that slight reduction in *T. cucurbitacearum* numbers comparing to control were obtained and recorded 8.29±0.582 and 2.58±0.435% in the initial effect of acetamiprid and thiamethoxam, respectively, followed by highly sudden increase in *T. cucurbitacearum* numbers reaching to -17.54±1.102 and -62.40±1.434%, in the general effect, respectively. Fixed elevation trend was observed in the activities of α-, β- and acetylcholine esterase enzymes against field strain of *T. cucurbitacearum*. Acetamiprid gave the highest significant increase reaching to (85.06, 36.96 and 71.10 % relative to control); while thiamethoxam expressed (51.07, 30.66 and 4.13 % compared to control), respectively.

Key words: Neonicotinoides, Tetranychidae, Phytoseiidae, esterse enzymes.

INTRODUCTION

Soybean, *Glycin max* L. is one of the most important legume crops all over the world. In the field, the plants are attacked by several serious pests of which the spider mite, *Tetranychus cucurbitacearum* (Sayed) is considered one of the major pests attacking different crops as field crops, vegetables, fruits and ornamental plants (Taha *et al.*, 2001 and Magouz and Saadoon, 2005). Although pesticides may be injurious to non-target plants and animals or humans, they are widely used in agricultural ecosystems (Ecobichon, 2001 and Marrs, 2012).

Neonicotinoides are new synthetic group of insecticides (Jeschke and Nauen, 2008), being derivatives of nicotine and classified as N-nitroguanidines (imidacloprid, thiamethoxam, dinotefuran, and clothianidin) and N-cyano-aminides (acetamiprid and thiaclopride). These active components determine the insecticidal potency and selectivity of the insecticides (Bolboaca and Jantschi, 2005) and (Goyal *et al.*, 2010).

They can act as contact, stomach and systemic compounds (Elbert *et al.*, 2008 and Anikwe, *et al.*, 2009). Neonicotinoides act selectively on insect nicotinic acetylcholine receptors (nAchR), accounting at least in part for the selective toxicity to insects over vertebrate (Silcox and Vittum, 2008 and Malev *et al.*, 2012). They act as agonists at the insect Nicotinic acetylcholine receptors, which plays an important role in synaptic transmission in the central nervous system (Muccio *et al.*, 2006).

Fundamental differences between the nACHRs of insects and mammals confer remarkable selectivity for the neonicotinoides (Tomizawa and Casida, 2003).

The objectives of this work were as follows: first to elucidate efficiency of two neonicotinoides (Acetamiprid and Thiamethoxam) belonging to two different groups; N-Cyano-aminides and N-nitroguanidines against *T. cucurbitacearum* under laboratory and field conditions. The second was to study their side effect on the predatory mite, *P. macropilis* and the third one was to determine their activities on the enzymes related to the nervous system of *T. cucurbitacearum*.

MATERIALS AND METHODS

1- Tested neonicotinoides:

1.1. Acetamiprid (Mospilan 20 % SP):

Chemical name: (*E*)-*N*-[(6-chloro-3-pyridinyl) methyl]-N'-cyano-N-methylethanimidamide, used at the rate of 25 g / 100 L. of water, a trademark of Sumitomo corporation under License Nippon SODA CO., Ltd-Tokyo- Japan.

1.2. Thiamethoxam (Pilot 25 % WG):

Chemicale name: 3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-4*H*-1,3,5-oxadiazin-4-imine, used at the rate of 50g/ 100 L. of water, a trademark of Rotam Agrochemical Co. LTD-Egypt under License of Rotam Agrochemical CO., LTD - Hong Kong.

2 - Rearing technique of *T. cucurbitacearum*:

Samples of egg plants, *Solanum melongena* L.

(Solanaceae) heavily infested with *T. cucurbitacearum* were collected from Zagazig district, Sharkia governorate. Pure culture of *T. cucurbitacearum* was initiated by transferring males and females using a fine hairbrush to fresh discs of mulberry leaves in Petri-dishes (10 cm in diameter). Each leaf was put on a pad of cotton saturated with water as a source of moisture and to prevent mite escaping, under laboratory conditions (27 ± 2 °C and 75 ± 5% R. H.).

