

## Some of Bio-Alternatives as Growth Enhancers for Seedlings and Controlling Leafminer in Citrus Nurseries

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### **ABSTRACT**

In citrus nurseries and orchards, the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a major and dangerous pest of citrus that severely damages the new leaf. In this respect, experiment was conducted under green-house in the citrus nursery of Horticulture Research Institute, Giza, Egypt. During two seasons (2021-2022) to examined the effect of four bio-alternatives were used viz; Lemongrass essential oil, Aquaprimo, Spinosad and Abamectin at 0.5 cm/L concentrations individual against citrus leaf miner infestation on enhance the growth, profitability of seedlings and increase grafting success percentage of sour orange (C.aurantum) rootstock in nurseries. Percent population infestation of citrus leafminer was recorded before the application and after 24, 48, and 72 hrs of spray. The gathered results indicated a significant increase in all evaluated parameters of leaf area, number of leaves / seedling, leaves and shoots dry matter percentage, percentage of increase in both seedling stem (length and diameter), grafting success percentage, leaf pigments contents, and mineral content of leaves as compared with the control. Moreover, the peak leafminer infestation occurred from the beginning of May to the end of September. An optimum result was obtained from the Lemongrass essential oil at 0.5 cm/L concentration in this study. In order to manage and reduce citrus leafminer infection on citrus nursery seedlings, lemongrass essential oil is advised.

Keywords: Citrus leafminer- lemongrass essential oil- Aquaprimo- Spinosad and Abamectin.

#### INTRODUCTION

Citrus produce fruits, including important crops such as oranges, mandarins, lemons, grapefruits, and limes. It is spread in the Middle East and the Mediterranean region. It renowns for their highly fragrant aromas and complex flavor, citrus are among the most popular fruits in cultivation. (Wu et al., 2011). It is one of the most popular fruit crops in Egypt, the total area is about 529405 Fed., their fruitful acreage reached over 486271 Fed., and yielding approximately 5142829 tons (Ministry of Agriculture and Land Reclamation, 2023).

Citrus leafminer (CLM), *Phyllocnistis* citrella Stainton (Lepidoptera: Gracillariidae), is one of the major pests of citrus, infesting almost all citrus cultivars causing damage in the form of mine on newly foliage (Arshad et al., 2019). When larvae cause damages on leaf it become the severe infestation, ultimately the plant can retard the growth and yield, adults emerge

about dawn and are active in the morning, late afternoon, and at night, females lay eggs in the evening and at night and damage includes the mining of the leaf, resulting in leaf drop and stem dieback (Prabhudev et al., synthetic 2021). Currently, chemical pesticides are frequently used to manage pest infestations that impact crop yield. These substances negatively impact human health, the agricultural ecosystem, and the frequency of environmental contamination; this led to a biological imbalance. Due to this, more research is being done on tactics for controlling insects. Certain pesticides known as bio-pesticides are made from naturally occurring substances like bacteria, plants, animals, and minerals (Zaka et al., 2019 and Salem et al., 2021).

The evergreen lemongrass plant, *Cymbopogon flexuosus*, is a tall shrub yields a citrusy, smokey essential oil that has several advantages. Whether applied



physically, utilized aromatically, or taken internally, the uses and benefits of Lemongrass essential oil are considerably diverse. The chemical makeup of Lemongrass includes aldehydes, which are known for their ability to maintain naturally repelling insects (Hassan et al., 2023).

Spinosad is a relatively non-polar compound synthesized from chemical compounds found in the bacterial species Saccharopolyspora spinosa containing a mixture of two spinosoids, spinosyn A, the main component, and spinosyn D, the secondary component. The spinosyns and spinosoids have a novel mode of action, primarily targeting the binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system, which differ from those of other insecticides. Spinal binding disrupts the neurotransmission of acetylcholine. Spinosad also has secondary effects as a γ-amino-butyric acid (GABA) neurotransmitter agonist (Esmat et al., 2022).

Abamectin is a bio-pesticide produced by the fermentation of soil microorganisms called *Streptomyces avermitilis* that contains more than 80% avermectin B1a and less

than 20% avermectin B1b. These two components are insect repellents. It works to open chlorine channels in the nervous system, which leads to increased secretion of an amino acid called GABA, which has a calming effect on the nervous system in insects that reduces their activity and increases the secretion of an amino acid called glutamic acid, which has a stimulating effect on the nervous system (Corzo et al., 2007).

Aquaprimo is a brand of bio-insecticide, specifically a water-based. It's designed to be effective against a wide range of insects. The bio-insecticide is known for its long-lasting action and low toxicity, making it suitable for various environments Ramadan (2016).

