More Effective, Selective and Safe Neonicotinoid: Efficacy of Selected Neonicotinoids on Cotton Aphid, *Aphis gossypii* (Homoptera: Aphididae) and Their impact on Natural Predators

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ABSTRACT: Recent limitations of European Food Safety Authority (EFSA) have been reported on imidacloprid, clothianidin and thiamethoxam uses at the request of European (EU) commision regulation. This research focused on safer alternatives for the restricted neonicotinoids in controlling cotton aphids. Semi-field experiments on laboratory cotton aphid strain revealed that the highest total residual efficacies of acetamiprid (61.50 and 64.00 %), clothianidin (50.50 and 51.50 %) and thiacloprid (58.00 and 57.00 %) during seasons of 2017 and 2018, respectively. Relative residual toxicity values of acetamiprid (1.87 and 1.98) and thiacloprid (1.15 and 1.55) altered the restricted neonicotinoids while dinotefuran (0.61 and 0.77) altered only thiamethoxam. The field experiments showed that the highest overall mean reductions of cotton aphid revealed in thiacloprid (90.91 and 87.96 %) and acetamiprid (87.36 and 81.48 %) during the seasons of 2017 and 2018, respectively. The field experiments on aphid's predators throughout 30 days post-treatments during the two seasons showed that dinotefuran was a super alternative that fulfilled equilibrium balance for predator/prey ratio and harmless effects based on the International Organization for Biological Control (IOBC). Thiamethoxam maintained equilibrium balances and slight harmfull by IOBC. The other tested insecticides were excluded from all safety margins.

Keywords: imidacloprid, clothianidin, thiamethoxam, dinotefuran, residual toxicity.

1. INTRODUCTION

The cotton aphid, *Aphis gossypii* (Homoptera - Aphididae), is one of the most important sap piercing-sucking pests that harms cotton yield and fiber quality in many growing areas world-wide (**Parker *et al*., 1995; Stoetzel *et al*., 1996; Daughtery *et al*., 1997; Gupta *et al*., 1997; Liu, 2000; Ajlan, 2001**). Direct feed and excretion of honeydew of cotton Aphid contains high content of mono-saccharides and many free amino acids that stimulate growth of fungi and viruses transmission that cause many pathogenic diseases on cotton plant **(Jech and Henneberry, 2001**). The unguided uses of insecticides since 1930's and until now develop resistance and outbreak in this pest (**Sarwar *et al*., 2014**).

Neonicotinoids (neonics) have been introduced into the market as a systemic selective insecticides instead of conventional insecticides, organophosphates and methyl-carbamates, that drive resistance in piercing sucking insect pests such as aphids (**Tomizawa *et al*., 2007**). Applications of foliar spray of acetamiprid, thiamethoxam, dinotefuran and imidacloprid caused a high significant reduction in the cotton aphid population under the cotton field conditions and efficiency residues of these insecticides persisted up to 21 days post-treatments (**Gaber *et al*., 2015**).

Exceptional admonition has been subjected to imidacloprid applications that should be used with caution by growers due to its long-term residues and the adverse effects on natural enemies (**Grafton-Cardwell *et al*., 2008; Gentz *et al*., 2010**). Thiamethoxam could adversely affect bee navigation. Chronic exposure of ingested doses of 1.96 – 2.90 ng / bee / day of field-relevant thiamethoxam could significantly cause declinations in durations and distances of flight approximately to half their rate as well as velocities decreased to 7 % after continuous exposure for either one or two days. Such exposure might corrupt foraging and homing, which were necessary to colony maintain and ecosystem (**Tosi *et al*., 2017**). These previous defects of some neonics came in accordance to assessment of European Food Safety Authority (EFSA), which had been released in February 2018 at the request of Commission Implementing Regulation (EU) concerning the three neonics of clothianidin, imidacloprid and thiamethoxam. The uses of these neonics in the open fields were submitted to restrictions for outdoor crops but the exception for neonics uses in greenhouses and after flowering applications were allowed. The restrictions and limitation of neonics applications aimed to protect the crop pollinators and bee's life **(EFSA, 2018)**.

In this respect, our objectives were directed to study the efficacy and residual toxicity of six selected neonics against cotton aphid in laboratory and under field condition. In addition, the research willing to elect alternative neonics instead of those submitted to EU restrictions in open field. Moreover, our work investigated some remarkable parameters to evaluate the

adverse and selective effects of the tested neonics on both cotton aphids as target pests and their beneficial insects. Eventually, the rational values of natural predator versus prey density in cotton field were performed as indicator for the status of the ecosystem balance.

2. MATERIALS AND METHODS

2.1. Rearing of cotton aphid:

Samples of *A. gossypii* were previously obtained from fields on broad weeds in late winter season of 2016. These samples were reared on cotton seedling plants (Giza 86) in plastic pots (diameter, 20 cm), inside cages (60 x 50 x 70 cm) covered with muslin cloth at 23±3 ºC, 60% RH and 16:8 light/dark regime in the laboratory of Plant Protection Research Institute, Al-Sabhia, Alexandria. This procedure simulated the rearing method performed by **Gaimari and Turner (1996)**. Disfigured plants were changed whenever needed by new healthy ones to avoid excessive crowding of cotton aphids. The obtained adult aphids were assigned for the toxicity tests as well as for semi-field experiments in seasons of 2017 and 2018.

2.2. Tested insecticides:

Table (1) shows the tested neonicotinoid insecticides.

Table (1): Overview of the selected neonicotinoid

Common name	Trade name	Produced company-Origin	Doses $100L^{-1}$
Imidacloprid	Imidact® 35% SC	Actra for Chemical Industries - Egypt	80 ml
Acetamiprid	Odax® 70% SP	Sharda Worldwide Export Limited - India	50 gm
Thiamethoxam	Lex® 25% WG	Higher Pesticides and Chemicals Group. Limited - China	25 gm
Dinotefuran	Oshin® 20% SG	Mitsui Chemicals Agro Incorporation-Japan	50 gm
Thiacloprid	Blanch® 48% SC	Jiangso Flag Chemical Industry Limited - China	30 ml
Clothianidin	Supertox® 48% SC	Jiangso Flag Chemical Industry Limited - China	15 ml

2.3. Laboratory studies:

Toxicity of imidacloprid, aceta-miprid, thiamethoxam, dinotifuran, thiacloprid and clothianidin were evaluated on *A. gossypii*. Six serial concentrations of each insecticide were prepared in distilled water. Cotton leaves of the same size were dipped in each concentration for 10 sec. and dried at room temperature. According to susceptibility test method of **Insecticide Resistance Action Committee (2016)** Two treated cotton leaves were placed in ventilated plastic container (7 cm in diameter) fitted with lids had gauze covered ventilation micro holes. Agar gel 1% was poured into the bases of the plastic container allow at least 10mm between the top of the agar and the rim of the petri-dish. Ten starved adults were transferred for each plastic container. Untreated cotton aphids were fed on cotton leaves dipped in distilled water only. Each concentration was replicated four times. The replicates incubated at room temperature and numbers of living and dead insects were observed after 24 hours of application. Mortality percentages were corrected by using formula of **Abbott (1925)** and subjected to probit analysis (**Finney, 1971**).

