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Efficacy of some Novel and Conventional Insecticides against *Aphis gossypii* Glover and their Side Effects on Non-Targeted Organisms and Plant Defense Enzymes of Cotton Plants

El-Sherbeni, A. D.[1]; S. A. Hamed[1]; E. S. El-Zahi[2] and S. K. M. Korish[2]
[1]Plant Protection Department, Faculty of Agriculture, Tanta University.
[2]Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

ABSTRACT

Cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is considered a key pest of cotton in Egypt. Hence, field and laboratory studies were conducted at Sakha Agriculture Research Station, Egypt during seasons 2016 and 2017. Efficiency of seven insecticides i.e., flonicamid, imidacloprid, thiamethoxam, emamectin-benzoate, chlorpyrifos, methomyl and deltamethrin against *A. gossypii* in cotton fields were evaluated. Their side effects on the associated predators, soil fauna and plant defense enzymes also were studied. Flonicamid was the most effective against *A. gossypii*. The efficacy of imidacloprid and thiamethoxam did not differ significantly from that of chlorpyrifos and methomyl recording from 83.28 – 93.27% reduction in *A. gossypii* infestation. Flonicamid, emamectin-benzoate, imidacloprid and thiamethoxam were the least harmful to the associated predators causing less than 50% mortality, while the others were highly toxic. Flonicamid exhibited the highest degree of safety to the soil micro-arthropods, followed by emamectin-benzoate, methomyl and deltamethrin. In contrast, chlorpyrifos and imidacloprid were the most toxic to the soil micro-arthropods. The conventional insecticides (chlorpyrifos, methomyl and deltamethrin) increased the activity of catalase and peroxidase causing physiological stress on the treated cotton plants, whereas the other tested insecticides recorded decreases in catalase and peroxidase activities inducing the plant defense response. Only imidacloprid and thiamethoxam increased the activity of polyphenol oxidase. Emamectin-benzoate and deltamethrin decreased the total soluble protein content, while the others tested insecticides caused increases in this criterion comparing to the control.

Keywords: Cotton, *Aphis gossypii*, insecticides, predators, soil fauna, antioxidant enzymes.

INTRODUCTION

Cotton aphid, *Aphis gossypii* Glover is considered one of the most serious cotton pests world-wide causing severe losses to the yield and the fiber quality (El-Kady, 2007). It is a polyphagous insect, infests cotton plants during the different stages of plant growth. Both nymphs and adults suck the cell sap from the lower leaf surfaces and secret the honey dew, encouraging the black sooty mold growth which reduces the leaves photosynthesis and contaminates the open boll lint (Sarwar *et al*., 2013). Insecticidal control is one of the common means against cotton aphid. The intensive use of insecticides over many years has led to development of aphid resistance to several classes of conventional insecticides (Tabacian *et al*., 2011). New generation insecticides such as flonicamid, imidacloprid and thiamethoxam with novel mode of action have obvious advantages in terms of effectiveness, specificity and safety to non-target organisms and environment components. Neonicotinoids have introduced into the market as effective substitutes of the organophosphates and carbamates (Tomizawa *et al*., 2007). Neonicotinoids act by binding to nicotinic acetylcholine receptors in the insect central nervous system and provide an excellent control either applied as seed or foliar treatments against a broad range of economically important sucking insects, such as aphids, whiteflies, thrips, jassid and others (Prasanna *et al*., 2004). Flonicamid is a novel systemic aphicide belongs to the pyridinecarboxamide group, and characterized with its antifeedant activities against aphid. It inhibits the feeding of aphid within 0.5 h of treatment without return or noticeable poisoning symptoms (Morita *et al*., 2007).

Plants tend to generate Reactive Oxygen Species (ROS), responding to biotic/a biotic stress condition, which cause oxidative stress to plants. Plant defense enzymes including catalase, peroxidase, super oxide dismutase and glutathione reductase which functioning under condition of overproduction of ROS reducing devastating damages occurred to plants (Chouldhury *et al*., 2013). Apart from their insecticidal activity, some insecticides, particularly neonicotinoids, exhibited positive impacts on translated in enhancement of foliage growth, plant vigor and drought-tolerance (Ford *et al*., 2010). At another cases, indiscriminate use of insecticides alters the activity of plant defense response and negatively affects the normal plant growth representing a physiological stress factors and reduces the yield quantity and quality (Garcia-Hernandez *et al.,* 2005)

Soil fauna is classified into four categories include: micro-fauna (nematodes and protozoa with body sizes of 20 -200µm), meso-fauna (mites and collembolans with body sizes of 200µm – 2mm, which make approximately 95% of soil micro-arthropods), macro-fauna (earthworms and millipedes with body sizes of 2mm – 20mm), and mega-fauna (some species of earthworms, snails, reptiles and amphibians with body sizes > 20mm) (Cole *et al*., 2006; Menta,2012). Mites and collembolans feed mainly on soil micro-biota (fungi, bacteria, actinomycetes, algae) and organic matter. Hence, they usually called "litter transformers" and are important in the formation of soil microstructure in several terrestrial ecosystems (Lavelle, 1997; Heneghan and Bolger, 1998). Only 5% of the pesticide applied to crops actually reaches the target pest; the rest enters the environment gratuitously causing adverse effects on the water, air and non targeted organisms (Pimentel and Levitan, 1986). Disturbance caused by pollutants in the soil result in both quantitative and qualitative changes in the fauna (Cortet and Poinsot-Balaguer, 1998). However, low data are available on the ecotoxicological effects of chemical insecticides on soil fauna. This study aimed to evaluate the activity of some novel and conventional insecticides on cotton aphid, *A. gossypii* and their side effects on associated predators, soil fauna and plant defense enzymes.