3- Rearing technique of *Phytoseiulus macropilis*:

Predatory mite species, *P. macropilis* was reared on the red spider mite, *T. cucurbitacearum* using methods modified by (McMurtry and Scriven, 1965). The predacious mite was reared in large plastic boxes 26×15×10 cm. cotton pad was placed in the middle of each box, leaving a space provided with water as a barrier to prevent predatory mite from escaping. Cotton leaves highly infested with *T. cucurbitacearum*, were provided every day as food source for the predacious mite. Water was added to the plastic pan whenever required, to prevent the mites from escaping. The culture units for all species were kept at room temperature 27±4 °C).

To obtain a new generation of adult females in the same age, graved females of *P. macropilis* were randomly selected from cultures (30 females) and released onto detached mulberry leaves (one predator for every 10 females of *T. cucurbitacearum*). The heavily infested mulberry leaves were placed up-side down on a filter paper (9×9 cm) placed on a piece of cotton wool (approximately 9×9×1 cm) to ensure a good supply of food for the predatory until three to four days after reaching adulthood. Gravid females were randomly selected for use in this experiment (Abdallah and El-Kawas, 2010).

4- Acaricidal activity of tested neonicotinoides against adult females of *T. cucurbitacearum* using two different methods:

To evaluate the toxic effects of some neonicotinoides on the adult females of *T. cucurbitacearum*, two different methods were used, leaf dip technique (feeding on treated leaves) and spraying technique. Two different concentrations of acetamiprid (50 and 25 ppm.) and thiamethoxam (125 and 62.5 ppm.) were chosen. Each treatment and the control were replicated 5 times (15 adult females per each). Control disks were prepared in both methods using water.

Adult females of *T. cucurbitacearum* were confined on the lower surface of mulberry leaf discs (3 cm. in diameter) which were dipped in tested concentrations of each treatment for 10 seconds using the leaf-dip technique method as described by Dittrich (1962). In the second method, the tested concentrations were sprayed on both mulberry leaves and adults of *T. cucurbitacearum*. Mortality was calculated after 72h. post treatment and corrected according to Abbott's formula (1925).

5- Acaricidal effects on eggs of *T. cucurbitacearum:*

The toxic effects on the egg stage was studied by confining four females that transferred to each leaf disc of mulberry using fine hairbrush (2.5 cm. in diameter) for 24 h. to deposit eggs on the lower surface of leaf discs, and then removed. These discs with deposited eggs (24 h. old) were sprayed with the same percent concentrations for 10 seconds and the excess solution was dried off by filter paper. Each concentration and control was replicated 3 times (50 eggs per each). The control was prepared using water only. Eggs were kept under laboratory conditions and hatchability of eggs on each concentration was recorded till 7 days post treatment.

6. Function response of the predatory mite, *P. macropilis*:

To estimate the number of devoured females of *T. cucurbitacearum* treated with the recommended rate of each tested neonicotinoid and the control that were consumed per predatory mite, *P. macropilis* until 72 h. of treatment, the closed cages called Huffaker cells were used (Huffaker, 1948). In each treatment, 10 Huffaker cells were handled as replicates, (10 treated females of prey/Huffaker cell). Additionally, the number of deposited eggs per predator were counted at 72 h. post treatment.

7. Field trails:

Field experiments were carried out at Zagazig district, Sharkia governorate, Egypt, during the soybean, season, 2014, in the first week of July. An area of about 1.5 feddans (6300 $cm^{2)}$ cultivated with soybean variety (Giza 111) was chosen for all treatments. Each area was divided into four experimental plots as replicates. Untreated belt was left between each two treatments as a border. A knapsack sprayer was used for spraying 200 liters solution per feddan to give a good coverage on the plants.

Numbers of moving stages of the red spider mite, *T. cucurbitacearum* were counted before and at 3, 7 and 15 days post treatment according to protocol (Anonymus, 2013). The reduction percentages at the 3^{rd} day were considered as initial kill effect; while the general mean of residual effect was calculated as the mean reduction percentages of individuals observed at 7^{th} and 15^{th} days post treatment for the tested compounds. Each compound was used at its recommended rate.