2.4. Field and semi-field experiments design:

Two field experiments were achieved at 24 and 27 of May during seasons of 2017 and 2018, respectively. The experiments were carried out on cotton variety Giza 86 at Ezbit-Mohseen Al-Kobra, Alexandria. All cultural practices tracked the guidance of optimal production processes of cotton crop. All treatments were assigned to 40 m^2 micro-plots in a randomized complete block design with four replicates. The treatments of the selected nionics were sprayed separately using Knapsack sprayer equipment (CP3) at their field recommended dosages in 2 liter per each micro-plot. Control treatment was sprayed by water only.

2.4.1. Semi-field evaluation on cotton aphid:

Young cotton leaves of apical shoots of treated and untreated (control) plants were collected from each plot and preserved in perforated bags at intervals of 1, 4, 7, 12 and 16 days after application and transferred to laboratory. Two equalized leaf disks were placed in each perti-dish. Ten of adult cotton aphids were mountain on the leaves of each perti-dish. Each treatment was replicated 4 times. The experiment was maintained under 25ºC and 60% RH. Mortality percentages of treated and untreated of cotton aphids (lab. strain) were recorded after 24 hrs of exposure and corrected according to equation of **Abbott (1925)**. Medium lethal time (LT_{50}) was used to express the time needed to realize 50% of mortality (**Abd El-Rheem, 2005; Patil, 2015**).

2.4.2. Field evaluation on cotton aphids:

In field, the treated and untreated plots were checked up for reduction percentages of cotton aphid after 1, 4 and 7 days post-treatment. In addition, population percentages of the survival predators were recorded at 1, 7, 16, 23 and 30 days post-treatment. Reduction percentages of cotton aphids and population percentages of the survival predators were calculated according to the formula **Henderson and Tilton (1955).**

2.4.3. Safety margins of the selected neonicotinoids on predators of cotton aphid in field:

Adverse effects of insecticides on natural enemies were classified by International Organization for Biological Control (IOBC) (**Hassan, 1992**). This classification system included class 1 that meant by harmless effect at mortality percentages < 25 %, class 2 meant by slightly harmful effect at mortality percentages of 25-50 %, class 3 meant by moderately harmful effect at mortality percentages of 51-75 % and class 4 meant by harmful effect at mortality percentages > 75 %. On the other hand, predator/prey ratio was based on beneficial arthropod index (BIx), developed by advisory systems and IPM in Belgium to estimate the possible biological control of aphids. This index included safety margin of (< 2) meant by unfavorable balance or cotton aphid outbreak, (range from 2 to 10) meant by equilibrium between cotton aphid and beneficial insects and (>10) meant by an efficient natural enemies overcome the aphids populations (**Naranjo *et al.*, 2004; Jansen *et al.*, 2014**).

2.5. Statistical analysis:

All the obtained results were subjected to analysis of variance (ANOVA). Means were determined for significance at 0.05 using LSD test using **SAS software (2002)**.

3. RESULTS

3.1. Toxicity of the selected neonicotinoids against adult stage of cotton aphid strain:

Results of toxicity in Table (2) showed that, the maximum LC_{50} values were recorded in the treatments of dinotefuran (0.32 mg L^{-1}) followed by imidacloprid (0.28 mg L^{-1}), clothianidin (0.25 mg L^{-1}) and thiamethoxam (0.23 mg L^{-1}) which showed moderate to higher toxicity. While, the minimum LC_{50} values were recorded in treatments of each of acetamiprid (0.11 mg L^{-1}) and thiacloprid (0.14 mg L^{-1}) that owned relative more toxic effects on *A. gossypii*. On the other hand, the maximum LC_{90} values were recorded in treatments of imidacloprid (5.82 mg L^{-1}) followed by thiamethoxam (4.51 mg L^{-1}), clothianidin (4.03 mg L^{-1}), dinotefuran (3.54 mg L^{-1}) and thiacloprid (2.85 mgL^{-1}). The minimum LC_{90} was recorded in treatment of acetamiprid (2.23 mg L^{-1}). According to LC_{50} values, the descending order of neonics toxicity were acetamiprid > thiacloprid > thiamethoxam > clothianidin > imidacloprid > dinotefuran on *A. gossypii* (Table 2).

Table (2): Toxicity of the selected neonicotinoids on adult stage of *Aphis gossypii* (lab. strain) at 24 hrs of exposure

Tested neonicotinoids	LC_{50} (mg L^{-1})	Confidence limits (mg L^{-1})	LC_{90} (mg L^{-1})	Confidence limits (mg L^{-1})	Slope ± SE*
Acetamiprid	0.11	0.07-0.18	2.23	0.97-11.54	0.99 ± 0.181
Thiacloprid	0.14	0.09-0.23	2.85	1.18-16.47	0.98 ± 0.180
Thiamethoxam	0.23	0.13-0.36	4.51	1.96-24.16	0.99 ± 0.184
Clothianidin	0.25	0.15-0.38	4.03	1.87-17.58	1.06 ± 0.187
Imidacloprid	0.28	0.17-0.46	5.82	2.41-35.38	0.98 ± 0.183
Dinotefuran	0.32	0.21-0.47	3.54	1.83-11.38	1.22 ± 0.193

*SE means standard error

3.2. Residuals toxicity of the selected neonicotinoids against *A. gossypii* (semi-field trials):

Regarding to the data of season 2017, acetamiprid, thiacloprid and clothianidin were considered to be the most potent neonic against adult stage of *A. gossypii* populations (Table 3). The results of overall mean of residual efficacy percentages revealed that acetamiprid and thiacloprid had the highest values of 61.50 and 58.00 %, respectively and followed by clothianidin at values of 50.50 %. Whereas, the data of LT_{50} were found in treatments of acetamiprid, clothianidin and lasted with thiacloprid at 9.12, 5.87 and 5.63 days, respectively. Acetamiprid, clothianidin and thiacloprid had calculated relative efficacies periods based on LT_{50} of imidacloprid at values of 1.87, 1.20 and 1.15, respectively as well as those based on LT_{50} of thiamethoxam were 3.1, 1.98 and 1.90, respectively. These values set the alternative orders for acetamiprid, clothianidin and thiacloprid at the 1st, 2nd and 3rd sequences, respectively. On the other hand, dinotifuran had the lowest values for each of overall mean of residual toxicity percentage of 41.50 % and LT_{50} of 3.00 days. The relative efficacy periods of dinotifuran based on LT_{50} of imidacloprid and thiamethoxam were 0.61 and 1.02, respectively and consequently had the 5th alternative order instead of thiamethoxam only. Finally, the data of overall mean of residual toxicity percentage of thiamethoxam had a medium value of 46.50 % compared to the tested neonics and the lowest value of LT_{50} at 2.96 days. Meantime, the result of overall mean of residual toxicity of imidacloprid had the lowest value of 43.00 % and LT_{50} at 4.88 days (Table 3).