MATERIALS AND METHODS

Chemicals used

Commercial formulations of flonicamid (Teppeki 50%WG, ISK Biosciences, Belgium), imidacloprid (Ecomida 30.5%SC, Bharat Insecticides Ltd., India), thiamethoxam (Actra 25%WG, Syngenta Agrosciences, Switzerland), emamectin-benzoate (Proclaim 5%SG, Syngenta Agrosciences, Switzerland), chlorpyrifos (Dursban 48%EC, Dow Agrosciences, USA), methomyl (Neomyl 90%SP, Rotam Agrochemical Co. Ltd., Hong Kong) and deltamethrin (Decis 2.5%EC, Bayer Crop Science, Germany) were used in this study based on their label recommended rates.

Field experiments

Experiment design

The field experiments were conducted at the farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt, during 2016 and 2017 cotton growing seasons. Cotton seeds (*Gossypium barbadense* var. Giza 86) were sown on April 9th in both seasons an area of about 2000 m^2 and divided into equal plots 42 m^2 for each. This area did not receive any insecticidal treatments before the start of the experiment. Eight treatments (seven insecticides and the control) were arranged in this area in a Randomized Complete Block Design with four replications. The tested compounds were sprayed once on August13th in both seasons at their label recommended rates. A Knapsack sprayer, CP_3 (Cooper Pegler Co. Ltd., Northumberland, England) was used for spraying. Irrigation water was used for dilutions. The final volume of spray solution was equal to 200 L/ Feddan (1Feddan = 0.42 hectare).

Sampling of *Aphis gossypii*, its associated predators and the soil fauna

To evaluate the effect of the tested insecticides against cotton aphid, 25 cotton leaves were randomly selected from each replicate (plot). The upper and lower leaf surfaces were examined carefully using lens 8X and the numbers of aphid were counted directly in the field. The sampling toke place before spraying and 1, 3, 7 and 10 days post spray. For sampling the associated predators, the predacious stages of the associated predators were counted i.e., larvae and adults of *Coccinella* spp. And *Scymnus* spp., adults of *Paederus alfierii*, larvae of *Chrysoperla carnea*, nymphs and adults of *Orius* spp., and true spiders. The predators' populations were counted visually on 10 randomly selected plants from both diagonals of the inner square area of each replicate at the same times of *A. gossypii* sampling.

Soil fauna were sampled before spray and 1, 7, 14 and 21 days after spraying. Soil samples were taken from 0 – 20 cm depth between cotton plants by steel cylinder (10 cm in its inner diameter and 20 cm tall), and the samples were collected in polyethylene bags and labeled according to Rajagopal *et al*. (1990). The samples were transmitted to the laboratory where the multifaceted extractor (Berlese Tullgren Funnels) was adopted. The soil micro-arthropods were collected and put into vials with 70% ethyl alcohol for identification. Species were identified under stereo binocular microscope. The numbers and types of soil micro-arthropods extracted from each treatment were recorded. The reduction percentages occurred in the populations of sampled insects was estimated using the formula of Henderson and Tilton (1955) **as follows:**

$$\%\ \text{Population change} = \left\{1 - \frac{\text{Post treatment population in treatment}}{\text{Pre treatment population in treatment}} \times \frac{\text{Pre treatment population in control}}{\text{Post treatment population in control}}\right\} \times 100$$

Laboratory experiment

Biochemical changes in cotton plants

To study the biochemical changes in catalase, peroxidase, polyphenol oxidase and total soluble protein occurred in cotton plants after application of the tested insecticides, cotton seedlings of 27 days age were sprayed with the tested insecticides at their recommended rates according to Cipollini *et al*. (2004). After four days of spray, newly maturated cotton leaves were collected from treated plants as well as control, and immediately frozen in liquid nitrogen. Half gram of leaves was homogenized in 3 ml of ice cold 0.1 M TRIS-HCL buffers (PH 7.8) containing 2-mercaptoethanol (5 mM), 1% polyvinylpyrolidone (PVP) and 0.5 mM EDTA. The homogenate was centrifuged at 7000 r.p.m. for 20 min at 4 ^oC. The supernatant was used as enzyme source (War *et al*., 2011). All spectrophotometric analyses were performed on HITACHI UV-2010 spectrophotometer. Catalase was determined by the method of (Aebi, 1984). Peroxidase was estimated by the method of (Hammerschmidt *et al*., 1982). The method of (Mayer and Harel, 1979) was adopted to determine polyphenoloxidase. Protein content was determined according to the method of (Bradford, 1976).

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and means were separated by Duncan Multiple Range Test (Duncan, 1955) to determine the significant differences among treatments means at 0.05 probability level using CoStat system for Windows, Version 6.311.

