EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

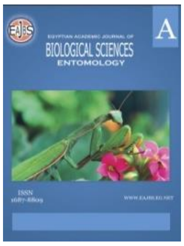
Vol. 14 No. 2 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(2) pp.19-26 (2021)*
DOI: 10.21608/EAJBSA.2021.165952

Egypt. Acad. J. Biolog. Sci., 14(2):19-26(2021)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Does Nano-Neonicotinoids Are More Efficient Than Commercial Sizes Against Leaf Miner, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) on Tomato?

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ARTICLE INFO

Article History
Received:5/3/2021
Accepted:29/4/2021
Keywords:
Leaf miner, Tomato, Nanoparticles, Neonicotinoids, nano-insecticides

ABSTRACT

The study conducted during (2017-2018 and 2018-2019) at Plant Protection Farm, Faculty of Agriculture, Assuit University, Assuit, Egypt on tomato variety 765 planted on the 10th of September for each season in an approximate area (315 m^2) in order to compare the efficiency of three Neonicotinoids [Thiamethoxam (Actara 25% WG), Imidacloprid (Best 25% WP), Acetamiprid (Mospildate 20% SP)] in Nano and commercial formulation size using recommended dose for them and the half-recommended dose for nano-insecticides against tomato leaf miner, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae). The randomized complete block design (RCBD) was applied with three replicates/ treatment and three control in a total of 21 plots. The replicate (plot) size was 3 ×3.5 m and consists of three rows planted with 5 plants/ row.

The nano and commercial insecticide sizes showed highly significant results in reducing the infestations of mines in tomato during the studied seasons in the first and second week after (1st and 2nd) spray compared with control. The nano-thiamethoxam 1x was the best for mines reduction followed by nano–acetamiprid, and nano-imidacloprid. However, there were no significant differences between commercial and nano-formulations in mines reduction % and the highest reductions showed by nano treatments. However, the treated tomato by the half-recommended rate of nano and commercial formulations showed the same reduction results.

INTRODUCTION

One of the most daily consumed crops all over the world is tomato, *Lycopersicon escuentum* Mill (Family: Solanaceae) which is characterized by high nutritional value (Singh, 2017). Tomato crops are vulnerable to attack by various insect pests such as leaf miner, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae), *Tuta absoluta* (Lepidoptera: Noctuidae), and whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) by (Patra *et al.*, 2016). The leaf miner, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae) is an example of tomato pest risk where the larvae cause mines in leaves of agricultural and ornamental plants by feeding on leaf tissues forming tunnels in the leaves for large variety of crops

(López *et al*., 2010). The adult's damage occurred by puncturing leaves for feeding and oviposition (Parrella *et al*., 1985). Mainly, insecticides are the major method for controlling tomato insect pests and their extensive applications led to developing resistance into insect pests towards insecticides. Moreover, tomato fruits are likely to retain high levels of pesticide residues that may not only be hazardous to consumers but may also affect the quality of exports (Patra *et al*., 2016). In the last decade, neonicotinoids and bio-insecticides were extensively used to control several sucking insects and leaf miners. However, imidacloprid has excellent systemic properties in a seed dressing, foliar, soil and stem treatments. Since several imidacloprid metabolites have been shown similar or better toxicity than the parent compound. The presence in the environment should be studied and thus considered in chemical analysis of future environmental studies (Shokr *et al*., 2006). The modification of insecticide size into nano-formulations with different dose applications might be a promising solution to overcome problems related to insecticide doses and efficiency with following up their residual levels in the tomato fruit.

The study aimed to compare the efficiency of three neonicotinoids [Thiamethoxam (Actara 25% WG), Imidacloprid (Best 25% WP), Acetamiprid (Mospildate 20% SP)] in nano and their commercial size formulations using recommended dose for them and the half-recommended dose for nano-insecticides against tomato leaf miner, *L. trifolii*.