Table (3): Residuals toxicity of the selected neonicotinoids against *A. gosssypii* (lab. strain) after 24hrs of exposure during cotton season of 2017

Tested neonicotinoids	Overall mean of residual toxicity %[1]	LT_{50}[2] (days)	Confidence limit (days)	Slope ± S.E[3]	Based on LT_{50} of imidacloprid		Based on LT_{50} of thiamethoxam	
					Relative residual toxicity [4]	Alternative Order	Relative residual toxicity	Alternative Order
Acetamiprid	61.50[a]	9.12	(7.91-10.57)	2.13±0.29	1.87	1	3.1	1
Thiacloprid	58.00[a]	5.63	(5.60-6.55)	2.24±0.40	1.15	3	1.90	3
Clothianidin	50.50[b]	5.87	(4.31-7.17)	1.52±0.28	1.20	2	1.98	2
Thiamethoxam	46.50[cb]	2.96	(0.69-4.64)	0.97±0.28	0.60	6	1.00	6
Dinotefuran	41.50[c]	3.00	(1.41-4.19)	1.41±0.30	0.61	5	1.02	5
Imidacloprid	43.00[c]	4.88	(4.05-5.60)	2.72±0.32	1.00	4	1.65	4

[1]Overall mean of residual efficacy percentages calculated within intervals of 1, 4, 7, 12, 16 days; [2]LT_{50} calculated within the intervals of 4, 7, 12, and 16 days; [3]S.E means standard error; [4]Relative residual efficacy period = LT_{50} of tested neonics / LT_{50} of restricted neonics (imidacloprid or thiamethoxam); Means of overall mortality with the same letter are not significantly different according to the $LSD_{0.05}$= 6.76.

The data of season 2018 showed that acetamiprid, thiacloprid and clothianidin were considered to be the most potent neonic against adult stage of *A. gossypii* populations. The results of overall mean of residual efficacy percentages revealed that acetamiprid, thiacloprid and clothianidin had the highest values of 64.00, 57.00 and 51.50 %, respectively corresponding to highest values of LT_{50} with values of 9.29, 7.28 and 5.30 days, respectively (Table 4). In addition, acetamiprid, thiacloprid and clothianidin had calculated relative toxicity periods based on LT_{50} of imidacloprid with values of 1.98, 1.55 and 1.13, respectively as well as those based on LT_{50} of thiamethoxam with values of 3.78, 2.96 and 2.15, respectively. These values set the alternative orders for acetamiprid, thiacloprid and clothianidin at the 1st, 2nd and 3rd sequences, respectively. Imidacloprid, dinotifuran and thiamethoxam had the lowest overall mean of residual efficacy percentages at similar values of 45.00 %. Likewise, dinotifuran and thiamethoxam had the lowest values of LT_{50} at 3.60 and 2.46 days, respectively. Dinotifuran had relative efficacy periods based on LT_{50} of imidacloprid and thiamethoxam at values of 0.77 and 1.46. Consequently, dinotifuran occupied the 5th alternative order instead of thiamethoxam only. On the other hand, the result of LT_{50} of imidacloprid was 4.70 days and its relative toxicity period based on LT_{50} of thiamethoxam was 1.91. Thus, imidacloprid occupied the 4th alternative order instead of thiamethoxam (Table 4).

Table (4): Residuals toxicity of the selected neonicotinoids against *A. gossypii* (lab. strain) after 24hrs of exposure during cotton season of 2018

Tested neonicotinoids	Overall mean of residual toxicity %[1]	LT_{50}[2] (days)	Confidence limit (days)	Slope ± S.E[3]	Based on LT_{50} of imidacloprid		Based on LT_{50} of thiamethoxam	
					Relative residual toxicity [4]	Alternative Order	Relative residual toxicity	Alternative Order
Acetamiprid	64.00[a]	9.29	(8.00-10.91)	1.99±0.29	1.98	1	3.78	1
Thiacloprid	57.00[b]	7.28	(5.59-8.96)	1.32±0.28	1.55	2	2.96	2
Clothianidin	51.50[cb]	5.30	(3.15-6.90)	1.172±0.28	1.13	3	2.15	3
Thiamethoxam	45.50[c]	2.46	(0.29-4.2)	0.89±0.29	0.52	6	1	6
Dinotefuran	45.00[c]	3.60	(1.89-4.89)	1.36±0.29	0.77	5	1.46	5
Imidacloprid	45.50[c]	4.70	(3.55-5.65)	1.99±0.30	1	4	1.91	4

[1]Overall mean of residual efficacy percentages calculated within intervals of 1, 4, 7, 12, 16 days; [2]LT_{50} calculated within the intervals of 4, 7, 12, and 16 days; [3]S.E means standard error; [4]Relative residual efficacy period = LT_{50} of tested neonics / LT_{50} of restricted neonics (imidacloprid or thiamethoxam); Means of overall mortality with the same letter are not significantly different according to the $LSD_{0.05}$.

3.3. Field efficacy of the selected neonicotinoids against *Aphis gossypii*:

During the first season of 2017, the data of overall mean of protection levels in table (5) showed that the most potent neonics against *A. gossypii* that achieved maximum reduction percentages and minimum population numbers per micro-plot were respectively obtained from the treatments of thiacloprid (90.91 % and 4.92 per micro-plot), acetamiprid (87.36 % and 3.42 per micro-plot) and thiamethoxam (83.64 % and 8.67 per

micro-plot). While clothianidin and dinotifuran had significant lower protection levels expressed by reduction percentages of69.57 and 62.51 %, respectively and population numbers of 13.92 and 10.75 per micro-plot, respectively. Finally, imida-cloprid had the lowest protection level expressed by reduction percentage of 61.17 % and population number of 12.92 per micro-plot.

Table (5): Protection levels of the selected neonicotinoids against *A. gossypii* under field conditions during cotton season of 2017.