RESULTS AND DISCUSSION

Three novel insecticides i.e., flonicamid, imidacloprid and thiamethoxam and other four insecticides actually recommended to control bollworms i.e., emamectin-benzoate, chlorpyrifos, methomyl and deltamethrin were all evaluated for their efficiency against *A. gossypii* on cotton. Their side effects on the associated predators, soil fauna and plant antioxidative enzymes were studied as well.

Activity of the tested insecticides on *Aphis gossypii*

Data presented in Table 1 indicated that the antifeedant insecticide, flonicamid, was significantly the most effective against *A. gossypii* recording 95.05 and 94.25% mean of reduction in the insect population in 2016 and 2017, respectively. The activity of the two neonicotinoid insecticides: imidacloprid and thiamethoxam did not differ significantly from that of chlorpyrifos and methomyl where all caused from 83.28 – 89.62% and from 81.82 – 93.27% mean of reduction in *A. gossypii* infestation in 2016 and 2017, respectively. Emamectin-benzoate and deltamethrin resulted in feeble effects against *A. gossypii* translated in 21.00 and 26.08% mean of reduction in 2016 and 16.35 and 29.00% mean of reduction in 2017, respectively. The results of the current study are in accordance with that of the previous investigations. Morita *et al.* (2007) clarified that flonicamid was very effective against *A. gossypii* and irreversibly inhibited the aphid feeding within 0.5 h after treatment. *Aphis gossypii* showed very low resistance to profenofos, chlorpyrifos and methomyl (Mushtaq and Arif, 2008). El-Zahi and Arif (2011) reported that methomyl, profenofos and chlorpyrifos demonstrated high activity against *A. gossypii* on cotton plants and were as potent as thiamethoxam and imidacloprid, while pyrethroid insecticides were the least effective. El-Naggar and Zidan (2013) demonstrated high efficiency of imidacloprid and thiamethoxam against *B. tabaci* and *A. gossypii* on cotton. Flonicamid showed high efficacy against *A. gossypii* followed by dinotefuran, while the pyrethroid insecticide bifenthrin was ineffective (Kumar *et* al., 2016). Since *A. gossypii* and bollworms infest cotton plants simultaneously during vegetative and fruiting stages of cotton growth, the obtained results suggest that chlorpyrifos and methomyl (as recommended insecticides against bollworms) could be used successfully to control *A. gossypii* and bollworms by one application of the fields infested with the two pests.

Side effects on some associated predators

Data presented in Table 2 showed the toxicity of the tested insecticide to the predacious stages of the *A. gossypii* associated predators i.e., larvae and adults of *Coccinella* spp. and *Scymnus* spp., adults of *Paederus alfierii,* larvae of *Chrysoperla carnea*, nymphs and adults of *Orius* spp., and true spiders. The tested novel insecticides (flonicamid, emamectin- benzoate, imidacloprid and thiamethoxam) were significantly the least harmful to the associated predators recording less than 50% mean of reduction in the associated predators populations in both seasons of study. In contrast, chlorpyrifos, methomyl and deltamethrin (conventional insecticides) were significantly the most toxic to the associated predators with mean of percent reduction ranged from 79.8- 88.3% and from 82.9- 90.1% in 2016 and 2017, respectively. Chlorpyrifos was comparatively the most harmful to the associated predators during 2016 and 2017.These results are in parallel with Abida *et al*. (2007) who found that methomyl and fenpropathrin caused more than 92% mortality in *C. carnea* second instar larvae. Flonicamid and methoxyfenozide exhibited no negative impacts on *Orius laevigatus* and *Amblyseius swirkii* in the green house (Colomer *et al*., 2011). Jansen *et al*. (2011) demonstrated the high selectivity of flonicamid and pymetrozine comparing to deltamethrin and pirimicarb against the predators: *Adalia bipunctata, Bembidion lampros* and *Episyrphus balteatus*. El-Zahi (2012) stated that emamectin- benzoate and thiamethoxam were less toxic than profenofos to *C. carnea* larvae.

Table 1. Efficiency of different treatments against *Aphis gossypii* on cotton under field conditions during 2016 and 2017 seasons.

Treatment	Application rate / L	season 2016						season 2017					
		Pre-spray	Mean number of *A. gossypii* / cotton leaf and percent reduction at days after spray				Mean	Pre-spray	Mean number of *A. gossypii* / cotton leaf and percent reduction at days after spray				Mean
			1	3	7	10			1	3	7	10	
Flonicamid	0.42 g	42.8	6.3 (87.6)	1.2 (96.7)	0.6 (98.5)	0.9 (97.4)	2.3 (95.05[a])	41.8	6.5 (79.5)	0.2 (99.2)	0.2 (99.2)	0.2 (99.1)	1.8 (94.25[a])
Emamectin-Benzoate	0.3 g	31.7	29.0 (25.6)	21.3 (22.1)	22.2 (23.2)	24.2 (12.9)	24.2 (21.00[c])	37.8	22.9 (19.8)	22.4 (15.2)	23.1 (11.3)	21.0 (19.1)	22.3 (16.35[e])
Imidacloprid	0.6 ml	38.3	7.8 (83.8)	2.4 (90.8)	2.4 (92.9)	3.0 (91.0)	3.9 (89.62[b])	40.1	3.4 (88.6)	1.3 (95.3)	1.5 (94.5)	1.3 (94.7)	1.9 (93.27[b])
Thiamethoxam	0.2 g	40.5	8.4 (83.2)	4.8 (86.7)	5.9 (84.0)	5.0 (86.2)	6.0 (85.03[b])	44.0	4.4 (86.0)	0.7 (97.9)	1.8 (93.7)	1.9 (93.0)	2.2 (92.55[b])
Chlorpyrifos	5 ml	44.5	11.4 (80.5)	6.8 (82.7)	4.6 (88.0)	5.2 (86.1)	7.0 (84.33[b])	43.0	3.2 (89.6)	2.2 (92.6)	2.7 (90.6)	2.9 (89.3)	2.8 (90.52[b])
Methomyl	1.5 g	32.4	5.3 (88.0)	3.0 (87.9)	6.0 (80.9)	6.8 (76.3)	5.3 (83.28[b])	46.5	4.0 (88.8)	4.8 (85.1)	8.8 (74.1)	6.0 (79.3)	5.9 (81.82[c])
Deltamethrin	2 ml	26.6	18.3 (45.1)	18.3 (24.0)	19.5 (19.2)	14.5 (16.0)	18.9 (26.08[c])	32.7	14.6 (41.7)	16.4 (29.7)	17.3 (25.0)	18.0 (19.4)	16.6 (29.00[d])
Control	—	44.6	55.2	39.8	40.7	39.6	43.8	42.4	32.7	29.8	29.0	28.4	30.0