MATERIALS AND METHODS

Experimental Design:

The study was carried out during (2017-2018 and 2018-2019) seasons at Plant Protection Experimental Farm, Faculty of Agriculture, Assuit University, Assuit, Egypt. The experimental area (approximately 315m^2) was planted with tomato variety 765 on the 10th of September for both seasons, respectively in a randomized complete block design (RCBD). Three blocks (10 plot/ block) were selected, and the plot size was 3 ×3.5 m and contains three rows (5 plants/ row) with a total (15 plants/plot). Each treatment was replicated three times in a total number of 27 plots for insecticide treatments and 3 plots were used as control.

Sampling:

Three plants were selected to collect leaflets from each treatment in polyethylene bags and transferred to the Laboratory of Economic Entomology, Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt). The upper and lower surfaces of leaflets were examined by binocular (Olympus VE-3- G20XT, made in Japan) and numbers of mines were counted and recorded from leaves of treated and controlled plots at the first and second week from spraying according to the method of (Mohan and Anitha, 2017).

Field and Efficacy of Selected Insecticides:

Six selected insecticides: acetamiprid, imidacloprid, thiamethoxam and their nanoformulations were applied in the first season, 2017-2018 using recommended and half-recommended dose for nano-insecticides. In the second season, 2018-2019 the six insecticides were replicated with the same doses. Additionally, the insecticides were applied two times and data was taken after one and second week from the application during seasons of study on the 1st and 28th of December of each season. The insecticides were diluted by tap water and the knapsack sprayer fitted with one nozzle adjusted with 200 liters of water /feddan. The control plots were sprayed only with water.

Nano Preparation:

The Three insecticides were prepared to nanoformulations under laboratory conditions in the Laboratory of Physics, Department of Physics, Faculty of Science, Assiut

University, Assiut, Egypt. The three insecticide powders were prepared depending on Up down approach according to the high ball milling technique (FRITSCH, PULVERISETTE -2) (Yadav & Vasu, 2016), which was used for size reduction. The average crystallite size (*D*) was determined by the equation of Scherrer (1918).

$$D = K\lambda / \beta.\cos\theta$$

D is a dimension of particle size; 0.89 is Scherrer's constant, **λ** is the wavelength of X-rays, (**θ**) is the Bragg diffraction angle, and (**β**) is the full width at half-maximum (FWHM) of the diffraction peak.

The x-ray diffraction pattern (Fig. (1): A, B & C) represents the treated powder and the average nanoparticle size was 21.75 nm for thiamethoxam (Actara 25% WG), 21.8 nm for imidacloprid formulations (Best 25% WP), and 21.1 nm for acetamiprid (Mospildate 20% SP).

Reduction Percentage of Nano and Commercial Formulations During (2017-2018 and 2018 -2019):

The reduction of mine percentages was calculated after 1 and 2 weeks after two sprays per treatment using the formula of Henderson & Tilton, (1955).

$$\text{Reduction\%} = \left(1 - \left(\frac{\text{no}\cdot\ \text{mines in treatment after} \times \text{no}\cdot\ \text{mines in control before}}{\text{no}\cdot\ \text{mines in treatment before} \times \text{no}\cdot\ \text{mines in control after}}\right)\right) \times 100$$

Statistical Analysis:

F-test and mean separation were used to compare the reduction among treatments according to Duncan's Multiple Range Test (DMRT) of significance at 5% by SASS program.