Tested neonicotinoids	Reduction % & (Population numbers) of *A. gossypii* per micro-plot							Overall mean of Protection levels
	Pre-treatments population No.	1-days		4-days		7-days		
Acetamiprid	(31.50)	87.93	(3.00)	86.73	(3.50)	87.41	(3.75)	87.36 (3.42^{e})
Thiacloprid	(48.00)	90.79	(5.00)	90.45	(5.50)	91.50	(4.25)	90.91 (4.92^{e})
Thiamethoxam	(37.00)	83.50	(8.75)	83.55	(7.00)	83.86	(10.25)	83.64 (8.67^{d})
Dinotefuran	(28.25)	62.78	(12.50)	62.54	(10.50)	62.20	(9.25)	62.51 (10.75^{cd})
Imidacloprid	(30.75)	61.30	(13.75)	60.80	(13.75)	61.41	(11.25)	61.17 (12.92^{cb})
Clothianidin	(36.25)	70.13	(14.00)	69.50	(13.25)	69.07	(14.50)	69.57(13.92^{b})
Control	(33.71)	-	(38.50)	-	(36.25)	-	(37.58)	- (37.44^{a})

Means of overall mean of population percentages with the same letter are not significantly different according to the $LSD_{0.05}$

Comparably, the data of overall mean of protection levels in the season of 2018 confirmed that the most potent neonics that achieved maximum reduction percentages and minimum population numbers against *A. gossypii* were respectively obtained from thiacloprid (87.96 % and 5.42 per micro-plot), acetamiprid (81.48 % and 4.33 per micro-plot) and thiamethoxam (81.00 % and 8.42 per micro-plot). Whereas, clothianidin and dinotifuran had significant lower protection levels expressed by reduction percentages of 67.27 and 61.54 %, respectively and population numbers of 14.17 and 10.58 per micro-plot, respectively. Eventually, imidacloprid had the lowest protection level expressed by reduction percentage of 58.68 % and population number of 12.83 per micro-plot (Table 6).

Table (6): Protection levels of the selected neonicotinoids against *A. gossypii* under field conditions during cotton season of 2018.

Tested neonicotinoids	Reduction % & (Population numbers) of *A. gossypii* in each micro-plot							Overall mean of Protection levels
	Pre-treatments population No.	1-days		4-days		7-days		
Acetamiprid	(27.25)	76.50	(5.25)	78.42	(5.00)	89.53	(2.75)	81.48 (4.33^{e})
Thiacloprid	(42.75)	85.34	(6.5)	86.40	(6.50)	92.15	(3.25)	87.96 (5.42^{e})
Thiamethoxam	(34.75)	77.58	(10.25)	80.20	(7.25)	85.21	(7.75)	81.00 (8.42^{d})
Dinotefuran	(25.00)	56.93	(13.5)	58.00	(10.50)	69.70	(7.75)	61.54 (10.58^{cd})
Imidacloprid	(26.50)	53.75	(15.25)	57.22	(13.50)	65.07	(9.75)	58.68 (12.83^{cb})
Clothianidin	(31.25)	59.05	(16.5)	69.60	(13.50)	73.17	(12.50)	67.27(14.17^{b})
Control	(34.71)	-	(39.50)	-	(37.79)	-	(39.04)	- (38.78^{a})

Means of overall mean of population percentages with the same letter are not significantly different according to the $LSD_{0.05}$

3.4. Residual efficacy of the selected neonicotinoids on the survival populations of aphid predators in the field:

Data of the two seasons showed that thiamethoxam and dinotifuran had clear harmful effects that prevailed within 24 hrs post-treatments coinciding with the approximate dissipations (≤ 25 %) in the mean survival population percentages of *C. carena*, *C. undecimpuctata* and *Orius* sp. according to the classification index developed by IOBC classification. In the same way, the harmful effects had been manifested for the treatments of imidacloprid, clothianidin, thiacloprid and acetamiprid that linked with aproximate dissipations for the populations of tested natural enemies. These dissipations grew up throughout more extended

periods reached 16 days or even sometimes 23 days post-treatment (Tables 7 and 8)..

In the first season of 2017, the data of field experiments of dinotifuran and thiamethoxam settled the margin of slight harmful effects based on the responses of their overall mean of the survival populations of *C. carena* with percentages of 66.04 and 71.32 %, respectively along the 30 days post-treatments. On the second rank, imidacloprid and thiacloprid had moderate harmful effects on the overall mean of populations of *C. carena* that reached survival percentages of 25.60 and 25.85 %, respectively. Finally, clothianidin and acetamiprid had harmful effects on the overall mean of populations of *C. carena* that reached survival percentages of 22.00 and 3.25 %, respectively. Particularly, treatments of dinotifuran and thiamethoxam reached the margin of harmless effects (treated *C. carena* population increased above 75%) within the periods from 16 to 30 days post-treatment. On the other hand, imidacloprid, clothianidin and thiacloprid realized the margin of slight harmful effects by the 30th day post-treatment. Finally, treatment of acetamiprid kept on the harmful effects throughout the 30 days post-treatment.

The data of the highest overall mean of survival population percentages of *C. undecimpuctata* treated with dinotifuran were 84.66 %. Thus, dinotifuran treatment possessed harmless effects. Treatment of thiamethoxam occupied the second rank due to its slight harmful effects on the overall mean of populations of *C. undecimpuctata* that reached survival percentages of 55.62 %. Whereas, thiacloprid treatments followed dinotifuran and thiamethoxam with a moderate harmful effects on *C. undecimpuctata* populations reached an overall mean of survival percentages of 26.13 %. Meantime, imidacloprid, clothianidin and acetamiprid had relative high harmful effects linked with significant lower percentages of 15.08, 17.45 and 2.35 %, respectively for overall mean of *C. undecimpuctata* populations. Particularly, treatments of dinotifuran and thiamethoxam had harmless effects (treated *C. undecimpuctata* population increased above 75%) within the periods from 16 to 30 days and at the 30th day post-treatment, respectively. Imidacloprid, clothianidin and thiacloprid realized the margin of slight harmful effects by the 30th day post-treatment. Finally, acetamiprid treatment kept on the harmful effects throughout the 30 days post-treatment.

The same trend of the field experiment revealed that the highest significant overall mean of survival populations of *Orius* sp were 74.40 and 56.43 % for the treatments of dinotifuran and thiamethoxam, respectively. Dinotifuran and thiamethoxam treatments possessed slight and moderate harmful effects, respectively. Meanwhile, overall mean of survival *Orius* sp populations treated with imidacloprid, clothianidin, thiacloprid and acetamiprid were 15.96, 16.57, 3.65 and 0.23 %, respectively. These treatments possessed harmful effects on the tested predators on cotton aphids. Particularly, treatments of dinotifuran, thiamethoxam and imidacloprid had harmless effects within the periods from 23 to 30 days, 16 to 30 days and at the 30th day post-treatment, respectively. Clothianidin treatment kept on the margin of moderate harmful effects as well as thiacloprid and acetamiprid treatments had harmful effects throughout the tested period of the field experiment. Finally, the data of overall mean of population percentages of the tested native predator of cotton aphid treated with dinotifuran and thiamethoxam in season of 2017 showed almost approximate accesses or even overpasses to the full recovery levels (≥100 %) within the period of 16 to 30 days and at the 30th days post-treatments, respectively.