Figures in parentheses refer to the percentages of reduction in *A. gossypii* population comparing to control. In the same column, means followed by the same letters are not significantly different at 0.05 probability level by Duncan (1955).

Table 2. Side effects of different treatments on *Aphis gossypii* associated predators* in cotton under field conditions during 2016 and 2017 seasons.

Treatment	Application rate / L	season 2016						season 2017					
		Pre-spray	Mean number of associated predators / cotton plant and percent reduction at days after spray				Mean	Pre-spray	Mean number of associated predators / cotton plant and percent reduction at days after spray				Mean
			1	3	7	10			1	3	7	10	
Flonicamid	0.42 g	25	14 (40.0)	19 (26.5)	20 (35.1)	13 (46.2)	16.5 (37.0^{d})	39	27 (35.0)	32 (27.3)	21 (47.8)	25 (44.8)	26.3 (38.7^{d})
Emamectin-benzoate	0.3 g	26	22 (29.9)	19 (29.3)	17 (31.4)	15 (40.3)	18.3 (32.7^{e})	37	28 (28.9)	23 (44.9)	29 (24.1)	26 (39.5)	26.5 (34.4^{d})
Imidacloprid	0.6 ml	20	11 (41.1)	9 (56.5)	11 (51.4)	12 (43.1)	10.8 (48.0^{c})	28	16 (46.3)	20 (36.7)	14 (51.6)	17 (47.7)	16.8 (45.6^{c})
Thiamethoxam	0.2 g	22	17 (51.3)	15 (34.0)	13 (52.1)	10 (20.1)	13.8 (39.4^{d})	45	28 (41.5)	29 (42.9)	30 (35.4)	26 (50.2)	28.3 (42.5^{c})
Chlorpyrifos	5 ml	27	2 (92.1)	6 (78.5)	2 (94.0)	3 (88.5)	3.3 (88.3^{a})	35	5 (86.6)	3 (92.4)	4 (88.9)	3 (92.6)	3.8 (90.1^{a})
Methomyl	1.5 g	24	5 (77.7)	7 (71.8)	3 (89.9)	2 (91.4)	4.3 (82.7^{b})	42	11 (75.4)	8 (83.1)	3 (93.1)	5 (89.7)	6.8 (85.3^{b})
Deltamethrin	2 ml	32	8 (73.2)	5 (84.9)	9 (77.2)	5 (83.8)	6.8 (79.8^{b})	40	10 (76.5)	9 (80.1)	5 (87.9)	6 (87.1)	7.5 (82.9^{b})
Control	—	30	28	31	37	29	31.3	31	33	35	32	36	34.0

Figures in parentheses refer to the percentages of reduction in associated predators population comparing to control. In the same column, means followed by the same letters are not significantly different at 0.05 level by Duncan (1955).

*** Associated predators included: larvae and adults of *Coccinella* spp. and *Scymnus* spp., adults of *Paederus alfierii,* larvae of *Chrysoperla carnea*, nymphs and adults of *Orius* spp., and true spiders.**

