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Efficiency Of Thiamethoxam and Acetamiprid Against Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

إعـــداد

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ABSTRACT

Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus and related Rutaceae and ornamental plants almost worldwide. Pesticides are considered an essential, effective, and efficient control tool for leafminer. This study was designed to assess the effectiveness of two neonicotinoid insecticides, thiamethoxam (0.25 g L^{-1}) and acetamiprid (0.25 ml L^{-1}) against citrus leafminer larvae. Two laboratory bioassay methods, leaf-dip, and topical techniques were used to evaluate the efficiency of tested pesticides. The results showed that thiamethoxam was more potent against citrus leafminer larvae than acetamiprid with percent mortality of (75%) and (100%) through leaf dip and topical techniques, respectively after 24-h of treatment. Acetamiprid was also effective against CLM with a percent of mortality up to 58.3% and 91.6% after 24h of application by both bioassays LDB and TB, respectively. The median lethal time (LT_{50}) values showed that the leaf-dip technique of thiamethoxam was more effective (LT_{50}=2.60-h) than the contact technique (LT_{50}=3.78-h)

Keywords: Pesticides, Thiamethoxam, Acetamiprid, Citrus leafminer (CLM), *Phyllocnistis citrella*

INTRODUCTION

Citrus spp. (Fam.: Rutaceae) is an economically valuable fruit crop worldwide, which contains orange, sweet orange, acid lime, and other related species of citrus. In Egypt, citrus is ranked as the largest fruit crop, with an annual production of about 4 million tons and occupying an area of about 184,390 hectares according to the Ministry of Agriculture and Land Reclamation (Annual Report 2013). However, citrus production has faced multiple problems, mainly related to diseases and pest infestations, which cause severe yield losses.

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is an important pest of citrus and related Rutaceae and ornamental plants almost worldwide (Achor et al., 1996). The CLM mines leaves, surface tissue of young shoots and stems, and less frequently the fruit (Sponagel and Diaz, 1994). Although citrus leafminer causes indirect damage to young leaves, which predisposes them to infection by canker so, controlling citrus leafminer is a vital component of canker management (Pena et al., 1996; Belasque et al., 2005). Severe infestations with an average of two or more mines per leaf can retard the growth and yield of nursery and newly planted trees, but their effect on mature trees is less serious. Such infestations usually only occur in late summer and autumn and are often related to low natural enemy activity. They rarely occur in spring because the production of new growth is prolific and synchronized, and quickly becomes immune to attack (Agfact, 2004).

Pesticides are considered an essential, effective, and efficient control tool for leafminer. Neonicotinoid insecticides such as thiamethoxam and acetamiprid are worthy to be comparably involved in the study. They are a relatively new class of systemic insecticides with relatedness to neonicotinoids which makes them act as the agonists to the nicotinic acetylcholine receptor (nAChRs) in a way different from other classes of insecticides. Furthermore, they show low toxicity to mammals, birds, and fish, but display significant toxicities to bees. However, the unwise and uneven use of pesticides leads to the pesticide resistance issue. In these

regards, new control strategies must be developed to respond to this problem (Doaa et. al., 2020).

The main objective of this study was to assess the effectiveness of two neonicotinoid insecticides, thiamethoxam and acetamiprid with recommended field rate against citrus leafminer larvae through two different methods of treatment, leaf-dip, and topical techniques.

MATERIALS AND METHODS

The experiments were performed in the laboratory of the department of pesticides, faculty of agriculture, menoufia university. Two pesticides 0.25 g L^{-1} thiamethoxam (Actara 25% WG, Syngenta) and 0.25 ml L^{-1} acetamiprid (Merva 20% SL, astrachem), along with control (distilled water) were selected to assess their effectiveness against CLM larvae, obtained from the Central Agricultural Pesticide Laboratory (CAPL), Dokki, Giza, Egypt.

For bioassay, the leaves with actively feeding of CLM third instar larvae were collected from citrus mandarins, horticulture farm, faculty of agriculture, menofia university. To keep the leaves moist during the bioassay, each petiole was covered by wet cotton. **For leaf-dipping technique**, leaves were dipped for 10 sec separately in each chemical and dipping in distilled water served as control. Then, leaves were air-dried for approximately 2 h and placed in clean plastic Petri dishes (9 cm diameter × 2.5 cm high) which were lined with wet filter paper and covered with a plastic lid. **For topical application technique**, leaves with actively feeding of CLM larvae were also treated through topical application method described by Shapiro et al., (1998). One drop (about 4µl) of each chemical was applied to the thorax of each CLM larvae using a sterilized micro-syringe. After application, leaves were transformed to clean plastic Petri dishes. Each treatment was replicated five times in both bioassays and five leaves were treated for each replicate, along with distilled water treated as a control group. Leaves were examined under a stereomicroscope (40x) to check the movement of CLM larvae. Data were recorded after 3, 6, 9, 12, 24 h after application and the numbers of live and dead larvae for each replicate were counted in the laboratory under a stereomicroscope.