Table (7): Mean percentages of survival predator's populations after sequent days of exposure to the selected neonicotinoids under field conditions in season of 2017.

Tested neonicotinoids	Mean of pre-treatments population	Survival population% & (population no.) of *Chrysoperla Carena*					Overall Mean% of survival populations	IOBC classification
		1-days	7-days	16-days	23-days	30-days		
Dinotefuran	-	14.03	35.56	92.31	93.15	95.17	66.04[a]	Slight harmful
	(22.75)	(2.25)	(8.25)	(20.25)	(19.50)	(22.00)	(14.45)	
Thiamethoxam	-	0.00	66.76	87.37	93.33	109.14	71.32[a]	Slight Harmful
	(22.75)	(0.00)	(9.00)	(18.25)	(20.00)	(21.00)	(13.65)	
Imidacloprid	-	0.00	0.00	0.00	67.97	60.03	25.60[b]	Moderate Harmful
	(22.25)	(0.00)	(0.00)	(0.00)	(8.75)	(10.75)	(3.90)	
Clothianidin	-	0.00	0.00	4.98	49.00	56.03	22.00[b]	Harmful
	(22.00)	(0.00)	(0.00)	(1.00)	(10.00)	(11.25)	(4.45)	
Thiacloprid	-	0.00	00.00	0.00	77.15	52.10	25.85[b]	Moderate Harmful
	(20.75)	(0.00)	(0.00)	(0.00)	(14.00)	(8.50)	(4.50)	

To be continued

Acetamiprid	- (22.75)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.16 (1.00)	11.07 (2.50)	3.25^{c} (0.70)	Harmful

Tested neonicotinoids	**Mean of pre-treatments population**	**Survival population% & (population no.) of *Coccinella undecimpuctata***					**Overall Mean% of survival populations**	**IOBC classification**
		1-days	**7-days**	**16-days**	**23-days**	**30-days**		
Dinotefuran	- (19.25)	7.11 (0.00)	49.78 (7.00)	109.07 (14.00)	155.41 (13.00)	101.96 (18.00)	84.66^{a} (10.40)	Harmful less
Thiamethoxam	- (18.75)	00.00 (1.25)	36.45 (9.75)	74.55 (20.75)	71.63 (20.75)	95.45 (20.50)	55.62^{b} (14.60)	Slight harmful
Imidacloprid	- (19.25)	00.00 (0.00)	00.00 (0.00)	4.14 (0.75)	20.86 (4.00)	50.40 (9.75)	15.08^{dc} (2.90)	Harmful
Clothianidin	- (22.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	24.20 (6.50)	63.04 (14.25)	17.45^{dc} (4.15)	Harmful
Thiacloprid	- (20.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	74.15 (9.00)	56.52 (12.00)	26.13^{c} (4.20)	Moderate harmful
Acetamiprid	- (20.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	11.77 (0.00)	2.36 (3.00)	2.35^{d} (0.60)	Harmful

Tested neonicotinoids	**Mean of pre-treatments population**	**Survival population% & (population no.) of *Orius* sp**					**Overall Mean% of survival populations**	**IOBC classification**
		1-days	**7-days**	**16-days**	**23-days**	**30-days**		
Dinotefuran	- (11.00)	23.23 (3.25)	62.73 (7.00)	70.31 (8.50)	101.73 (12.25)	114.00 (14.25)	74.40^{a} (9.05)	Slight harmful
Thiamethoxam	- (11.25)	00.00 (0.00)	29.73 (1.50)	92.63 (5.25)	60.37 (6.25)	99.42 (9.25)	56.43^{a} (4.45)	Moderate harmful
Imidacloprid	- (13.75)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	79.78 (8.50)	15.96^{b} (1.70)	Harmful
Clothianidin	- (12.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	39.49 (3.00)	43.36 (4.75)	16.57^{b} (1.55)	Harmful
Thiacloprid	- (12.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.30 (0.75)	9.96 (1.00)	3.65^{b} (0.35)	Harmful
Acetamiprid	- (11.25)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	1.17 (0.25)	0.23^{b} (0.05)	Harmful

*(**International Organization for Biological Control**) performed classification system (**Hassan 1992**).
Means of overall survival population% of each predator species with the same letter are not significantly different at $LSD_{0.05}$.

In the second season of 2018, the results of field experiments of dinotifuran and thiamethoxam had unique slight harmful effects based on their overall mean of survival populations of *C. carena* with percentages of 73.25 and 61.76 %, respectively within the 30 days post-treatments. Treatments of imidacloprid and clothianidin came on the second rank with moderate harmful effects on the overall mean of population of *C. carena* that reached survival percentages of 34.42 and 26.40 %, respectively. Finally, thiacloprid and acetamiprid had harmful effects on the overall mean of population of *C. carena* that reached survival percentages of 19.35 and 18.56 %, respectively. Particularly, treatments of both of dinotifuran and thiamethoxam as well as imidacloprid accessed the margin of harmless effects within the periods from 16 to 30 days post-treatment for dinotifuran and thiamethoxam and at the 30th days post-treatment for imidacloprid treatment. On the other hand, clothianidin and acetamiprid realized the margin of slight harmful effects not before the 30th day post-treatment. Finally,

treatment of thiacloprid reached the moderate harmful effects at the 30th day post-treatment (Table 8).

The overall mean population of *C. undecimpuctata* treated with dinotifuran had the highest survival percentage of 71.47 %. Therefore, dinotifuran treatment realized slight harmful effect. Treatment of thiamethoxam occupied the second rank due to its slight harmful effect on the overall mean of populations of *C. undecimpuctata* that reached survival percentage of 54.05 % then followed by imidacloprid with moderate harmful effects expressing the survival percentage of 31.64 %. Whereas, clothianidin, thiacloprid and acetamiprid possessed harmful effect on *C. undecimpuctata* populations that reached an overall mean of survival percentages of 22.04, 19.07 and 15.23 %, respectively. Particularly, treatment of dinotifuran had harmless effects within the periods from 16 to 30 days post-treatment. On the other hand, thiamethoxam treatment had variable effects swing between harmful less and slight harmful effects. Clothianidin, thiacloprid and acetamiprid realized the margin of slight harmful effects not before the 30th day post-treatment.