Side effects on soil fauna

Data concerning the toxicity of flonicamid, emamectin-benzoate, imidacloprid, thiamethoxam, chlorpyrifos, methomyl and deltamethrin to the soil micro-arthropods in 2016 and 2017 are presented in Tables 3 and 4, respectively. Survey of micro-arthropods populations per 200 g dry soil in pre-spray inspection of all treated and control plots indicated that the collembolan group was the most dominant (5.7 – 9.0 and 6.8 – 15.2 individuals) comparing to miscellaneous mites (3.7- 6.3 and 4.8 – 11.1 individuals) and predacious mites (4.7- 6.7 and 4.2 – 11.3 individuals) in 2016 and 2017, respectively. The antifeedant insecticide, flonicamid, was significantly the most harmless to all groups of soil micro-arthropods causing 4.1, 3.2 and 1.3% mean of reduction in 2016 and 3.5, 1.8 and 0.6% mean of reduction in 2017 in the populations of miscellaneous mites, predacious mites and collembolan, respectively. In the other hand, chlorpyrifos proved to be the most toxic to the soil fauna recording 55.9, 54.2 and 52.9 % mean of reduction in 2016 and 54.4, 63.5 and 57.4% mean of reduction in 2017 in miscellaneous mites, predaceous mites and collembolan, respectively. The neonicotinoid insecticides, imidacloprid and thiamethoxam, ranked the second order of toxicity against the soil fauna, where they gave 35.2 & 29.3%, 50.0 & 43.5% and 51.9 & 33.0% mean of reduction in 2016 and 52.8 & 29.7%, 60.1 & 45.9% and 53.6 & 42.9% mean of reduction in 2017 in the population density of miscellaneous mites, predacious mites and collembolan, respectively. Emamectin-benzoate, methomyl and deltamethrin comparatively demonstrated moderate toxicity to the soil micro-arthropods. The obtained results indicated that the predacious mites were the most affected comparing to miscellaneous mites and collembolan. This may be because predacious mites were more exposure to the applied insecticides via either direct contact or feeding on poisoning arthropods. As shown in Tables 3 and 4, collembolan recolonized the treated areas faster than other species. This may be due to elimination of predators and parasites that prey or parasitize on this group of soil fauna. Soil micro- arthropods are functionally essential in soil nutrient cycling and play vital roles in the process of litter decomposition and humus formation (Partsch *et al*., 2006). Only 5% of the pesticide applied to crops actually reaches the desirable targeted pest, while the rest enters the environment gratuitously, contaminating soil, water and air and creating adversely effects on non-target organisms (Pimentel and Levitan, 1986). Sur and Stork (2003) and Goulson (2013) reported that plants uptake 1.6% of the neonicotinoids dosage applied as seed dressing, leaving around 98% of the compound in the exposure of soil invertebrates. Our results are in agreement with that of Endlweber *et al*., (2006) and Kamoun *et al*. (2018) who found that chlorpyrifos was the most toxic to the soil arthropods (collembolan and predatory mites) comparing to deltamethrin and dimethoate. Imidacloprid and thiamethoxam resulted in significant reductions in the populations of soil micro- arthropods either under or between treated plants (El- Naggar and Zidan, 2013; De Lima e Silva *et al*., 2017). In the current study, the high toxicity of imidacloprid to different groups of the soil fauna compared to thiamethoxam may be attributed to: 1) Imidacloprid is more persistent in the soil than thiamethoxam (Hilton *et al*., 2016; De Lima e Silva *et al*., 2017). 2) The low concentration of the organic matter in the cotton fields in this time of season, where cotton plants consume it in the growth, since imidacloprid binds to the soil organic matter in a reversible way (Cox *et al*., 1997; Knoepp *et al*., 2012), accordingly the absence of organic matter increases the imidacloprid availability to soil organisms. Peck (2009) noticed reductions in collembolan populations in the rooting zone, where uptake of imidacloprid from the soil occurs. The low toxicity of deltamethrin, emamectin- benzoate and methomyl obtained in this study is in accordance with results of the previous investigations. Badji *et al*. (2007) did not detect deltamethrin by gas chromatography analysis of soil samples 24 h after it was sprayed on maize fields and found its impact on collembolan and mites was lower than expected. Also, Burkhard *et al*. (2015) reported that emamectin- benzoate is sensitive to light and has a rapid degradation rate in the soil.

Table 3. Effect of different treatments on micro- arthropods (miscellaneous mites, predacious mites and collembolans) found between cotton plants under field conditions during season 2016.