The percentage mortality of CLM larvae was calculated and corrected by Abbot's (1952) formula:

$$percent\ mortality = (1 - \text{n in T after treatment}/\text{n in Co after treatment})_x 100$$

Where, T is treatment and Co is control

Values of LT_{50}, 95% confidence limits (CLs), and slope ± SE using probit and logit analysis software program (Polo Plus, ver. 2.0.2008) based on Finney analysis, (Finney 1971).

RESULTS

The mortality percentages of CLM larvae on mandarin leaves treated with selected insecticides presented in Figs. (1 and 2). The results showed that thiamethoxam was more effective in controlling citrus leafminer larvae than acetamiprid with a percent mortality of 75% and 100% through leaf dip bioassay and topical bioassay, respectively after 24-h of application. The contact topical application was more effective compared to leaf-dip technique with 12.5% mortality after 9h and 25% after 24-h. Acetamiprid was also effective against CLM with larvae percent of mortality up to 58.3% and 91.6% after 24h of application via both methods (LDB and TB), respectively. The data in Fig.3 showed that TB was more effective than LDB with (25% and 33.3%) mortality after (12 and 24h), respectively.

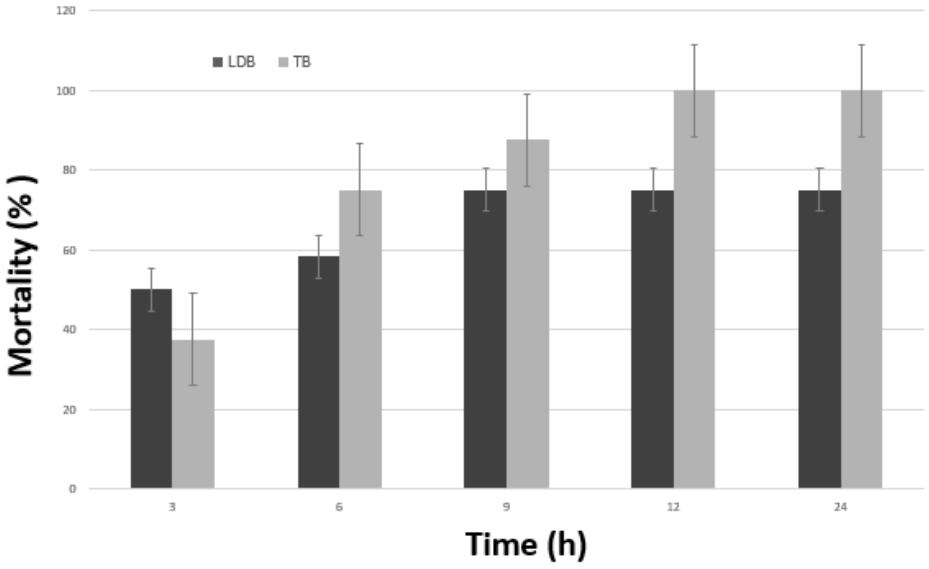

Fig.1. Percent mortality of CLM larvae treated with thiamethoxam through leaf dip bioassay (LDB) and topical bioassay (TB) after 3,6,9,12 and 24h.

