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Seasonal incidence and efficacy of nano-thiamethoxam on tomato leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

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Abstract

Seasonal incidence of tomato leaf miner, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), and evaluation of the new nano-formulation of thiamethoxam insecticide under climatic factors that supplying the vital knowledge for successful integrated pest management (IPM) programs. This study was conducted in two successive seasons 2017-18/ 2018-19 on variety (no. 765) of tomato that cultivated at the plant protection experimental Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt. The numbers of mines on the tomato leaf were recorded from the first of November to the first of the February in the two seasons. The highest peak of mines was 28.34 mines/ plant in the 13th of December, 2018, while, it was 41.8/ plant in the January1st, 2019. Further, the climatic factor analysis during the two studied seasons demonstrated that the relative humidity was the most efficient factor (52.49 % out of 91.46 %), while the temperature was the least effective one (8.67% out of 91.46%). The nano thiamethoxam at the recommended dose (1x) of neonicotinoid insecticide significantly reduced the mines compared to traditional thiamethoxam. Furthermore, the half recommended dose of nano thiamethoxam in the two tested seasons was equal in the efficacy to the traditional thiamethoxam (1x). The reduction percentages were 86.87, 75.81 and 68.67 for nano-thiamethoxam (1x), thiamethoxam (1x), and nano- thiamethoxam (½ x), respectively, in 2017-18 for the first spray. In the same corresponding treatments, the values recorded were 78.94, 65.61and 62.75% in the second spray. Results of the second season (2018-19) were in the same line, where, the reduction percentages were 84.23, 76.43 and 62.43% at first spray, while the second spray were 72.95, 65.40 and 59.31% for nano-thiamethoxam (1x), thiamethoxam (1x) and nano- thiamethoxam (½ x), respectively. These results suggested that, the relative humidity was the main climatic factor effect on the mines population of *Lirimyza trifolii*. The nano-thiamethoxam was significantly reduced the pest population. Results of this study highly recommend using nano formulation in applying of IPM leaf miner programs.

Keywords: seasonal abundance, tomato, leaf miner, climatic factors, thiamethoxam, nano insecticide.

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1. Introduction

Tomato, *Lycopersicon escuentum* Mill (Family Solanaceae) is one of the most daily consumed crops and has spread widely all over the world due to high nutritional value(Singh, 2017). This crop is attacked by various insect pests such as leaf miner, *Liriomyza trifolii* Burgess (Diptera: Agromyzidae), thrips, *Frankfiniella schuftezi* Trybon (Thysanoptera: Thripidae), fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), whitefly, *Bemisia tabaci* Genn. (Homoptera: Aleyrodidae) and tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Noctuidae), (Patra et al., 2016; El-Aassar et al., 2015). The leaf miner, *L. trifolii*, is one of the most important insect pest that the larvae cause mines in the 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; El Bouhssini et al., 2008; Hossain & Poehling, 2006). Background information on relative incidence and population dynamics is essential before a strategy for managing any insect pest. *L. tritolii* has been studied with respect to population dynamics on tomatoes, bean, and cucumber (Chakraborty et al., 2004; Saradhi & Patnaik, 2004; Jyani, 1999). Several studies have been conducted on the relationship between occurrence of *L. tritolii* and weather parameters on tomato (Chakraborty et al., 2004; Saradhi & Patnaik, 2004; Zoebisch et al., 1992). Chemical control is still the first line of defense against various insect pests of tomatoes. Extensive insecticide applications have led to developing resistance issue of tomato insect pest to insecticide. 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 to exports (Patra et al., 2016). Thiamethoxam is one of insecticides from a neonicotinoid group. Due to the high potency and persistence of neonicotinoid insecticides, it had extensively used in last decade for controlling several sucking insects and leaf miner insect pests (Abdu-Allah & Mohamed, 2017; Abd-Ella, 2014; Abdu-Allah, 2012, 2011, 2010). The mode of action of this group is binding agonistically with the nicotinic acetylcholine receptors (nAChRs) in the central nervous system of pest insects (Nauen et al., 2001). It is necessary to investigate for new ways to reduce the pesticide residues of inside the fruits and preserve the environment from pollution (Tabikha & Hassan, 2015). Therefore, the use of nano-pesticides in the present study is a modern way could be reduced the resistance of pests to pesticides and may be enhance the speed of their degradation in the environment,. The objectives of this study were to evaluate the seasonal incidence of serpentine leaf miner, *L. trifolii* on tomato and to study the field efficacy of thiamethoxam at nano size with different rates compared with their traditional use at the recommended rate against leaf miner, *L. trifolii* on tomato.