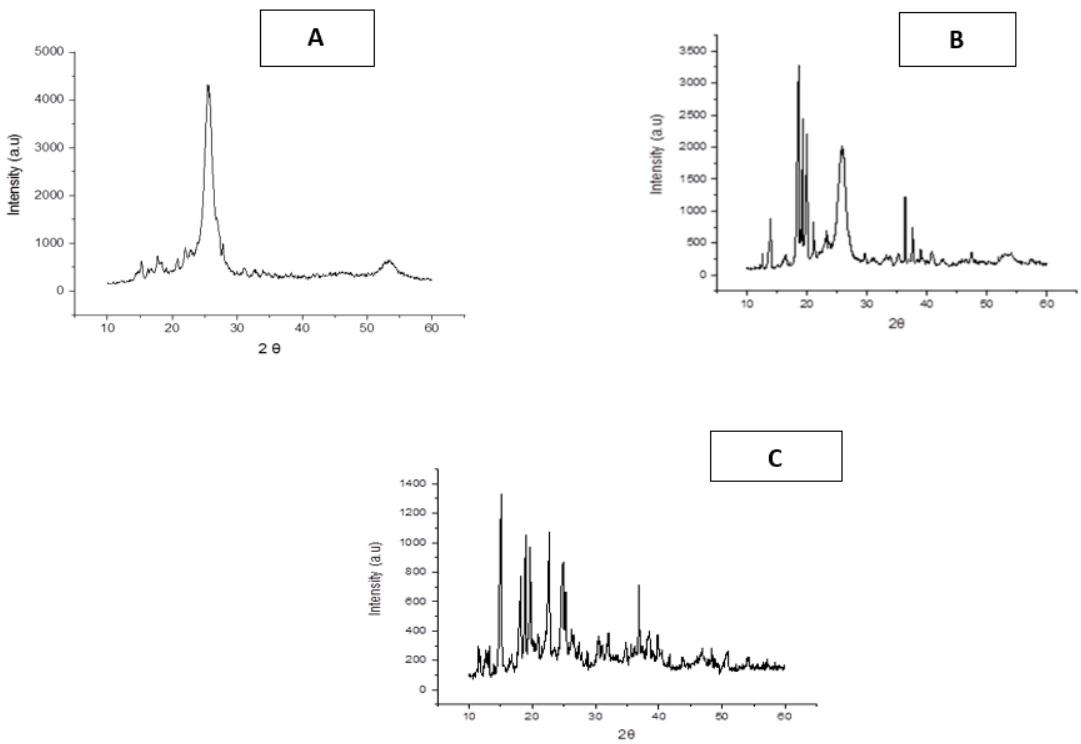

Fig.1. X-ray diffraction (XRD) of treated neonicotinoid insecticides (thiamethoxam 25%WG (A), imidacloprid 25%WP (B), and acetamiprid 20%SP (C).

RESULTS

Efficacy of Insecticides Against *L. trifolii* during (2017/2018 and 2018/ 2019):

1. First Spray:

In the 1st season, the mean numbers of mines (Table 1) were significantly increased in control (28.34 mines/ plant) compared to insecticides treatments (5.56, 4.89, 10.22, 6.50, 4.44, 6.83, 6.00, 4.11and 7.11 mines/plant) with an reduction percentages (81.78, 86.18, 65.75; 82.43, 87.20, 71.92; and 76.29, 87.55, 76.52 %; respectively) for imidacloprid 1x, nano imidacloprid 1x, nano imidacloprid 0.5x, thiamethoxam 1x, nanothiamethoxam1x, nano thiamethoxam 0.5x, acetamiprid 1x, nano acetamiprid 1x, and nano acetamiprid 0.5 x, respectively. After two weeks of post spray for same corresponding treatments of mines numbers and reduction percentage were (9.11; 68.58 %, 8.00; 76.21 %, 15.33; 45.98 %, 10.83; 69.20 %, 4.44; 86.54 %, 8.00; 65.43 %, 10.22; 57.52 %, 10.75; 65.77 %, and 13.44; 53.32 %; respectively) for the same corresponding order of insecticides in the first treatments. The grand means of mines and reduction % for the same corresponding treatments were (7.33; 75.18%, 6.44; 81.19%, 6.44; 55.87%, 12.78; 75.81, 8.67; 86.87%, 4.44; 86.87%, 7.42; 68.67%, 8.11; 66.90%, 7.43; 76.66% and 10.28; 64.92%, respectively).

Table 1: The efficacy of insecticides against tomato leaf miner *L. triforlii* under field conditions at 1st spray during (2017-2018).