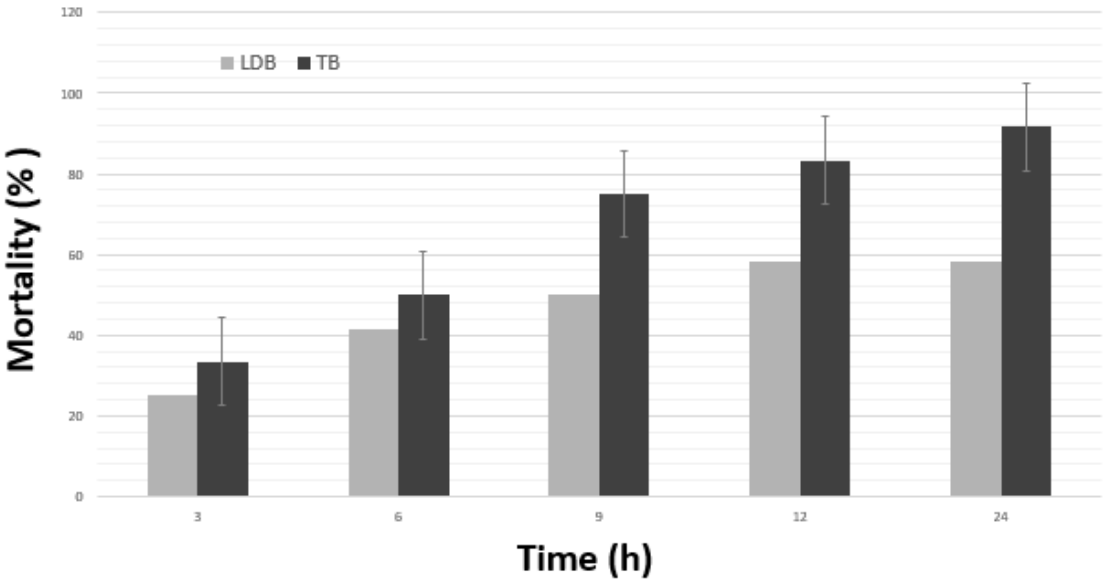

Fig.2. Percent mortality of CLM larvae treated with acetamiprid through leaf dip bioassay (LDB) and topical bioassay (TB) after 3,6,9,12 and 24h.

The results summarized in Table (1) showed the comparative toxicity data of selected insecticides based on LT_{50} values (the median lethal time) using two bioassay methods. The results of leaf-dip bioassay demonstrated that thiamethoxam was significantly more efficient (LT_{50}=2.60h) than acetamiprid (LT_{50}=10.51h). For the contact-topical method, the toxicity data showed insignificant difference between thiamethoxam (LT_{50}=3.78h) and acetamiprid (LT_{50}=4.96h).

Pesticides	Bioassay method	
	Leaf-dip bioassay (LDB)	**Topical bioassay (TB)**
Thiamethoxam (TH)	Slope = 0.857± 0.570 LT_{50} = 2.605 (٠.875-4.33)	Slope = 3.649 ± 1.291 LT_{50} = 3.789 (1.987-5.063)
Acetamiprid (AC)	Slope = 1.747± 0.597 LT_{50} = 10.516 (5.031-12.30)	Slope = 2.187 ± 0.655 LT_{50} = 4.964 (2.796-6.812)

Table 1. LT50 (with 95% FL) and slopes of time-mortality regressions thiamethoxam (TH) and acetamiprid (AC) larvae using two bioassay methods.