Treatment	Rate per L	Mean number/ 200 g soil and percent reduction of micro- arthropods																	
		Miscellaneous mites [1]						Predacious mites [2]						Collembolan [3]					
		Pre-Spray	Mean number and percent reduction at days after spray				Mean	Pre-spray	Mean number and percent reduction at days after spray				Mean	Pre-spray	Mean number and percent reduction at days after spray				Mean
			1	7	14	21			1	7	14	21			1	7	14	21	
Flonicamid	0.42 g	5.7	13.1 (6.5)	11.6 (4.2)	13.5 (3.0)	12.5 (2.8)	12.7 (4.1[e])	4.7	5.0 (3.7)	6.0 (0.3)	6.3 (4.5)	6.9 (3.8)	6.1 (3.2[e])	7.3	7.4 (1.9)	8.3 (0.7)	8.8 (0.0)	8.7 (2.5)	8.3 (1.3[e])
Emamectin-benzoate	0.3 g	5.3	9.5 (27.6)	9.0 (18.6)	11.0 (15.0)	10.6 (11.3)	10.0 (18.1[c])	5.3	5.5 (6.1)	6.4 (5.7)	6.0 (19.3)	7.2 (11.0)	6.3 (10.5[d])	5.7	4.2 (28.7)	5.5 (15.7)	5.6 (21.8)	6.2 (11.0)	5.4 (19.3[c])
Imidacloprid	0.6 ml	4.3	6.4 (40.7)	5.3 (41.4)	6.4 (39.0)	8.0 (19.5)	6.5 (35.2[b])	6.7	3.0 (59.5)	3.3 (61.5)	4.3 (54.3)	7.7 (24.7)	4.6 (50.0[a])	7.0	3.3 (54.4)	4.0 (50.1)	3.7 (57.9)	4.7 (45.1)	3.9 (51.9[a])
Thiamethoxam	0.2 g	3.7	6.5 (30.1)	5.0 (37.4)	7.0 (22.5)	5.9 (27.3)	6.1 (29.3[b])	5.3	3.2 (45.4)	3.6 (47.0)	4.2 (43.5)	5.0 (38.2)	4.0 (43.5[b])	7.7	4.3 (46.0)	5.3 (39.9)	7.3 (24.5)	7.4 (21.4)	6.1 (33.0[b])
Chlorpyrifos	5 ml	6.3	5.9 (61.1)	5.5 (57.6)	6.7 (56.4)	7.3 (48.6)	6.4 (55.9[a])	5.3	2.8 (52.2)	3.1 (54.3)	3.0 (59.7)	4.0 (50.6)	3.2 (54.2[a])	6.7	3.0 (56.7)	2.7 (64.8)	3.5 (58.4)	5.6 (31.6)	3.7 (52.9[a])
Methomyl	1.5 g	4.7	9.3 (21.1)	8.9 (11.4)	9.8 (14.6)	10.3 (2.7)	9.5 (12.5[d])	4.7	4.0 (23.0)	5.2 (13.6)	6.0 (9.0)	6.7 (6.6)	5.5 (13.1[d])	8.7	6.2 (31.0)	8.3 (16.6)	9.7 (11.2)	9.5 (10.7)	8.4 (17.4[c])
Deltamethrin	2 ml	6.0	11.3 (23.0)	10.0 (22.1)	13.3 (11.2)	11.7 (13.6)	11.6 (17.4[c])	5.0	3.4 (38.5)	5.3 (17.2)	6.0 (14.5)	6.8 (10.9)	5.4 (20.3[c])	8.0	6.5 (21.4)	8.1 (11.5)	10.0 (0.4)	9.5 (2.8)	8.5 (9.0[d])
Control	—	4.3	10.8	9.2	10.5	9.7	10.1	5.7	6.3	7.3	8.0	8.7	7.6	9.0	9.3	10.3	11.3	11.0	10.5

Figures in parentheses refer to the percentages of reduction in micro- arthropods population comparing to control. In the same column, means followed by the same letters are not significantly different at 0.05 probability level by Duncan (1955).

[1] **Miscellaneous mites included:** ***Pygmephorus*** **sp.,** ***Hypochthonius*** **sp.,** ***Tydeus*** **sp.,** ***Haplozetes*** **sp.,** ***Belba*** **sp.,** ***Oribatula*** **sp.,** ***Tarsonemus*** **sp.,** ***Stenotarsonemus*** **sp.,** ***Tyrophagus putrescentiae*****,** ***Rhizoglyphus echinopus*****.**

[2] **Predacious mites included:** ***Rhodacarus*** **sp.,** ***Macrochelus*** **sp.,** ***Cheyletus malaccensis, Amblyseius*** **sp.,** ***Cunaxa capreolus*****.**

[3] **Collembolan included:** ***Tulbergia callipygos*****,** ***Hypogastrura armatus*****,** ***Onychurius absoloni*****,** ***Proistoma minuta*****,** ***Freisea claviseta*****,** ***Isotomiella*** **sp.**

decrease or increase = (treatment – control) / control × 100%

Table 4. Effect of different treatments on micro- arthropods (miscellaneous mites, predacious mites and collembolan) found between cotton plants under field conditions during season 2017.