The data the highest overall mean of survival populations percentages of *Orius* sp were 101.20 % for the treatment of dinotifuran that possessed harmful less effects. Meanwhile, overall mean of survival populations percentages of *Orius* sp treated with thiamethoxam was 47.97 %, which expressed by its moderate harmful effect. Imidacloprid, clothianidin, thiacloprid and acetamiprid were 15.51, 14.12, 6.11 and 6.84 %, respectively. These treatments output harmful effects on the overall mean of the tested predators of cotton aphids. Particularly, treatments of dinotifuran and thiamethoxam had harmless effects within the periods from 7 to 30 days and at the 30th day post-treatment, respectively. Imidacloprid, clothianidin and thiacloprid treatments reached the margin of harmless effects not before the 30th day post-treatment. Meanwhile, acetamiprid kept on the margin of harmful effects throughout the tested period of the field experiment. Finally, the data of dinotifuran treatment showed almost approximate values or even accessed full recovery levels for the overall mean of the tested native predator populations in season of 2018 within the period of 16 to 30 days post-treatment. While the treatment of imidacloprid brought out both of *C. carena* and *C. undecimpunctata* populations to reach their full recovery levels at the 30th day post-treatments. Meantime, thiamethoxam treatment led *C. carena* populations to access their full recovery levels not before the 30th day post-treatments.

Table (8): Mean percentages of survived predator's populations after sequent days of exposure to the selected neonicotinoids under field conditions in season of 2018.

Tested neonicotinoids	Mean of pre-treatment population	Survival population% & (population no.) of *Chrysoperla Carena*					Overall Mean% of survival populations	IOBC* classification
		1-days	7-days	16-days	23-days	30-days		
Dinotefuran	-	14.78	48.97	96.17	107.00	99.34	73.25[a]	Slight harmful
	(27.75)	(3.50)	(12.50)	(25.50)	(26.25)	(26.50)	(18.85)	
Thiamethoxam	-	0.00	44.54	76.76	87.64	99.85	61.76[b]	Slight harmful
	(26.00)	(0.00)	(11.25)	(21.25)	(24.25)	(26.75)	(16.70)	
Imidacloprid	-	0.00	0.00	21.09	44.51	106.51	34.42[c]	Moderate harmful
	(27.00)	(0.00)	(0.00)	(3.25)	(5.50)	(23.25)	(6.40)	
Clothianidin	-	0.00	0.00	4.19	63.53	64.26	26.40[dc]	Moderate harmful
	(25.75)	(0.00)	(0.00)	(1.00)	(16.00)	(15.50)	(6.50)	
Thiacloprid	-	0.00	0.00	2.82	56.41	37.51	19.35[d]	Harmful
	(26.50)	(0.00)	(0.00)	(0.75)	(18.75)	(12.00)	(6.30)	
Acetamiprid	-	0.00	0.00	0.00	34.66	58.11	18.56[d]	Harmful
	(26.50)	(0.00)	(0.00)	(0.00)	(9.25)	(15.00)	(4.85)	

To be continued

Tested neonicotinoids	Mean of pre-treatments population	Survival population% & (population no.) of *Coccinella undecimpuctata*					Overall Mean% of survival populations	IOBC* classification
		1-days	7-days	16-days	23-days	30-days		
Dinotefuran	-	13.49	38.75	92.39	99.89	112.81	71.47[a]	Slight harmful
	(24.75)	(3.00)	(13.75)	(28.00)	(28.75)	(34.25)	(21.55)	
Thiamethoxam	-	0.00	34.50	76.45	69.01	90.27	54.05[b]	Slight harmful
	(24.50)	(0.00)	(7.50)	(19.00)	(18.00)	(22.50)	(13.40)	
Imidacloprid	-	0.00	0.00	0.00	55.58	102.59	31.64[c]	Moderate harmful
	(25.25)	(0.00)	(0.00)	(0.00)	(6.50)	(18.75)	(5.05)	
Clothianidin	-	0.00	0.00	1.06	38.84	70.29	22.04[dc]	Harmful
	(26.75)	(0.00)	(0.00)	(0.25)	(11.75)	(19.25)	(6.25)	
Thiacloprid	-	0.00	0.00	2.64	37.73	54.95	19.07[d]	Harmful
	(25.75)	(0.00)	(0.00)	(0.75)	(13.00)	(16.00)	(5.95)	
Acetamiprid	-	0.00	0.00	00.00	16.26	59.90	15.23[d]	Harmful
	(26.00)	(0.00)	(0.00)	(0.00)	(4.50)	(16.25)	(4.15)	

Tested neonicotinoids	Mean of pre-treatments population	Survival population% & (population no.) of *Orius* sp					Overall Mean% of survival populations	IOBC* classification
		1-days	7-days	16-days	23-days	30-days		
Dinotefuran	-	35.29	90.90	106.07	126.40	147.31	101.20[a]	Harmful less
	(13.00)	(5.25)	(11.50)	(12.25)	(17.00)	(16.75)	(12.55)	
Thiamethoxam	-	0.00	26.97	66.10	56.61	90.16	47.97[b]	Moderate harmful
	(12.50)	(0.00)	(2.75)	(6.00)	(7.00)	(9.75)	(5.10)	
Imidacloprid	-	0.00	0.00	0.00	30.00	47.53	15.51[c]	Harmful
	(15.50)	(0.00)	(0.00)	(0.00)	(4.00)	(4.75)	(1.75)	
Clothianidin	-	0.00	0.00	0.00	28.58	42.00	14.12[c]	Harmful
	(14.25)	(0.00)	(0.00)	(0.00)	(4.25)	(7.50)	(2.35)	
Acetamiprid	-	0.00	0.00	0.00	13.73	16.83	6.11[c]	Harmful
	(13.00)	(0.00)	(0.00)	(0.00)	(2.00)	(1.50)	(0.70)	
Thiacloprid	-	0.00	0.00	0.00	4.82	29.37	6.84[c]	Harmful
	(13.50)	(0.00)	(0.00)	(0.00)	(0.75)	(2.25)	(0.60)	

*(**International Organization for Biological Control**) performed classification system (**Hassan 1992**).
Means of overall survival population % of each predator species with the same letter are not significantly different at $LSD_{0.05}$.

3.5. Safety margins of the selected neonicotinoids based on authorized classification indexing:

The data of the selected neonics effects on the total survival populations of natural enemies represented by their percentage values per micro-plot were classified within safety margins based on classification indexing of IOBC on the 30th day and ratio of predators / cotton aphid (preys) on the 7th day represented by their total numbers per micro-plot (Fig. 1). The obtained results of dinotifuran treatments in seasons of 2017 and 2018 brought out the total mean of survival predators populations at percentage values of 75.04 and 81.97 %, respectively.