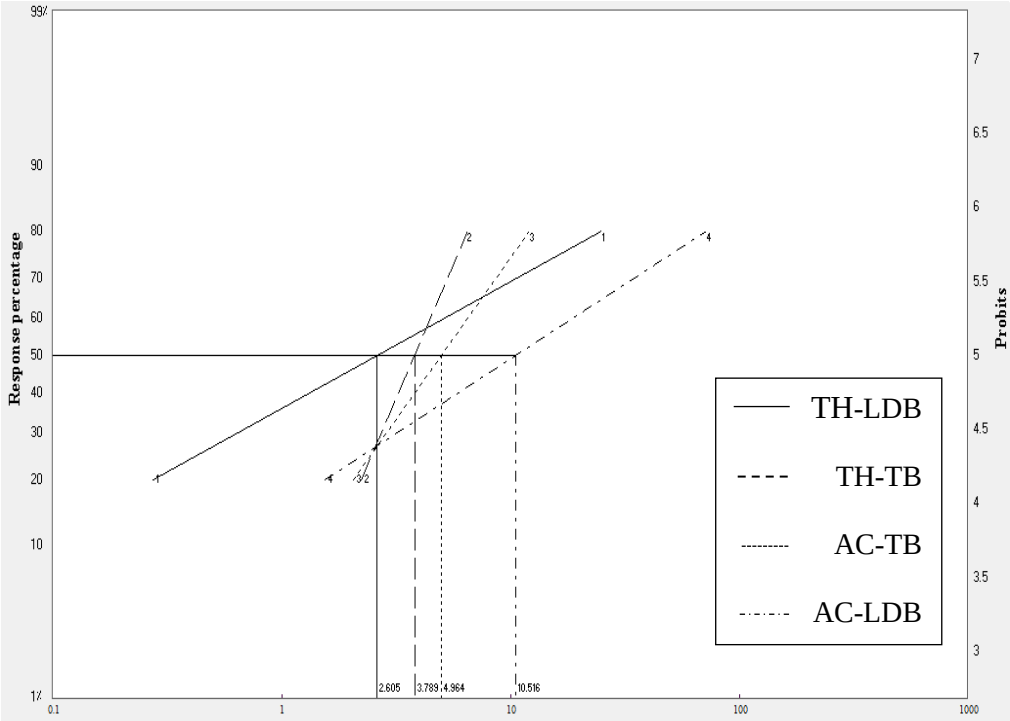

Fig. 3. LT_{50} of thiamethoxam and acetamiprid using two bioassay techniques.

DISCUSSION

Citrus leafminer is a very important insect pest of the citrus crop all over the world, as a result of intensive use of pesticides CLM became resistant to a lot of pesticides. In these regards, new control strategies must be developed to respond to this problem. Two different commercial pesticides and two different bioassays were tested for the management of CLM in this study. Leaf dip bioassay was tested to check the ability of tested chemicals for the penetration into the leaf, and topical bioassay was tested to check the direct activities of chemicals against CLM larvae. As seen from the results, both insecticides tested at field recommended dose showed good response against CLM larvae, but thiamethoxam was more effective. Thiamethoxam and acetamiprid belong to a new class of systemic insecticides with similarity to neonicotinoids which makes them act as the agonists to the nicotinic acetylcholine receptor (nAChRs) in a way different from other classes of conventional insecticides. Furthermore, they show low toxicity to mammals, birds, and fish, but display significant toxicities to bees.

Similar findings were reported by (Raga et al. 2001), thiamethoxam was effective for controlling *P. citrella* when used at 10g a.i. ha^{-1} in water, however, this concentration gave maximum mortality of CLM when used in petroleum oil. Thiamethoxam 25WG (0.06 %) was the most significant treatment for controlling citrus leafminer (Jadhav, 2015). Likewise, *P. citrella* was controlled significantly with systemic insecticides i.e., imidacloprid, and thiamethoxam (Saravanan and Savithri 2005). In soil application of imidacloprid, acetamiprid and thiamethoxam revealed that all these insecticides were effective for controlling CLM up to 20 days after application (Chadda et al. 2009).

Muhammad et. al., (2019) reported that Insecticide application may be quite useful at the time of new flushes when the leaves are most susceptible to CLM damage. In additions, both synthetic and botanicals gave better response against third instar CLM larvae through topical bioassay compared to leaf dip bioassay, but there was no significant

difference between these two bioassays. Mafi and Ohbayashi (2006) found a 3 to 44% mortality of citrus leafminer eggs due to insecticides exposure by using dip method, but the mortality of 1st instar CLM larvae was almost over 90%.

In conclusion, our study suggests that thiamethoxam and acetamiprid pose interesting perspectives for the leafminer management program. However, further investigations should be conducted to explore the effectiveness of these insecticides under field conditions and to demonstrate their effect on natural enemies, honeybees, and humans.

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ملخص

تعتبر حشرة صانعات الأنفاق من أهم آفات التي تصيب أشجار الموالح والعديد من نباتات الزينة في مناطق كثرة على مستوي العالم، وتعتبر المبيدات من العوامل الرئيسية الهامة وذات الفعالية العالية في مكافحة هذه الآفة. ولذلك صممت هذه الدراسة لتحديد فعالية اثنان من المبيدات الجديدة التابعة لمجموعة نيونيكوتينويد وهما مبيد اسيتامبريد، ومبيد ثياموكسام باستخدام معدل التطبيق الحقلي على يرقات حشرة صانعات الأنفاق. تم إجراء هذه الدراسة باستخدام نوعين من طرق التقييم الحيوي المعملي، (طريقة غمر الأوراق وطريقة المعاملة الموضعية). وأظهرت النتائج أن مبيد ثياموكسام كان أكثر فعالية من مبيد اسيتامبريد بمعدل موت ٧٥٪، ١٠٠٪ لطريقة غمر الأوراق والمعاملة الموضعية، على التوالي بعد ٢٤ ساعة من المعاملة. أيضاً تم حساب الزمن اللازم لموت ٥٠٪ من الحشرات المعاملة، وأظهرت النتائج أن طريقة غمر الأوراق لمبيد ثياموكسام كانت أفضل من طريقة المعاملة الموضعية بزمن ٢,٦٠س، ٣,٧٨س لكلا منهما على التوالي.