Treatment	Rate per L	Mean number/ 200 g soil and percent reduction of micro- arthropods																	
		Miscellaneous mites [1]						Predacious mites [2]						Collembolan [3]					
		Pre-spray	Mean number and percent reduction at days after spray				Mean	Pre-spray	Mean number and percent reduction at days after spray				Mean	Pre-spray	Mean number and percent reduction at days after spray				Mean
			1	7	14	21			1	7	14	21			1	7	14	21	
Flonicamid	0.42 g	7.4	13.2 (3.8)	11.3 (6.3)	15.7 (1.3)	15.9 (2.6)	14.0 (3.5[d])	4.9	6.3 (1.9)	7.0 (0.8)	7.7 (2.3)	7.9 (2.1)	7.2 (1.8[e])	6.8	8.2 (0.1)	11.4 (0.5)	13.1 (1.6)	22.0 (0.0)	13.7 (0.6[e])
Emamectin-benzoate	0.3 g	6.7	9.1 (26.8)	8.7 (20.3)	12.1 (15.0)	13.2 (10.5)	10.8 (18.2[c])	6.5	7.7 (8.3)	9.0 (4.5)	8.6 (16.9)	9.6 (10.3)	8.7 (10.0[d])	8.9	7.4 (30.0)	10.9 (24.3)	14.6 (15.4)	15.7 (13.8)	12.2 (20.9[c])
Imidacloprid	0.6 ml	6.5	6.1 (49.4)	4.8 (54.7)	5.0 (65.0)	8.1 (42.1)	6.0 (52.8[a])	6.9	3.1 (66.7)	2.1 (77.3)	5.3 (52.8)	6.2 (43.6)	4.2 (60.1[a])	14.1	9.7 (41.9)	9.4 (59.0)	10.3 (61.4)	14.1 (52.1)	10.9 (53.6[a])
Thiamethoxam	0.2 g	11.1	14.8 (28.1)	11.6 (35.8)	16.9 (29.3)	18.7 (25.5)	15.5 (29.7[b])	11.3	7.0 (54.5)	10.4 (35.7)	10.0 (47.0)	10.5 (46.2)	9.5 (45.9[b])	9.5	5.8 (50.0)	8.9 (40.5)	11.7 (36.8)	10.6 (44.4)	9.3 (42.9[b])
Chlorpyrifos	5 ml	6.9	4.3 (66.4)	5.4 (53.9)	6.7 (56.3)	9.0 (41.1)	6.4 (54.4[a])	6.7	3.0 (65.0)	3.1 (68.4)	3.7 (62.2)	4.5 (58.3)	3.1 (63.5[a])	15.2	8.3 (53.3)	9.7 (59.2)	9.6 (67.2)	15.0 (50.0)	10.7 (57.4[a])
Methomyl	1.5 g	4.8	6.9 (22.5)	6.2 (18.5)	9.3 (10.6)	10.1 (5.3)	8.1 (14.2[c])	4.2	4.1 (26.4)	5.7 (6.5)	6.3 (7.4)	6.6 (5.0)	5.7 (11.3[d])	9.7	7.0 (38.0)	12.3 (24.5)	13.9 (24.6)	16.8 (18.9)	12.5 (26.5[c])
Deltamethrin	2 ml	8.7	13.1 (18.8)	12.5 (12.3)	15.9 (13.8)	17.2 (10.8)	14.7 (13.9[c])	5.8	4.0 (44.0)	5.4 (36.4)	8.2 (9.7)	8.4 (12.3)	6.5 (25.6[c])	12.3	11.8 (18.3)	19.0 (9.9)	22.8 (7.4)	24.0 (5.8)	19.4 (10.4[d])
Control	–	6.2	11.5	10.1	13.4	13.7	12.2	7.8	10.2	11.3	12.5	12.9	11.7	10.4	12.6	17.4	20.5	21.3	18.0

Figures in parentheses refer to the percentages of reduction in micro- arthropods population comparing to control .In the same column, means followed by the same letters are not significantly different at 0.05 probability level by Duncan (1955).

[1] **Miscellaneous mites included:** ***Pygmephorus*** **sp.,** ***Hypochthonius*** **sp.,** ***Tydeus*** **sp.,** ***Haplozetes*** **sp.,** ***Belba*** **sp.,** ***Oribatula*** **sp.,** ***Tarsonemus*** **sp.,** ***Stenotarsonemus*** **sp.,** ***Tyrophagus putrescentiae*****,** ***Rhizoglyphus echinopus*****.**

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Side effect on plant defense enzymes and total protein content

Catalase, peroxidase and polyphenol oxidase activities and total soluble protein content influenced by application of different insecticides on cotton plants are discussed in Table 5. It is obvious that catalase and peroxidase activity increased by the conventional insecticides (chlorpyrifos, methomyl and deltamethrin), while flonicamid, emamectin-benzoate, imidacloprid and thiamethoxam decreased catalase and peroxidase activity.

Only neonicotinoid insecticides (imidacloprid and thiamethoxam) significantly increased the activity of polyphenol oxidase, whereas the other tested insecticides caused decreases in its activity. Cotton plants treated with flonicamid, imidacloprid, thiamethoxam, chlorpyrifos and methomyl showed increases in their total soluble protein content. In the other side, plants treated with emamectin-benzoate and deltamethrin indicated decrease in total soluble protein content comparing to the control. These results could be explained with the findings of the previous investigations. Responding to biotic/a biotic stress, plants tend to generate Reactive Oxygen Species (ROS) which cause devastating damages to plants, and consider the substrate of antioxidant enzymes (catalase, peroxidase, superoxide dismutase and glutathione reductase), which functioning under overproduction of ROS (Chouldhury *et al*., 2013). Peroxidase activity was significantly increased in hot pepper plants after application of the organophosphorus insecticides: gusation, parathion and tamaron, coincided with negative impacts on plant growth and yield as a result of physiological stress (Garcia-Hernandez *et al*., 2005). Due to a biotic stress caused by insecticides application, the plants become unable to uptake the essential micronutrients retarding the plant growth (Chauhan *et al.,* 2013). Kerns and Gaylor (1993) found that sulprophos treated-cotton leaves had higher levels of total essential amino acids. Moreover, chlorpyrifos increased soluble protein in cotton leaves twice more than the control on 10th and 14th days of the treatment (Asrorov *et al.,* 2014). Imidacloprid is bio-activated to 6- chloro-2- hydroxypyridinyl-3-carboxylic acid, which is potent inducer of pathogen proteins and inhibitor of salicylic acid – sensitive enzymes (catalase and peroxidase) associated with enhanced stress tolerance in *Arabidopsis thaliana* (Ford *et al*., 2010). Our obtained results indicated that the conventional tested insecticides (chlorpyrifos, methomyl and deltamethrin) caused physiological stress on the treated cotton plants, whereas the other tested insecticides induced the plant defense response and enhanced the cotton growth. Chlorpyrifos had a particular case where it increased the defense enzymes activity (physiological stress) and increased the total soluble protein.

Table 5. Determination of catalase, peroxidase and Polyphenoloxidase activities and total soluble protein content in cotton plants after treatment of the tested insecticides.