Before and after treatments, 100 leaves were investigated randomly (25 leaves × 4 replicates) for each treatment and control. This technique consists of cutting five leaves from three levels of soybean plants which selected randomly in each replicate (25 leaves/ replicate) and taken to the laboratory to determine the number of alive immature stages with the aid of a binocular microscope (Naranjo and Flint, 1994). The reduction percentages in *T. cucurbitacearum* moving stages were calculated according to the equation of Henderson and Tilton (1955).

8. Biochemical assay:

The preparation of samples involved the use of *T. cucurbitacearum* after 3 days of treatment with all tested compounds and control. The field populations of mites were collected from soybean leaves during growing season 2014, when the climate is suitable for the development of *T. cucurbitacearum.* Treated soybean leaves and control were transferred in paper bags to the laboratory and checked under a binocular microscope, to exclude dead mite individuals, then placed in clean jars. Mite masses were homogenized in distilled water using a teflon homogenizer surrounded with jacket of crushed ice for 3 minutes. The homogenate was centrifuged at 3500 r.p.m. for 10 min. at 5° C to remove the cellular debris. The samples were divided into three small portions and kept in a deep freezer at (-20 °C) until required. The supernatant was used to determine the activity of some selected enzymes.

8.1. Non specific esterases (Alpha and Beta esterases):

Alpha and β- esterases were determined according to the method of Van Asperen (1962) using α – naphthyl and β-naphthyl acetate as substrates, respectively.

8.2. Acetyl cholin esterase:

Acetyl choline-esterase activity was measured according to the method described by Simpson *et al.*, (1964), using acetycholine bromide (AChBr) as substrate.

9- Statistical analysis

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) (Snedecor and Cochran, 1980). Data were subjected to statistical analyses using a software package CoStat® Statistical Software (2005) a product of Cohort Software, Monterey, California.

RESULTS AND DISCUSSION

Toxicity on adult females:

The tested neonicotinoides, acetampirid and thiamethoxam at the two tested concentrations showed low toxicity on adult females of *T. cucurbitacearum* at 72 h. post treatment, Table (1). The mortality percentages using spray technique ranged between 5.00 % for acetamiprid at the lowest concentration (25 ppm.) to 22.50 % for thiamethoxam at the highest concentration (125 ppm.). While, using dipping technique, they ranged between 7.50 % for the lowest concentration of both neonicotinoides to 12.50 % for the highest concentration of thiamethoxam.

Toxicity on one-day old eggs:

The same trend was obtained against one-day old eggs of *T. cucurbitacearum* after 7 days post treatment. According to unhatchability percentages, the spraying method was more toxic than the dipping technique that ranged between 22.22 to 28.89 % for acetamiprid at 25 ppm. to thiamethoxam at 125 ppm., respectively, comparing to 8.22 to 21.11 % for thiamethoxam at 62.50 ppm. to acetamiprid at 50 ppm., respectively in the second method, Table (2).

Impact on function response of the predatory mite, *P. macropilis*:

The number of prey (devoured females of *T. cucurbitacearum*) treated with tested neonicotinoides consumed per predator *P. macropilis* and number of deposited eggs per predator were considered as a functional response of *P. macropilis*.

All tested neonicotinoides affected significantly the rate of consumption of *T. cucurbitacearum* exhibited by *P. macropilis* than control, recording 15.28±0.34, 11.17±0.89 and 28.22±0.70 females of *T. cucurbitacearum* for acetamiprid, thiamethoxam and control, respectively, $P= 0.0000^{***}$ (as initial effect after three days after spray), Fig. (1). The direct proportional established between the mean consumption values for *P. macropilis* on treated females of *T. cucurbitacearum* and number of deposited eggs by *P. macropilis* is represented in Figs (1, 2). The mean numbers of deposited eggs were 3.00±0.27, 2.33±0.35 and 6.33±0.06 eggs/female predator, respectively. All the tested neonicotinoides caused significant decrease in deposited eggs than the control, $P=0.0001^{***}$.