2. Materials and methods

2.1 Experimental design

The present study was conducted in the 10th of September during (2017-18 and 2018-19) at Plant Protection Experimental Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt under traditional agricultural practices. The randomized complete block design (RCBD) was used for an area of approximately 126 m^2 planted with tomato variety 765 in 12 plots (replicates). The plot size was 3×3.5 m (replicate 10.5 m^2) included three terraces; each terrace was planted with 5 seedlings with considering three replicates per insecticide treatment as well as for the control.

2.2 Sampling and determination of season incidence of *L. trifolii*

Observation the number of mines/ plant was recorded on three randomly selected plants each treatment was at the first and second week after spraying **(**Mohan & Anitha, 2017). The collected samples transferred to the Laboratory of Economic Entomology. The upper and lower surfaces of leaflets were examined by binocular (Olympus VE-3- G20XT, made in Japan) and the number of mines were counted in the treated and controlled plots in order to determine the reduction percentages of nano and traditional formulation beside the incidence of *L. trifolii* under free insecticide treatment. The reduction percentages of nano and traditional formulations of thiamethoxam were calculated after 1 and 2 weeks after two sprays per treatment using the formula of Henderson and Tilton (1955).

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

2.3 The field efficacy of nano and traditional formulation of thiamethoxam

The traditional formulation of thiamethoxam (Neonicotinoids; trade name: Actara 25% WG, 20g/ 100 L water, Syngenta Agro, Egypt) was used according to the recommended dose (1 gm/ 5L water). The nano formulation was applied as the same recommended dose and its half amount (1 and 0.5 gm/ 5L water) using Knapsack hand spray fitted with one nozzle and the control plots were sprayed with water.

2.4 Nano preparation

The nano insecticides were prepared depending on Top Down approach according to the high energy ball milling technique (FRITSCH, Pulversette- 2) (Yadav & Vasu, 2016), was utilized for size reduction. The average crystallite size (D) was determined using Scherrer's equation (1918):

$$D = \mathrm{k}\lambda \,/\, \beta.\cos\theta$$

The thiamethoxam average crystallite size (D) was 12.4 ±1.1 nm.

2.5 The meteorological data

The experimental considered climatic data were: the daily maximum temperature °C (DMxT), daily minimum temperature (DMnT) °C, relative humidity (RH %), daily soil maximum temperature (DSMxT) °C, and daily soil minimum temperature °C (DSMnT) which obtained from the metrological station at the Farm of Faculty of Agriculture, Assiut University, Assiut, Egypt from November to February period of 2017-2018 and 2018-2019 seasons.

2.6 Statistical analysis

Data were pooled to statistical analysis using F-test and mean separation was compared according to Duncan's multiple Range Test (DMRT) of significance at 5% by SASS program. Simple, partial and multiple analysis were carried out by mean of Advanced Statistical Analysis Package (ASAP) program. Figures were done using Graph Pad Prism 5^{TM} software (San Diego, CA).

3. Results and Discussion

3.1 Incidence of *L. trifolii* during the first season (2017/ 2018)

In the beginning of the season when the plant age was 74 days after transplanting, the mean numbers of mines for *L. trifolii* was recorded the lowest in the peak fluctuation with 21.83 mines /plant (Table 1 and Figure 1). The climatic factors were maximum temperature 24.96°C, minimum temperature 10.82°C, maximum RH 80.21 %, minimum RH 25.93 %, soil maximum temperature 33.99 °C and soil minimum temperature 16.89°C. Afterwards, the population when the plant age was 97 days, it recorded the highest peak in the 13th of December with 28.34 mines/ plant; when the climatic factors: maximum temperature (23.35°C), minimum temperature 8.94°C, maximum RH 85%, minimum RH 28.24%, soil maximum temperature 29.39°C and soil minimum temperature 12.99°C. After that the population fluctuate decline till the end of the season (24.67 mines /plant) in the 6th of February, 2018 maximum temperature 25.2°C, minimum temperature 7.4°C, maximum RH 74.4 %, minimum RH 20.6 %, soil maximum temperature 29.97 °C, soil minimum temperature 10.63 °C; while the plant age was 141 days (Table 1). The data in Table 2 showed the effect of both maximum and minimum relative humidity on the population of *L. trifolii* where the highest mines peak recorded under 85% and 28.24 %; respectively for maximum and minimum temperature, as well as, the correlation values were positive (r = 0.315* and 0.662*; respectively), for maximum and minimum humidity. On the other side, the plant age played an important role in the fluctuation, as the mean of mines were in low fluctuation due to the shortage of humidity and the content of chlorophyll in the seedlings in the beginning of the season which makes the plants un favorable for pest attack. Therefore, the peak was recorded when the plants are in 97 days after transplanting (r = 0.435 *). These results are in agreement with the findings of Dhillon and Sharma (2010) on *L. trifolii.* They stated that there was a significant positive association with relative humidity (r = 0.46**), while these results are not in agreement with the findings of Shilpakala and Murali Krishna (2016) who found positive association with maximum temperature and minimum temperature while negative association with relative humidity.