Treatments	Application rate / L	Catalase (µ Mole/g protein)	% decrease or increase from control	Peroxidase (µ Mole/g protein)	% decrease or increase from control	Polyphenoloxidase (µ Mole/g protein)	% decrease or increase from control	Total protein mg /g fresh weight	% decrease or increase from control
Flonicamid	0.42 g	6.84^{bc}	-20.74	0.0086^{bc}	-25.86	0.0017^{c}	-19.05	6.4^{ab}	+52.40
Emamectin-benzoate	0.3 g	7.64^{bc}	-11.47	0.0106^{b}	-8.62	0.0015^{c}	-28.57	3.4^{c}	-19.05
Imidacloprid	0.6 ml	3.02^{d}	-65.05	0.0030^{d}	-74.14	0.0026^{ab}	+23.81	6.4^{ab}	+52.40
Thiamethoxam	0.2 g	2.90^{d}	-66.63	0.0046^{c}	-60.35	0.0038^{a}	+80.95	5.6^{ab}	+33.33
Chlorpyrifos	5 ml	10.30^{a}	+19.37	0.0176^{a}	+51.72	0.0016^{c}	-23.81	7.7^{a}	+83.33
Methomyl	1.5 g	9.12^{ab}	+5.65	0.0153^{ab}	+31.89	0.0017^{c}	-19.05	4.5^{b}	+7.14
Deltamethrin	2 ml	9.21^{ab}	+6.74	0.0163^{ab}	+40.52	0.0014^{c}	-33.33	2.7^{c}	-35.71
Control	—	8.63^{b}	—	0.0116^{b}	—	0.0021^{b}	—	4.2^{b}	—

In the same column, means followed by the same letters are not significantly different at 0.05 probability level by Duncan (1955).

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كفاءة بعض المبيدات الحديثة والتقليدية ضد منّ القطن وتأثيراتها الجانبية علي الكائنات الحية غير المستهدفة والإنزيمات النباتية الدفاعية في نبات القطن

عبد الحكيم الدمرداش الشربيني[١] ، صبحي عبد الرحيم حامد[١] ، الزاهي صابر الزاهي[٢] و سامي كمال محمد قريش[٢]

[١] قسم وقاية النبات، كلية الزراعة، جامعة طنطا

[٢] معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الجيزة، مصر

تعتبر حشرة منّ القطن من الآفات الهامة التى تصيب نباتات القطن في مصر, و من ثم أجريت دراسات حقلية ومعملية في محطة البحوث الزراعية بسخا، مصر خلال موسمى ٢٠١٦ ، ٢٠١٧ لتقييم كفاءة سبعة مبيدات وهى الفلونيكاميد، الإميداكلوبريد، الثياميثوكسام، إيمامكتن-بنزوات، الكلوربيريفوس، الميثوميل، الدلتامثرين على حشرة منّ القطن في الحقل. كذلك تمت دراسة التأثيرات الجانبية لهذه المبيدات على المفترسات المصاحبة والكائنات الحية الأرضية والإنزيمات الدفاعية النباتية. كان مركب الفلونيكاميد الأكثر فاعلية ضد حشرة المنّ . لم تختلف معنويا فاعلية مركبات الإميداكلوبريد و الثياميثوكسام عن فاعلية مركبات الكلوربيريفوس و الميثوميل مسجلة من ٨٣.٢٨ – ٩٣.٢٧ % حفض في الإصابة بحشرة منّ القطن. و كانت مركبات الفلونيكاميد، إيمامكتن-بنزوات ، الإميداكلوبريد، الثياميثوكسام الأقل ضرراً على المفترسات المصاحبة مسببة أقل من ٥٠% نسبة موت، في حين كانت المركبات الأخرى سامة جداً. أثبت الفلونيكاميد درجة عالية من الأمان على مفصليات الأرجل الدقيقة التى تسكن التربة متبوعاً بالإيمامكتن-بنزوات و الميثوميل و الدلتامثرين . على العكس من ذلك, كان الكلوربيريفوس و الإيميداكلوبريد هما الأكثر سمية على مفصليات الأرجل الدقيقة التى تسكن التربة. زادت المبيدات التقليدية (الكلوربيريفوس، الميثوميل، الدلتامثرين) من نشاط إنزيمات الكاتاليز والبيروكسيديز مسببة ضغط فسيولوجي على نباتات القطن المعاملة ، في حين أن المركبات الأخري المختبرة سجلت خفض في نشاط إنزيمات الكاتاليز والبيروكسيديز مستحسة بذلك إستجابة النبات الدفاعية. زاد نشاط إنزيم البوليفينول أكسيديز بواسطة مركبى الإميداكلوبريد و الثياميثوكسام فقط. أدت المعاملة بمركبات الإيمامكتن-بنزوات و الدلتامثرين إلى إنخفاض محتوى النباتات المعاملة من البروتينات الكلية الذائبة، بينما المعاملة بالمركبات الأخرى المختبرة زادت من محتوى النباتات المعاملة من هذه البروتينات الكلية الذائبة مقارنة بالنباتات غير المعاملة.

كلمات مفتاحية: القطن، حشرة منّ القطن، المبيدات، المفترسات، كائنات التربة ، الإنزيمات المضادة للأكسدة.