So, the aim of the present work was to use environmentally safe preventive and curative measures to investigate the possibilities of using some types of biological resources due to their great importance in combating the leafminer insect on sour orange seedlings with the aim of reaching a seedling with high quality characteristics.

#### MATERIALS AND METHODS

This experiment was performed in a greenhouse was clad with single skin plastic film at the citrus nursery of the Horticulture Research Institute in Giza, Egypt, over two consecutive seasons, (2021/2022), focusing on seedlings growth of the citrus rootstock sour orange (Citrus aurantium). In first stage, in both studied seasons at March sour orange rootstock seedlings that were virtually uniform, healthy, and free of infections were carefully chosen, grown individually in plastic bags filled with the growing media consists of (sand + compost of plant origin) at 1:1 ratio. The experiment utilized a complete randomized block design. The estimated total number of seedlings used in the experiment was as follows:three hundred seedlings were prepared from

one citrus rootstock × five treatments × three replicates × twenty seedling / replicate. The bags were immediately irrigated and thereafter, tap water was used for irrigation once every two days. Every treatment was administered Lemongrass essential oil at 0.5cm/L, Aquaprimo at 0.5cm/L, Spinosad at 0.5cm/L and Abamectin at 0.5cm/L as a foliar spray (Monthly) from first-May to first-September. In second stage, rootstock seedlings were carefully selected in late-June of the following year based on their consistency, health, and suitability for the graft process with Washington navel orange. After the successful grafting process, ninety transplants were prepared from one citrus verity (Washington navel orange transplants)  $\times$  three treatments  $\times$  three replicates  $\times$  ten



transplants / replicate. A mere two treatments were chosen Lemongrass essential oil at 0.5 cm/L and Spinosad at 0.5 cm/L to be foliar spray (every two weeks) to the transplants four times in (August and September). Triton B was used as a wetting ingredient in all foliar solutions at a rate of 0.1% and spraying continued until runoff. During the two consecutive seasons of 2021-2022, agricultural techniques, such as irrigation and fertilization were carried out in accordance with land reclamation recommendations and the Citrus Res. Dept. of the Ministry of Agriculture.

### The experiment involved treatments as follows:

**First stage** (sour orange rootstock seedlings before budding).

T<sub>1</sub> Control (Synthetic insecticides).

T<sub>2</sub> Lemongrass essential oil at 0.5cm/L.

T<sub>3</sub> Aquaprimo at 0.5cm/L.

T<sub>4</sub> Spinosad at 0.5cm/L.

T<sub>5</sub> Abamectin at 0.5cm/L.

**Second stage** (Washington navel orange transplants after budding).

T<sub>1</sub> Control (Synthetic insecticides).

T<sub>2</sub> Lemongrass essential oil at 0.5cm/L.

T<sub>3</sub> Spinosad at 0.5cm/L.

### Extraction and preparation of formulated Lemongrass essential oil:

The Lemongrass essential oil was extracted according to Cavalcanti et al. (2004) technique with fresh green foliage C. citratus leaves were procured from the fields of the Medicinal and Aromatic Plants Research Department in El-Kanater El-Khairiya, Kalubeia Governorate, Egypt. Using a Clevenger-type equipment (Salem et al., 2014), hydrodistillation for 100 g of fresh material was hydro-distilled to extract C. citratus EO for 2.5-3.0 hours after water was heated to 70 °C, or until no more increase was noted. Fresh Lemongrass leaves yielded an essential oil of pale yellow, with a 2.65% yield (fresh weight basis) and kept at 4°C in dark glass tubes or airtight

containers before being used in analytical and bioassay tests.

### **Extraction and preparation of Spinosad:**

Spinosad is extracted Saccharopolyspora spinosa fermentation broth. First, adjust the pH of the fermentation liquid to an alkaline range (above pH 8) by adding an appropriate alkali. Following this, perform filtration to separate the solid material (filter cake) from the liquid. Next, soak the filter cake in an organic solvent, such as a methanol-aqueous solution. Treat the resulting extract with activated carbon to remove color impurities. Afterward, add acidic water to the organic solution to transfer spinosad into the water layer. Collect this water layer, which now contains the spinosad, and decolorize it again. Subsequently, adjust the pH of the layer water to an alkaline range (approximately pH 9.0 to 10.0) to induce the crystallization of spinosad. Finally, separate the spinosad crystals from the solution through filtration, and dry the crystals to obtain the final spinosad product (Yuanli et al., 2014).