The two neonicotinoides exhibited poor acaricidal effect. Based on mortality percentages of *T. cucurbitacearum* adult females and unhatchability percentages of its one day old eggs, the tested neonicotinoids were more toxic against both adult females and eggs of *T. cucurbitacearum* using

Table (1): Toxicity of two neonicotinoides to adult females of *Tetranychus cucurbitacearum* (Sayed) after 72 h. post treatment

Treatments	Rate (ppm.)	Mortality (%)	
		Spray technique	Dipping technique
Acetamiprid	50	12.50	10.50
	25	5.00	7.50
Thiamethoxam	125	22.50	12.50
	62.5	15.00	7.500

Table (2): Ovicidal activity of some neonicotinoides on one day old eggs of *T. cucurbitacearum* after 7 days post treatment.

Treatments	Rate (ppm.)	Unhatchability (%)	
		Spray technique	Dipping technique
Acetamiprid	50	25.56	21.11
	25	22.22	18.25
Thiamethoxam	125	28.89	12.22
	62.5	26.67	8.22

Table (3): Effect of neonicotinoides; Acetamiprid and Thiamethoxam on *T. cucurbitacearum* numbers in soybean fields during season 2014

Treatments	Rate gm / 100 L. water	Mean no. of moving stages before spray	Mean number and % reduction in moving stages after spray at indicated periods:						General mean of residual effect %
			Initial effect		Residual effect				
			3 days		7 days		15 days		
			No.	% R.	No.	% R.	No.	% R.	
Acetamiprid	25	6.40	14.50	8.29±0.58	23.20	-15.19±0.91	12.30	-19.89±0.89	-17.54±1.10
Thiamethoxam	50	5.90	14.20	2.58±0.43	29.30	-57.80±2.81	15.80	-67.00±2.52	-62.40±1.43
Control	-	6.80	16.80	-	21.40	-	10.90	-	-
L.S.D. $_{0.05}$	-	-	-	2.017	-	8.206	-	7.412	5.025
P	-	-	-	0.0014^{**}	-	0.0001^{***}	-	0.0001^{***}	$.0000^{***}$

- Data expressed as (mean ± standard error) at the given intervals post treatment.
- Means under each variety sharing the same letter in a column are not significantly different at P<0.05.
- (-) values referred to increase in the number of *T. cucurbitacearum* comparing to control.

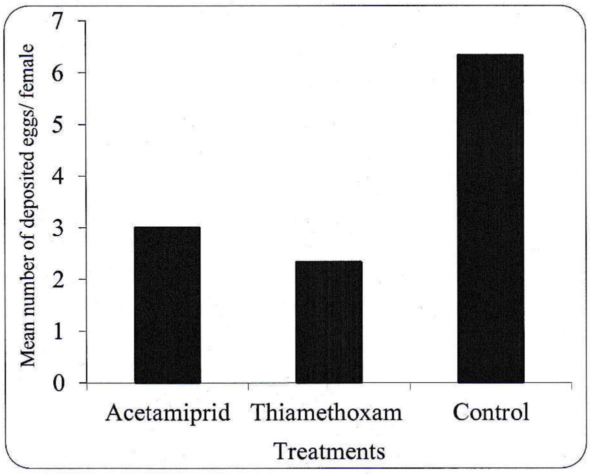

Fig. (1): Mean number of devoured *T. cucurbitacearum* females treated with some neonicotinoides by *P. macropilis* after 72h.

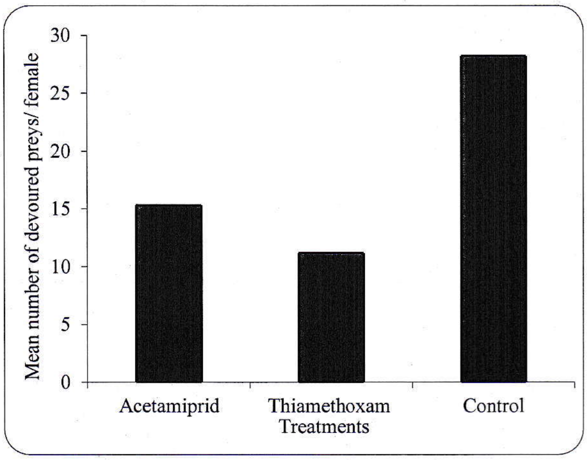

Fig. (2): Mean number of deposited eggs of *P. macropilis* female, after feeding on *T. cucurbitacearum* females treated with some neonicotinoides after 72h.

spraying method than dipping technique method. Anikwe *et al.*, (2009) reported that the effect of thiamethoxam was contact, stomach and systemic activity, whereas, it was only contact and systemic with acetamiprid.