3.2 Incidence of *L. trifolii* during the second season (2018/ 2019)

The occurrence of *L. trifolii* in the second of the season when the plant age was 79 days after transplanting recorded in low levels with mean numbers of mines (25.5 mines/ plant). Gradually, the mean of mines increased to moderate levels of abundance during the times of sampling in December. The highest peak was occurred in the 1st of January, 2019 (41.8 mines/ plant). Then, by the end of the season the population was in high levels of fluctuation (36.8 mines/ plant) (Table 1). The population fluctuation *of L. trifolii* was controlled by both the maximum and minimum temperatures of weather and soil temperature, as they showed negative correlation with the mean number of mines r= -0.753*, -0.569* and -0.671*, -0.566*; respectively, for the maximum and minimum temperatures of weather and soil maximum and minimum temperature. This might be occurred because the considered temperatures are mainly affect plant humidity which directly influences the dynamics of *L. trifolii* number. These results were in agreement with Variya and Bhut (2014) who reported that the correlation studies indicated that number of mines, larvae as well as percent damaged leaves had significant negative correlation with maximum temperature (-0.68162**, -0.71533** and -0.71308**, respectively) and minimum temperature (-0.78761**, -0.82541** and -0.82630**, respectively). While these results are not in agreement with the findings of Shalaby et al. (2012) on *L. trifolii*, *Aphis craccivora* and *Empoasca discipiens* who reported that, the results indicated that significant correlation between the populations of insects and daily mean of maximum and minimum temperatures (r= 0.511 and 0.793), during 2008/2009 season.

Table 1: Incidence of *L. trifolii* in relation to plant age and climatic factors on tomato cultivar 765 under free insecticides during 2017/ 2018 and 2018/ 2019 seasons at Assiut, Egypt.

Season	Sampling date	Mean mine numbers / plant	Plant age	Temperature (°C)		Relative Humidity RH (%)		Soil Temperature at 5 cm (°C)	
				Max.	Min.	Max.	Min.	Max.	Min.
2017/ 2018	Nov., 29th, 2017	21.83	74	24.96	10.82	80.21	25.93	33.99	16.89
	Dec.,6th ,2017	26.94	80	25.80	9.20	82.20	26.60	31.29	13.56
	Dec .,13th , 2017	28.34	97	23.35	8.94	85.00	28.24	29.39	12.99
	Jan .,22th, 2018	23.61	127	20.38	6.48	80.57	26.76	27.14	9.66
	Jan .,29th , 2018	26.75	134	19.68	6.31	84.25	32.13	26.71	10.09
	Feb .,6th , 2018	24.67	141	25.2	7.40	74.40	20.60	29.97	10.63
2018/ 2019	Nov .,27th , 2018	25.50	79	26.23	12.81	76.77	26.46	35.85	17.91
	Dec .,4th , 2018	28.80	86	23.86	10.71	79.14	28.00	31.86	15.89
	Dec .,11th , 2018	26.90	93	21.00	8.43	86.71	35.86	29.89	13.54
	Dec .,25th , 2018	35.40	107	21.29	7.79	84.21	34.57	28.94	12.11
	Jan .,1st , 2019	41.80	114	18.86	7.00	84.00	39.14	26.97	10.91
	Jan .,8th , 2019	36.80	121	17.86	5.86	80.43	31.29	26.77	9.49

3.3 Efficiency percentage of considered factors on the fluctuations of *L. trifolii* during the seasons (2017/ 2018 and 2018/ 2019)

The considered of abiotic factors (Table 2) was significantly participated together in regulating the population abundance of *L. trifolii* with 91.46 %. The influence of these factors could be arranged according to the efficiency from the most to the least efficient factor as following: maximum relative humidity (30.06 %), minimum relative humidity (22.88 %), plant age (17.86 %), weather minimum temperature (8.62 %), soil minimum temperature (7.13 %), soil maximum temperature (5.02 %), and weather maximum temperature (0.05 %) out of 91.46 %.