Thus, dinotifuran treatments in both two seasons belonged to the safety margins of class 1 (harmless effects) indicated by of IOBC. Moreover, the results of dinotifuran treatments had ratio of predator/ prey with values of 6.67 and 13.73 per micro-plot that overpassed all the safety margins (more than 10) in 2017 and 2018, respectively. On the second rank, the results of thiamethoxam treatments in the two seasons of 2017 and 2018 gave rise the total mean of survival predator populations with percentages of 61.12 and 54.59 %, respectively, which expressed by the safety margins of class 2 (slight harmful effects) in IOBC. Furthermore, the results of thiamethoxam treatment in both two seasons

affected the ratio of predator/prey in micro-plot to owned values of 4.12 and 6.62 that belonged to the margins of equilibrium balance between cotton aphids and their predators.

On the other hand, the obtained results of acetamipride, clothianidin and thiaclopride treatments in both seasons of 2017 and 2018 almost realized class 4 expressed harmful effects on total predator's populations and had the ratio of predator/prey that belonged to the margin of unfavorable levels (< 2).

Finally, imidacloprid treatments in seasons of 2017 and 2018 were varied between the margins of class 4 (harmful effects) and class 3 (moderate harmful effects), respectively.

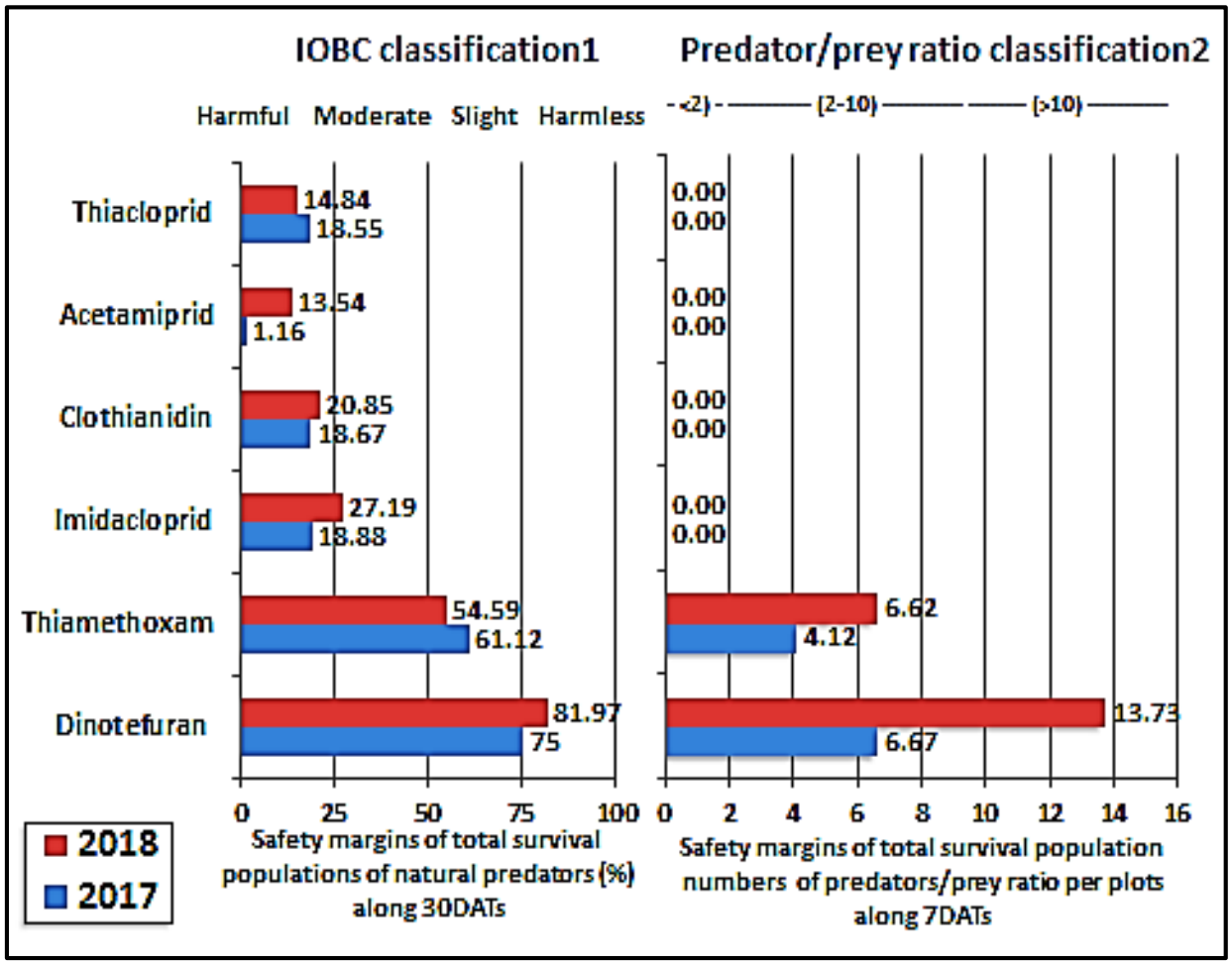

Fig. (1): Classifications of the selected neonicotinoid in terms of safety on the total tested predators of cotton aphids based on IOBC and predator/prey ratio during the seasons of 2017 and 2018

[1](International Organization for Biological Control) performed classification system based on field studies (**Hassan 1992**).

[2]:Calculated according to beneficial arthropod index (BIx), which developed by advisory systems and IPM in Belgium to estimate to possible biological control of aphids (**Naranjo *et al*. 2004; Jansen *et al*. 2014**).

4. DISSCUSIONS

The outlook of this research towards the tested neonics came in accordance to the limitations imposed by EFSA report in 2018 on the uses of imidacloprid, clothianidin and thiamethoxam at the request of Commission Implementing Regulation (EU) No. 485/2013 to protect the life of the bees. These decisions aimed to elaborate the limitations for foliar applications of these neonics in open field on crops attractive to bees. An exception had been demonstrated for greenhouses and after flowering applications. Moreover, the assessments of EFSA, included an evaluations of non-insecticide alternatives and alternative active substance to these neonics for several crop/pest combinations studies (**Commission Implementing Regulation (EU), 2013; EFSA, 2018**). In this research, we selected the restricted neonics of imidacloprid and thiamethoxam as base to calculate the relative residual efficacy periods compared to the other tested neonics, referring to their common uses against aphid pests also both of them had relative short effectiveness times required to fulfill the

half reduction percentages of *A. gossypii*. Thus, an alternative neonics could be established for the restricted ones. Referring to efficacies of the tested neonics in semi-field and field experiments on cotton aphids and regardless to their safety standards on natural enemies, sensible alternative neonics to imidacloprid and thiamethoxam could be determined. In this case, semi-field experiments on cotton aphids (lab. strain) in the two growing seasons revealed that acetamiprid, clothianidin and thiacloprid had the highest overall mean of reduction percentages and frontier orders of relative residual efficacy periods. Thus, acetamiprid, clothianidin and thiacloprid considered as reasonable alternatives to imidacloprid and thiamethoxam against *A. gossypii*. In addition, dinotifuran had reasonable order for relative residual efficacy periods based on thiamethoxam only and spontaneously considered as one of the good alternative neonics to thiamethoxam. These findings were only agreed with the uses of dinotifuran that considered as the most effective alternatives against imidacloprid-resistant *A. gossypii* but the use of nitenpyram, acetamiprid and thiacloprid should be avoided on imidacloprid-resistant populations of *A. gossypii* (**Shi *et al*., 2011**).