Treatments	Pre-treatment	Post- treatment				General mean	
		1st Week	R* (%) ±SE	2nd Week	R* (%) ± SE	No	R* (%) ± SE
Control	21.83	28.34	------	26.94	------	27.64	------
Imidacloprid 1x	23.50	5.56	81.78 ± 5.70 ab	9.11	68.58 ± 8.18 bc	7.33	75.18 ± 5.33 abc
Nano imidacloprid 1x	27.25	4.89	86.18 ± 3.13 a	8.00	76.21 ± 3.88 ab	6.44	81.19 ± 3.18 ab
Nano-Imidacloprid 0.5 x	23.00	10.22	65.75 ± 6.89 c	15.33	45.98 ± 1.34 e	12.78	55.87 ± 5.37 d
Thiamethoxam 1x	28.50	6.50	82.43 ± 4.22 ab	10.83	69.20 ± 1.58 bc	8.67	75.81 ± 3.51 abc
Nano-Thiamethoxam 1x	26.75	4.44	87.20 ± 1.95 a	4.44	86.54 ± 2.89 a	4.44	86.87 ± 1.56 a
Nano-Thiamethoxam 0.5 x	18.75	6.83	71.92 ± 1.07 bc	8.00	65.43 ± 5.31 bc	7.42	68.67 ± 2.85 bcd
Acetamiprid 1x	19.50	6.00	76.29 ± 2.28 ab	10.22	57.52 ± 8.14 cd	8.11	66.90 ± 5.73 bcd
Nano-Acetamiprid 1x	25.44	4.11	87.55 ± 2.35 a	10.75	65.77 ± 3.05 bc	7.43	76.66 ± 5.11 ab
Nano-Acetamiprid 0.5 x	23.33	7.11	76.52 ± 1.87 bc	13.44	53.32 ± 2.30 de	10.28	64.92 ± 5.32 cd

* Mean Reduction Percentage (±SE) 1x=Recommended rate 0.5x=Half of recommended rate
Means, in the same column followed by the same letter are not significantly different from each other at 5%probability level Duncan's Multiple Range Test.

In the 2nd season, the mean number of mines (Table 2) were significantly increased in control (26.90 mines/ plant) compared to treated plots. The mines number recorded (6.06, 4.25, 7.72, 5.67, 6.08, 8.78, 5.83, 4.08, and 7.06 mines/ plant) with reduction percentages of (71.49, 82.18, 69.93, 78.31, 79.83, 64.73, 76.16, 83.78, and 74.11%; respectively for imidacloprid 1x, nano imidacloprid 1x, nano imidacloprid 0.5 x, thiamethoxam 1x, nano thiamethoxam1x, nano thiamethoxam 0.5 x, acetamiprid 1x, nano acetamiprid 1x, and nano acetamiprid 0.5 x). The same corresponding treatments after two weeks post spray, the mines number and reduction% were (5.83; 74.33%, 5.25; 79.42%, 9.44; 65.62%, 7.11; 74.55%, 3.67; 88.63%, 10.61; 60.14%, 12.50; 52.24%, 9.44; 64.92% and 15.56;46.70%, respectively to the above treatments order). The grand means of mines and reduction % for the same corresponding treatments were (5.94; 72.91%, 4.75; 80.80%, 8.58; 67.77%, 6.39; 76.43%, 4.88; 84.23%, 9.69; 62.43%, 9.17; 64.20%, 6.76; 74.35 and 11.31; 60.41, respectively).

Table 2: The efficacy of insecticides against tomato leaf miner *L. triforlii* under field conditions at 1st spray during (2018-2019).