In the spraying method, adults and eggs of *T. cucurbitacearum* are vulnerable to not only feeding on treated leaves but also to direct contact with neonicotinoides that penetrate through its cuticle, thus increase the toxicity than dipping. In another study, Mead (2012) found that essential oil of lemon grass; *C. citratus* was more toxic to *T. urticae* adults and eggs using spraying method than dipping technique.

P. macropilis is a predator with a special feeding habit, consuming exclusively mites that belong to the genus *Tetranychus* (McMurtry and Croft, 1997). The use of functional response of *P. macropilis* to contaminated *T. cucurbitacearum* adults with neonicotinoides was adequate to evaluate the toxicity of these insecticides. Similarly, Poletti *et al.*, (2007) reported that all the tested neonicotinoides caused a significant reduction in *P. macropilis* consumption compared to control.

Positive relationship was obtained between mean variation in consumption and mean number of deposited eggs per *P. macropilis.* Therefore, a reduction in the predatory capacity on the contaminated adults of *T. cucurbitacearum* after spraying with acetamipirid and thiamethoxam might affect its reproductive capacity, inhibiting its population growth and consequently affecting its mites predatory behavior. Additionally, Poletti *et al.*, 2007 conducted similar work to evaluate the impact of neonicotinoides on the predatory capacity of the phytoseiid mite, *P. macropilis* on the tetranychid mite, *T. urticae* eggs at densities of 5, 10, 20, 40, 60 and 80 eggs / arena. They found acetamiprid detrimental to the performance, of *P. macropilis* from a density of 20 *T. urticae* eggs per arena, while thiamethoxam significantly reduced *P. macropilis* consumption from a density of 40 *T. urticae* eggs per arena. The consumption exhibited by *P. macropilis* was always higher in the case of thiamethoxam than the value estimated for acetamiprid.

Field studies:

Table (3) summarized the efficacy of the two tested neonicotinoides, acetamiprid and thiamethoxam against *T. cucurbitacearum* moving stages infesting soybean fields during season 2014.

The initial effects recorded slight reduction in *T. cucurbitacearum* moving stage number compared to control that recorded 8.29±0.582 and 2.58±0.435 % for acetamiprid and thiamethoxam, respectively, following by sudden outbreak in *T. cucurbitacearum* numbers relative to control that reached to (-15.19±0.909 and -19.89±0.890%) for acetamiprid and (- 57.80±2.812 and -67.00±2.517%) for thiamethoxam after 7 and 15 days, respectively. The general means of residual effect recorded - 17.54±1.102 and - 62.40 ±1.434 % for acetamiprid and thiamethoxam, respectively.

Acetamiprid significantly reduced the population of *T. cucrbitacearum* than thiamethoxam in both initial and general mean of residual effect, P= 0.0014 and 0.0000, respectively.

As a general trend, acetamiprid and thiamethoxam treatments resulted increase of *T. cucurbitacearum* populations compared to untreated check at different time intervals with the exception of the initial effect that gave slight reduction in *T. cucurbitacearum* numbers than the control that didn't exceed 8.29±0.582 %. It's noteworthy that outbreak of *T. cucurbitacearum* populations occurred in this study is intriguing. Several authors postulated many theories to explain the relationship between increase of mite populations and neonicotinoides application. Szczepaniec *et al.*, (2011) demonstrated that imidaclopride (neonicotinoide) increased spider mite fecundity through a plant mediated mechanism. James and Price (2002) added that neonicotinoides have a hormoligant effect on mites, causing increase in fecundity. Additionally, the massive break of *T. urticae* that are frequently seen on Hops in Washington may be a consequence of imidacloprid stimulation of mite reproduction with suppression of natural enemies (James and Coyle, 2001).