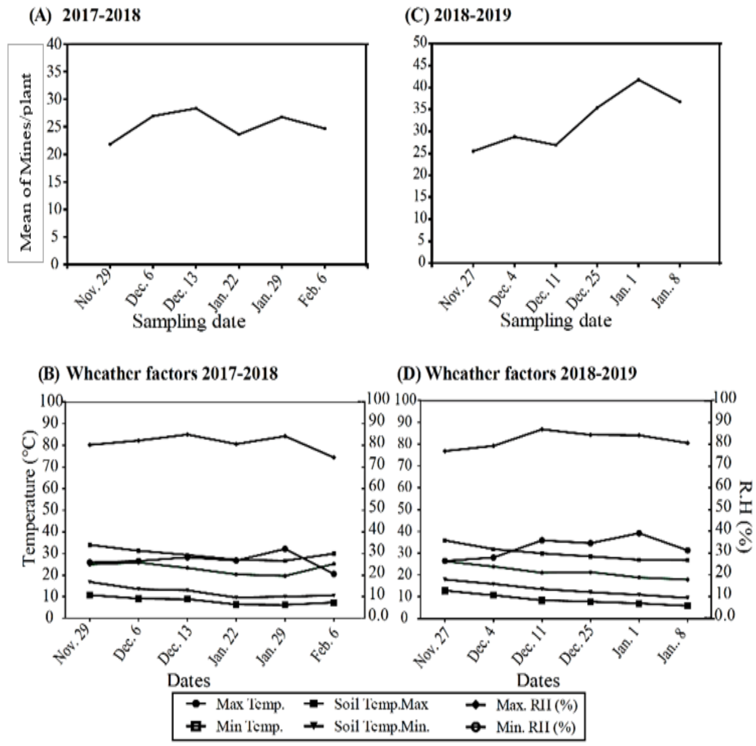

Figure 1: Mean of mines for *L. trifolii* (A) 2017/2018, (C) 2018\2019 and its correlation with abiotic factors (B) 2017/2018, (D) 2018/2019 on different sampling dates.

The significant effect of the tested climatic factors was obvious along the year of study and in the same line with the results of Tabikha and Hassan (2015) who found that the combined effect of four climatic factors as a group was 34.09% (F= 8.274***, P=0.0001) and 35.76% (F= 8.908***, P=0.0001) during 2013 and 2014 seasons, respectively on population density of tomato leaf miner males of *Tuta absoluta.*

3.4 Efficacy of nano and traditional formulations of thiamethoxam against *L. trifolii* during 2017-2018 / 2018-2019 seasons

The grand mean reduction percentages of mines were decreased significantly with nano-thiamethoxam (1x) than the traditional formula. Also, the mine reduction of half rate of nano-thiamethoxam (0.5x) had no significant difference from the reduction caused 1x rate of traditional thiamethoxam. The mines reduction % were 86.87, 75.81, 68.67and 78.94, 65.61, 62.75% for1x rate of nano-thiamethoxam, 1x rate of traditional thiamethoxam and 0.5x of nano-thiamethoxam at first and second spray, respectively in the 1st season 2017/2018. In the second season 2018-2019, the same trend of reduction was observed, meanwhile the nano1x rate of thiamethoxam showed the highest mine reduction % than 1x traditional of thiamethoxam. Also, the half rate of nano could give the same efficiency as the 1x rate of traditional thiamethoxam. The grand mean of reduction % were 84.23, 76.43, 62.43 and 72.95, 65.40, 59.31% for nano 1x of thiamethoxam, traditional 1x and 0.5x nano for 1st and 2nd spray, respectively (Table 3).

Table 2: Multiple-regression analysis between the mean numbers of mines for *L. trifolii* and the considered climatic factors during 2017/ 2018 and 2018/ 2019 seasons at Assiut, Egypt.

Variables	The correlation coefficient values of *L. trifolii*			
	r	R	$R^2 \times 100$	Efficiency (%)
None	--	0.956	91.46	--
Plant age	0.435*	0.895	80.10	17.68
Max. Tem	-0.753*	0.956	91.43	0.05
Min. Tem	-0.569*	0.926	85.93	8.62
Max .RH	0.315*	0.876	76.76	22.88
Min .RH	0.662*	0.849	72.15	30.06
S. Max. Tem	-0.671*	0.939	88.23	5.02
S. Min. Tem	-0.566*	0.932	86.88	7.13

r= Simple correlation coefficient, R= Multiple regression coefficient, R^2=Coefficient of determination. *Significant at 5 % level of probability.