Treatments	Pre-treatment	Post-treatment				General mean	
		1st Week	R* (%) ± SE	2nd Week	R* (%) ± SE	No	R* (%) ± SE
Control	25.47	26.90	------	28.78	------	27.84	------
Imidacloprid 1x	20.11	6.06	71.49 ± 3.07 Bcd	5.83	74.33 ± 2.02 Bc	5.94	72.91 ± 1.78 abc
Nano imidacloprid 1x	22.58	4.25	82.18 ± 2.77 A	5.25	79.42 ± 2.50 Ab	4.75	80.80 ± 1.76 a
Nano-Imidacloprid 0.5 x	24.31	7.72	69.93 ± 4.43 Cd	9.44	65.62 ± 4.63 Cd	8.58	67.77 ± 3.02 bcd
Thiamethoxam 1x	24.73	5.67	78.31 ± 0.88 Ab	7.11	74.55 ± 2.66 Bc	6.39	76.43 ± 1.56 ab
Nano-Thiamethoxam 1x	28.56	6.08	79.83 ± 1.96 Ab	3.67	88.63 ± 1.88 A	4.88	84.23 ± 2.32 a
Nano-Thiamethoxam 0.5 x	23.56	8.78	64.73 ± 4.99 D	10.61	60.14 ± 7.06 Cd	9.69	62.43 ± 3.91 cd
Acetamiprid 1x	23.17	5.83	76.16 ± 2.19 Abc	12.50	52.24 ± 2.67 De	9.17	64.20 ± 5.63 bcd
Nano-Acetamiprid 1x	23.83	4.08	83.78 ± 4.49 A	9.44	64.92 ± 3.12 Cd	6.76	74.35 ± 4.79 Abc
Nano-Acetamiprid 0.5 x	25.83	7.06	74.11 ± 1.89 Abc	15.56	46.70 ± 5.82 E	11.31	60.41 ± 6.82 d

*Mean Reduction Percentage (±SE) 1x=Recommended rate 0.5x=Half of recommended rate
Means, in the same column followed by the same letter, are not significantly different from each other at 5%probability level Duncan's Multiple Range Test.

2. Second Spray:

In the 1st season, the mean numbers of mines were significantly increased (Table 3) in the control (26.75 mines/ plant) compared to insecticides treated plants (4.78, 5.44, 7.89, 7.83, 4.78, 8.67, 3.50, 2.83and 4.78 mines/ plant) with reduction percentages (80.68, 77.30, 72.87, 68.73, 83.57, 69.06, 85.81, 88.46, and 81.77%, respectively), for imidacloprid 1x, nano imidacloprid 1x, nano imidacloprid 0.5 x, thiamethoxam 1x, nano thiamethoxam1x, nano thiamethoxam 0.5 x, acetamiprid 1x, nano acetamiprid 1x, and nano acetamiprid 0.5 x, respectively. Respect to the results of after two-week posts sprays for the same corresponding treatments, the mines numbers and reduction% were (8.89; 61.03%, 7.25; 67.21%, 11.00; 58.98%, 8.67; 62.48%, 6.89; 74.31%, 11.25; 56.44%, 5.67; 75.09%, 5.00; 77.91% and 7.33; 69.66%; respectively). The grand means of mines and reduction % for the same corresponding treatments were (6.83; 70.86%, 6.35; 72.26%, 9.44; 65.92%, 8.25; 65.61%, 5.83; 78.94%, 9.96; 62.75%, 4.58; 80.45%, 3.92; 83.18% and 6.06; 75.72%; respectively).

In the 2nd season, the means of mines (Table 4) were significantly increased in control (41.78 mines/plant) compared to treated plants. The mines means were (8.33, 8.17, 10.39, 9.22, 6.50, 11.00, 12.67, 7.50 and 10.83 mines/ plant with reduction % of 71.08, 73.58, 71.47, 69.65, 76.59, 67.19, 62.94, 77.54 and 55.77%; respectively for imidacloprid 1x, nano imidacloprid 1x, nano imidacloprid 0.5 x, thiamethoxam 1x, nano thiamethoxam1x, nano-thiamethoxam 0.5 x, acetamiprid 1x, nano acetamiprid 1x, and nano acetamiprid 0.5 x). After two weeks post spray, the mines number and reduction% were (10.33; 59.26%, 8.50; 68.76%, 14.17; 55.80%, 10.39; 61.16%, 7.50; 69.31%, 14.33; 51.44%, 12.33; 59.01%, 9.17; 68.82% and 10.78; 50.01%, respectively for the same corresponding treatments). The grand means of mines and the reduction % of the same order treatments were (9.33; 65.17%, 8.33; 71.17%, 12.28; 63.63%, 9.81; 65.40%, 7.00; 72.95%, 12.67; 59.31%, 12.50; 60.98%, 8.33; 73.18 and 10.81; 52.89%, respectively).