From our laboratory experiments, the tested neonicotinoides showed significantly reduced reproduction capacity of the mite predator, *P. macropilis*. Debilitation of key predators had been suggested as a likely cause of mite out break (Sclar *et al.*, 1998).

Acetamiprid was more effective insecticides than thiamethoxam against *T. cucurbitacearum* infesting soybean fields. On contrary, Al-Kherb, (2011) showed that the tested neonicotinoides significantly suppressed the population of immature stages and adults of whitefly. The nitro-substituted neonicotinoides insecticides (thiamethoxam and imidacloprid) were the most toxic to whitefly and some predators infesting summer and autumn plantations of cucumber and tomato plants than the cyano-substituted neonicotinoid (acetamiprid) which exhibited a much lower toxicity. The same arrangement was obtained by Iwasa *et al.*, (2004) when tested the same neonicotinoides against honey bee, *Apis mellifera*. This contradiction may be due to

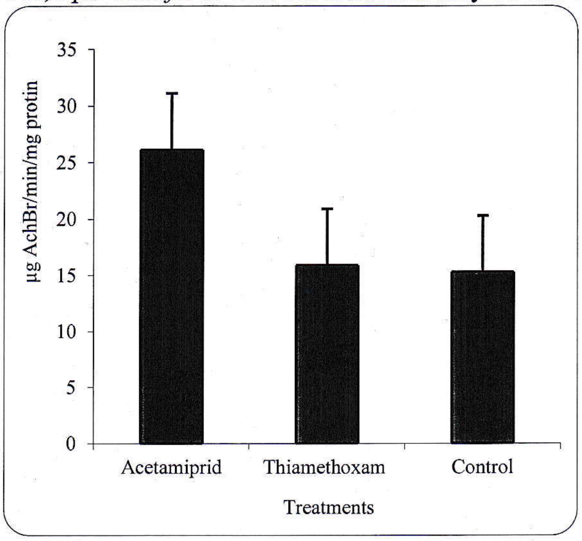

Fig. (3): α-esterase activity in the adult female homogenates of *T. cucurbitacearum* after being sprayed with tested neonicotinoides.

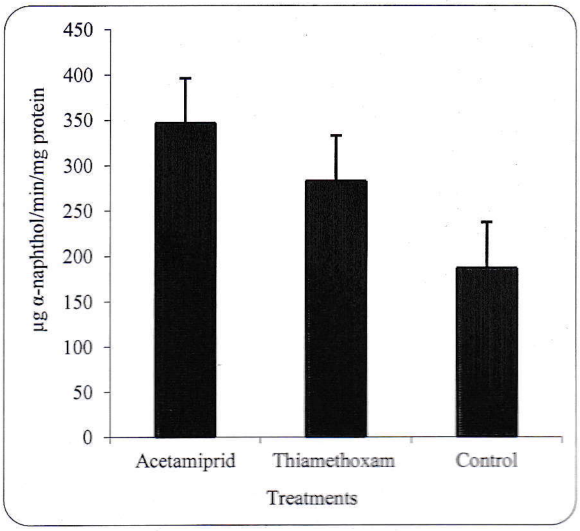

Fig. (4): β-esterase activity in the adult female homogenates of *T. cucurbitacearum* after being sprayed with tested neonicotinoides.

*Each column depict mean of value recorded in three separate replicates

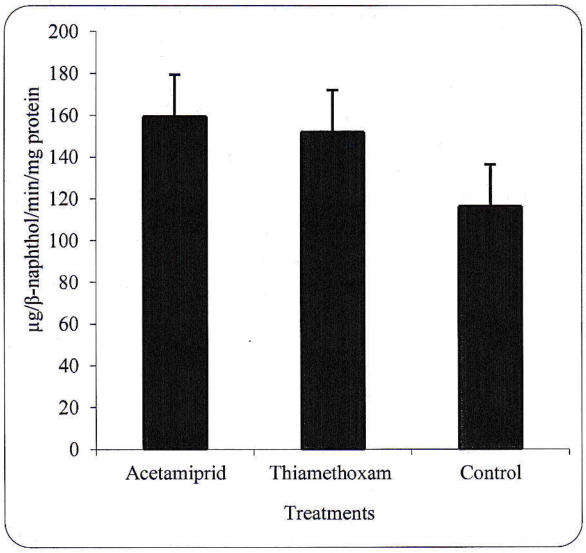

Fig. (5): AchE activity in the adult female homogenates of *T. cucurbitacearum* after being sprayed with tested neonicotinoides.