Results of the first and second spray during 2017-2018/ 2018-2019 indicated that nano- thiamethoxam insecticide significantly reduced the mines of *L. trifolii* larvae when the recommended dose was used. It is of interest to point herein that the tested insecticide showed great effect on *L. trifolii*; this may be due to the differences between the particle size, penetration of the plant, and the prevailing environmental conditions during experimental 2017-2019 seasons. All these factors can play a role on the pesticide disappearance among plants and influencing the efficiency of the tested insecticide.

Table 3: Reduction percentages after treatments with grand means for nano and traditional formulation of thiamethoxam against *L. trifolii* on tomato cultivar 765 under field conditions during 2017-2018 and 2018-2019 seasons.

Season	No. Spray	Treatments	Pre-Treatments	Post- Treatments				Grand means (1st and 2nd week)	
				1st Week	R (%)*	2nd Week	R (%)*	No.	R (%)*
2017/ 2018	1st Spray	Control	21.83	28.34	--	26.94	--	27.64	--
2017/ 2018	1st Spray	Thiamethoxam 1x	28.50	6.50	82.43 a	10.83	69.20 b	8.67	75.81 b
2017/ 2018	1st Spray	Nano-Thiamethoxam 1x	26.75	4.44	87.20 a	4.44	86.54 a	4.44	86.87 a
2017/ 2018	1st Spray	Nano –Thiamethoxam 0.5x	18.75	6.83	71.92 b	8.00	65.43 b	7.42	68.67 b
2017/ 2018	2nd Spray	Control	23.61	26.75	--	24.67	--	25.71	--
2017/ 2018	2nd Spray	Thiamethoxam1x	22.11	7.83	68.73 a	8.67	62.48 a	8.25	65.61 b
2017/ 2018	2nd Spray	Nano-Thiamethoxam1x	25.67	4.78	83.57 a	6.89	74.31 a	5.83	78.94 a
2017/ 2018	2nd Spray	Nano –Thiamethoxam 0.5x	24.72	8.67	69.06 a	11.25	56.44 a	9.96	62.75 b
2018/ 2019	1st Spray	Control	25.47	26.90	--	28.78	--	27.84	--
2018/ 2019	1st Spray	Thiamethoxam1x	24.73	5.67	78.31 a	7.11	74.55 ab	6.39	76.43 a
2018/ 2019	1st Spray	Nano-Thiamethoxam1x	28.56	6.08	79.83 a	3.67	88.63 a	4.88	84.23 a
2018/ 2019	1st Spray	Nano –Thiamethoxam 0.5x	23.56	8.78	64.73 b	10.61	60.14 b	9.69	62.43 b
2018/ 2019	2nd Spray	Control	35.44	41.78	--	36.78	--	39.28	--
2018/ 2019	2nd Spray	Thiamethoxam1x	25.78	9.22	69.65 a	10.39	61.16 a	9.81	65.40 ab
2018/ 2019	2nd Spray	Nano-Thiamethoxam1x	23.56	6.50	76.59 a	7.50	69.31 a	7.00	72.95 a
2018/ 2019	2nd Spray	Nano-Thiamethoxam 0.5x	28.44	11.00	67.19 a	14.33	51.44 b	12.67	59.31 b

*Reduction percentage. 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 other hand nano-formulation increases the efficiency of insecticides and also reduces the dose level required to control. In addition, pesticide permeability during the layers outside the surface of the plant depends on the amount of pesticides used in the plant tissues, and on the rate of demolition, which also varies greatly depending on the chemical composition of the compound tested in the field conditions (Farha et al., 2016). As for as it is known according to available published research the nano-thiamethoxam is used for the first time on *L. trifolii*. The above findings coincided with findings of that Wankhade et al. (2014) who found that the minimum leaf damage caused by tomato leaf miner at 3 and 14 days' post spraying, and the leaf damage % was 21.56, 15.48% and 32.22, 27.94% for thiamethoxam 25 WG 0.003 % and the control, respectively. Also, thiamethoxam 25 WG 0.06 % recorded the lowest leaf infestation 5.47% against the citrus leaf miner (Dabhi & Barad, 2018). As well as, thiamethoxam 25% WDG significantly decreased the mines of serpentine leaf miner than the control to be 9.64 and 14.69 mines/ plant, respectively in tomato fields (Singh, 2017). Our results concluded that the nano-thiamethoxam was significantly reduced the leaf miner infestations and showed more protection to tomato crop than the traditional thiamethoxam formulation. Further studies showed are applied to other neonicotinoid insecticides such as imidacloprid and acetamiprid and the residual of nano-neonicotinoid insecticides compared the traditional one in agriculture foods. Results of this study could be highly recommend using nano-neonicotinoid insecticides formulation in applying IPM leaf miner programs with caution about the probability side effects of this formulation.

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