Table 3: The efficacy of insecticides against tomato leaf miner *L. triforlii* under field conditions at the 2nd spray during (2017-2018).

Treatments	Pre-treatment	Post-treatment				General mean	
		1st Week	R* (%) ± SE	2nd Week	R* (%) ± SE	No	R* (%) ± SE
Control	23.61	26.75	------	24.67	------	25.71	------
Imidacloprid 1x	21.83	4.78	80.68 ± 1.97 Ab	8.89	61.03 ± 1.94 bc	6.83	70.86 ± 4.50 Bcd
Nano imidacloprid 1x	21.17	5.44	77.30 ± 3.87 Abc	7.25	67.21 ± 2.70 ab	6.35	72.26 ± 3.06 Abcd
Nano-Imidacloprid 0.5 x	25.67	7.89	72.87 ± 5.63 Bc	11.00	58.98 ± 0.56 bc	9.44	65.92 ± 3.89 Cd
Thiamethoxam 1x	22.11	7.83	68.73 ± 9.67 C	8.67	62.48 ± 5.27 bc	8.25	65.61 ± 5.08 Cd
Nano-Thiamethoxam 1x	25.67	4.78	83.57 ± 5.02 Ab	6.89	74.31 ± 3.75 a	5.83	78.94 ± 3.43 Ab
Nano-Thiamethoxam 0.5 x	24.72	8.67	69.06 ± 1.53 c	11.25	56.44 ± 6.04 c	9.96	62.75 ± 4.14 D
Acetamiprid 1x	21.78	3.50	85.81 ± 0.68 a	5.67	75.09 ± 2.95 a	4.58	80.45 ± 2.72 Ab
Nano-Acetamiprid 1x	21.67	2.83	88.46 ± 2.37 a	5.00	77.91 ± 1.62 a	3.92	83.18 ± 2.60 A
Nano-Acetamiprid 0.5 x	23.14	4.78	81.77 ± 1.75 ab	7.33	69.66 ± 1.98 ab	6.06	75.72 ± 2.93 Abc

*Mean Reduction Percentage (±SE) 1x=Recommended rate 0.5x=Half of recommended rate
Means, in the same column followed by the same letter, are not significantly different from each other at 5%probability level Duncan's Multiple Range Test.

Table 4: The efficacy of insecticides against tomato leaf miner *L. triforlii* under field conditions at 2nd spray during (2018-2019).

Treatments	Pre-treatment	Post-treatment				General mean	
		1st Week	R* (%) ± SE	2nd Week	R* (%) ± SE	No	R* (%) ± SE
Control	35.44	41.78	------	36.78	------	39.28	------
Imidacloprid 1x	24.44	8.33	71.08 ± 0.29 Ab	10.33	59.26 ± 3.59 Cd	9.33	65.17 ± 3.17 abc
Nano imidacloprid 1x	26.22	8.17	73.58 ± 2.44 Ab	8.50	68.76 ± 3.19 Ab	8.33	71.17 ± 2.09 ab
Nano imidacloprid 0.5 x	30.89	10.39	71.47 ± 2.02 Ab	14.17	55.80 ± 1.12 Cde	12.28	63.63 ± 3.63 bc
Thiamethoxam 1x	25.78	9.22	69.65 ± 6.22 Bc	10.39	61.16 ± 2.45 Bc	9.81	65.40 ± 3.42 abc
Nano-Thiamethoxam 1x	23.56	6.50	76.59 ± 2.17 A	7.50	69.31 ± 0.90 A	7.00	72.95 ± 1.91 a
Nano thiamethoxam 0.5 x	28.44	11.00	67.19 ± 2.88 Bc	14.33	51.44 ± 2.90 De	12.67	59.31 ± 3.90 cd
Acetamiprid 1x	29.00	12.67	62.94 ± 0.74 C	12.33	59.01 ± 1.72 Cd	12.50	60.98 ± 1.25 cd
Nano-Acetamiprid 1x	28.33	7.50	77.54 ± 0.86 A	9.17	68.82 ± 1.61 Ab	8.33	73.18 ± 2.18 a
Nano -Acetamiprid 0.5 x	20.78	10.83	55.77 ± 2.43 D	10.78	50.01 ± 4.78 E	10.81	52.89 ± 2.75 d