the used neonicotinoides at field recommended rates were not the sole factor contributing to toxicity of mites but differences in varieties of tested pest or crop, climate, agronomic practice and pest resistance can also affect the efficacy of neonicotinoides.

Biochemical studies:

The data showed changes in the activity of non specific esterase (α- and β-esterase) and acetylcholine esterase in the homogenated adult females of *T. cucurbitacearum* treated with the two neonicotinoides; acetamiprid and thiamethoxam. The data were expressed as percentage activity relative to control.

Alpha esterase:

A highly significant increase in α- esterase was detected in the present work that reached 85.06 and 51.07 % for acetamiprid and thiamethoxam compared to control, respectively. It increased from control 187.33±2.905 µg α-naphthol/min/mg protein to 346.67±9.27 and 283.00±5.13 for acetamiprid and thiamethoxam, respectively, P= 0.0000***, Fig. (3).

Beta esterase:

Fig. (4) showed remarkable significant increase in the enzyme activity in *T. cucurbitacearum* compared to control as affected by acetamiprid (36.96 %) followed by thiamethoxam (30.66%), P= 0.0005***.

Acetylcholine esterase:

Dealing with acetylcholine esterase and its relation to neonicotinoides, data showed an increase from 15.26±0.381 µg AchBr/min/mg protein in the homogenated of *T. cucurbitacearum* control to 26.11±0.735 for acetamiprid. AchE showed a lowest the same activity in both thiamethoxam treated and untreated mites. This elevation expressed as (71.10 and 4.13 % compared with control, respectively), Fig. (5). Highly significant increase was obtained as affected by acetamiprid, P=0.0000***.

In the present work, fixed elevation trend was observed in the activity of general esterases against field strain of *T. cucurbitacearum*, suggesting that, they are involved in the contribution of the detoxification mechanism. Several studies have demonstrated that esterases play an important role in conferring or contributing to insecticides detoxications in insect and other arthropod species (Mouches *et al.*, 1986). Esterase may contribute to resistance by hydrolyzing the pesticide (Devonshire and Moores, 1982) or by temporary binding to pesticide when the catalytic activity is relatively low (Devonshire, 1989).

Acetylcholin esterase (AchE) is a key enzyme in the nervous system, terminating nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. Neonicotinoides act selectively on insect nicotinic acetylcholine receptors (n AchR), accounting at least in part for the selective toxicity to insects over vertebrate (Silcox and Vittum, 2008 and Malev *et al.*, 2012). They act as agonists at the insect nicotinic acetylcholine receptors which plays an important role in synaptic tramission in the central nervous system (Muccio *et al.*, 2006). This assumption may be able to explain the over production of both AchE and non specific esterase. Elevating the activity of α- and β- esterases was also observed by El-Kawas *et al.*, 2009 when tested hexaflumuron (IGR) and chlorfenapyr against field strain of *T. urticae*. Furthermore, Farag and Mead, 2009 noticed increase in the activity of AchE after treating 4th instar larvae with Spinotram (as it appearing to be of a unique mechanism, with a primary site of attack being the nicotinic acetylcholine receptors and a second site of attack being GABA receptors).

Finally, it can be verified that, although acetamiprid and thiamethoxam showed low toxicity on both adults and eggs of *T. cucurbitacearum* and elevation of some esterase enzymes compared to control, they reduced significantly the reproductive capacity and functional response of its mite predator, *P. macropilis*. This response may be one of the reasons for the increase of *T. cucurbitacearum* populations under field conditions. Therefore, furthermore work remains to be done particularly under field conditions in order to spot light on the factors which may interact together with neonicotinoides to lead to increase mite populations.

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