On the other hand, the results of overall mean of protection levels of cotton crops against cotton aphids FS under field condition investigated that thiacloprid and acetamiprid could be used as super alternative applications for thiamethoxam against *A. gossypii*. Moreover, thiacloprid, acetamiprid, clothianidin and dinotifuran were considered as alternative applications for imidacloprid in controlling *A. gossypii* in fields. These results came in accordance with the highest significant mortalities for the treatments of seven tested neonics on the populations of *A. gossypii* (lab. strain) collected from cucumber and sweet pepper crops. Whereas, the collected cotton aphid FS populations had higher significant mortality rates in treatments for acetamiprid and thiacloprid compared to low mortalities for the treatments of imidacloprid, dinotifuran, clothianidin, thiamethoxam and nitenpyram (**Matsuura and Nakamura, 2014**).

Regarding to the necessity of natural enemies ecosystem's protection, all the tested neonics were submitted to classified system and margins indicated by IOBC (**Hassan, 1992**) and predator/prey ratio (**Naranjo *et al*., 2004; Jansen *et al*., 2014**) for *C. carena*, *C. undecimpuctata and Orius* species. the foliar spray applications in open field during the two seasons with dinotifuran so far accomplished the safety margin of class 1 (harmful less effects) indicated by IOBC and fulfill the equilibrium balance levels of predator/prey ratio for all the tested predators on cotton aphids. Thiamethoxam came on the second rank according to their safety margins of class 2 (slight harmful effects) indicated by IOBC as well as to the equilibrium balance levels of predator/prey ratio for all the tested predators on cotton aphids during the two seasons. On the other hand, the foliar application of thiacloprid, acetamiprid and clothianidin treatments surpassed the tests of effectiveness and protection levels in open field trials against *A. gossypii* but failed in equilibrium balance levels for predator/prey ratio and IOBC as they shifted to class 4 (harmful effects). These variable results on the tested natural enemies came in accordance to the systemic uptake evaluation in laboratory for the impacts of imidacloprid and thiamethoxam by on parasitoid species of *Aphytis melinus*, *Gonatocerus ashmeadi*, *Eretmocerus eremicus* and *Encarsia formosa* and predators species of *Geocoris punctipes* and *Orius insidiosus*. These beneficial arthropods systemically subjected to treated leaves of imidacloprid and thiamethoxam had adverse effects on adult stage in all survival parasitoids populations, with higher potency against *A. melinus*. In addition, *G. ashmeadi*, *E. eremicus* and *E. formosa* had high mortalities but not before 48 hrs of exposure. The two predators of *G. punctipes* and *O. insidiosus* had variably susceptibilities to these neonics after 96 hrs of exposure. Thus, toxic effect on these predators may be related to their feeding on treated plant leaves as well as to their contact exposures to surface residues (**Prabhaker *et al*., 2011**).

CONCLUSION

Dinotifuran could consider as a super alternative to restricted neonics as it overpassed laboratory and opened field trials on *A. gossypii* and realized safety margins to their predators.

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مبيد النيونيكوتينويد الاكثر تأثيرا، إختيارية وآمان : التأثير الإبادى لمبيدات النيونيكوتينودات على من القطن والمفترسات الطبيعية

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الملخص العربى:

بناء على المحددات الاخيرة التى أعدت من وكالة سلامة الغذاء الاوروبية بطلب مسبق من منظمة الاتحاد الاوروبى بخصوص إستخدام مبيدات مجموعة النيونيكوتينويد وهى الإميداكلوبراید، الكلوثيانودين والثيامثوكسام نتيجة تأثيراتهم الضارة على الملقحات الزهرية. لذى فأن هذا البحث ركز على إيجاد مبيدات أخرى تتبع نفس المجموعة كبدائل أكثر امان من نظيرتها السابقة لمكافحة آفات من القطن. هذا وقد أظهرت الاختبارات النصف حقلية على سلالة من القطن المعملية بأن الاثر الإبادى الكلى كان فى أعلى مستوياته لمبيدات الاسيتاميبريد (٦١,٥٠ و٦٤,٠٠ %)، الكلوثيانيدين (٥٠,٥٠ و٥١,٥٠%) والثياكلوبراید (٥٨,٠٠ و٥٧,٠٠ %) خلال موسم ٢٠١٧ و٢٠١٨ على التوالى. وتبين أن قيم النسبية الزمنية النصف قاتلة لمبيد الأسيتاميبراید (١,٨٧ و١,٩٨) والثياكلوبراید (١,١٥ و١,٥٥) تفوق النيونيكوتينويدات التى تم تحديد استخدامها بينما مبيد الدينوتيفيوران تفوق على مبيد الثيامثوكسام فقط. هذا وأوضحت التجارب الحقلية بأن المتوسط الكلى لنسب الخفض فى من القطن كانت فى أعلى مستوياتها فى الثياكلوبراید (٩٠,٩١ و٨٧,٩٦%) و الاسيتاميبراید (٨٧,٣٦ و٨١,٤٨%) خلال موسم ٢٠١٧ و٢٠١٨ على التوالى. وقد أظهرت نتائج الحقلية على مدار ٣٠ يوم من بدء المعاملة خلال الموسمين بأن الدينوتيفيوران كان من البدائل الاكثر امانا على المفترسات الطبيعية لمن القطن التى تعرضت للمعاملات وذلك من خلال إستيفاءها لمعايير معادلة نسب الاتزان للمفترسات الطبيعة\الآفات المفترسة وأيضا معاييرحساب التأثيرات الضارة التابعة للمنظمة العالمية للمكافحة البيولوجية. وتبين النتائج حفاظ الثيامثوكسام على بعايير منظمة العالمية للمكافحة البيولوجية بينما باقى المبيدات المختبرة قد أخلت بجميع معايير حدود الأمان.