*Mean Reduction Percentage (±SE) 1x=Recommended rate 0.5x=Half of recommended rate
Means, in the same column followed by the same letter, are not significantly different from each other at 5%probability level Duncan's Multiple Range Test.

DISCUSSION

Overall seasons of study, the tested insecticides showed highly significant results in reducing grand means of mines at 1st-week spray than 2nd week. However, nano-formulations showed more effectiveness in decreasing leaf mines than the commercial size at the same recommended rate in both seasons. These results could occur because nano-formulation increases the efficiency of insecticides and reduces the dose level required to control due to the small sizes of nanoparticles which makes insecticides more penetrative into leaf tissue and easily reach to target leaf miner. The obtained results are in agreement with the findings of (Ahmed *et al*., 2020) and (Memarizadeh *et al.*, 2014) who found that mortality percentages of *Glyphodes pyloalis's* larvae was increased in leaf dip bioassay experiment by nano-imidacloprid with an obvious reduction in insecticide concentration and LC_{50} decreased to 4.82 and 9.05-fold less than the commercial size of imidacloprid; respectively.

A comparison between the efficiency rates into a nano-insecticides group for both seasons, nano-thiamethoxam 1x showed the highest reduction (84.23-86.87%) in the first spray and for the second sprays nano-acetamiprid 1x by recommended dose (83.18-73.18%). These results could be explained due to the lower water solubility of thiamethoxam (4.1g/L) than acetamiprid (4.25 g/L) which could make it more concentrated (Tomizawa and Casida 2005). The half-recommended rate of nano and commercial insecticide showed similar results in the reduction percentage and nano-thiamethoxam 1x showed the best efficiency towards mines reduction followed by nano–acetamiprid, nano-imidacloprid, Neonicotinoids are outstanding plant systemic activity acts quickly at low doses. Moreover, most insects are sensitive to these groups and have less ability to be resistant to these groups. These results are in the same line with (Chuster and Morris, 2002) who stated that neonicotinoids have high potency and a significant reduction in mines of *L. trifolii* in tomato resulted from the applications of imidacloprid 2F (16 oz/acre) and thiamethoxam 2SC (8 oz/acre) insecticides. However, the findings of (Radwan and Taha, 2012) that imidacloprid was the superior toxicant against *T. absluta* due to exposure of moths and 3rd instar larvae to LC_{30}, LC_{50} and LC_{80} of imidacloprid, dinotefuran, phenthoate insecticides caused significant reduction (51.11and 25.00%) or increasing (41.78 and 28.77%) in the activity of AChE, respectively.

Generally, nano-neonicotinoid insecticides reduced leaf miner infestations and could be promising for more protection to tomato crops than commercial formulation. Further studies are needed for the probability of other side effects such as residual nano-insecticides compared to commercial sizes in aspects of agriculture food Safety, natural enemies, bees, and wildlife before final recommendations for using these new insecticide formulations.

All insecticide formulations showed highly significant effectiveness in reducing mines infestations in tomatoes during two tested seasons at one week as well as the second week after spraying compared to the control. The nano-thiamethoxam 1x showed the best reduction results for mines reduction followed by nano–acetamiprid, and nano-imidacloprid. Although there was no significant difference found among commercial and nano-formulation treatments in mines reduction %, but nano treatments showed better results in mines reductions. The half-recommended rate of nano treatments showed a similar reduction as the recommended rate of commercial formulation size.

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