### Fermentation, extraction and purification of Abamectin:

Begin by culturing the Streptomyces avermitilis strain. Once cultured, introduce the strain into a culture solution and allow it to ferment. Following the fermentation process, heat the resulting liquid to a temperature between 60 and 95°C as part of the pre-treatment phase. Next, filter the fermentation liquid to separate the wet mycelium from the liquid broth. The wet mycelium is then dried and ground into a fine powder. The dried mycelium powder is subjected to leaching using a suitable solvent such as ethanol or sec-butyl acetate. After leaching, filter the liquid to separate the solvent-rich part. Extract the abamectin from the remaining solid material using an appropriate solvent. Remove the solvent to concentrate the extract and, if needed, add



activated carbon to eliminate color impurities. Subsequently, cool the solution to induce crystallization. Once crystals form, filter them out, wash thoroughly, and dry them to obtain the final abamectin product (Wislocki et al., 1989).

### Preparation of Aquaprimo:

Aquaprimo is a bio-insecticide with imidacloprid as its active ingredient. The preparation of imidacloprid involves a reaction between 2-nitroiminoimidazolidine and 2-chloro-5-chloromethyl pyridine in the presence of an alkali carbonate within an organic solvent. The process requires the gradual addition of a stoichiometric amount of 2-chloro-5-chloromethyl pyridine to a mixture containing equivalent an ofstoichiometric quantity nitroiminoimidazolidine and the organic solvent, maintained under reflux conditions. (Francisco et al., 2012).

#### Horticultural study:

### 1. Measurements of vegetative growth.

At the end of both experiments, the effectiveness of treatments was assessed using the results of the following measurements:

a) The increase (%) in seedling stem length (cm) and diameter (cm); samples was taken at the end of 1<sup>st</sup> experiment only, as delineated by **El-Harouny** *et al.*, (2023). Length at time end - Length at time beginning

Length at time end - Length at time beginning
Increase in seedling stem length (%) =\_\_\_\_\_ x 100
Length at time beginning

Diameter at time end - Diameter at time beginning
Increase in seedling stem diameter (%) =  $\frac{1000}{100}$  x 100
Diameter at time beginning

- b) Ten mature leaves from each replicate's spring cycle-roughly six months old—were selected from the center of sour orange rootstock seedlings. Using a portable area meter model LI 3000 made in U.S.A, the leaf area was calculated in (cm<sup>2</sup>).
- c) Number of leaves / plant.
- d) Leaves and shoots dry matter percentage: random samples (5g) were taken in

- September and dry matter percentage was assessed after drying at 70°C for 24hour.
- e) Grafting success percentage: Grafting was performed during March for the1<sup>st</sup> experiment of study, for sour orange rootstock seedlings with stem diameter over from 0.5 inch, which represent suitable diameter for budding citrus rootstocks (Albrecht et al., 2017). Grafting success percent was recorded 15 days after grafting using the following equation (Patel et al., 2010).

Grafting Success (%) =  $\frac{\text{Number of sprouted grafts}}{\text{Total number of grafts}} \times 100$ 

The total number of grafts represented the number of sour orange rootstock seedlings that attained the stem thickness appropriate for grafting on late-March alternative for both seasons.

### **2. Bio-chemical contents:** Leaf pigments contents at the end of two stages.

Chlorophylls a & b contents (mg/100g) of leaves fresh weight were estimated. Chlorophylls were extracted with dimethyl formaldehyde solution and placed overnight at (5°C). Chlorophyll A and B as well as carotenoid were measured by spectrophotometer at wave lengths 663, 647 and 470 nm respectively according to the equation which described by Norani (1982).

#### 3. Mineral content of leaves:

0.5 g of dried leaves at the end of 2<sup>nd</sup> stage samples were digested using the H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> as described by Cottenie (1980). The extracted samples were used to determine the following minerals:

- a) Nitrogen using the semi-micro Kjeldahl technique, as ascribed to Pregl (1945).
- b) Phosphor using spectrophotometer according to Murphy and Riely (1962) technique.
- c) A flame photometer was used to assess potassium according to Brown and Lilleland (1946).
- d) Total Carbohydrates (g/100g D.W.): were determined in dried samples of leaves and



shoots according to the method of (Dubois et al., 1956).

#### 4. Economical evaluation:

The profitability of the present treatments which indicated the cost and the net income per treatment were calculated with consideration of the form gate price of the grafted seedlings as (Hudson and Gregorion, 2010). Chemicals, labors and constant costs were assessed for control and treatments. Constant costs include: electricity for irrigation, fertilizers, pruning, pesticides and grafting.

Total cost = Chemicals + labors + constant costs Seedling yield = Grafted seedlings x seedling price

Net income = Seedling price – Total cost

### 5. Statistical analysis:

A complete randomized block design with (five treatments) in 1st stage and (three treatments) in 2nd stage with three replicates for each stage were established. The obtained data of each season were subjected to one-way analysis of variance (ANOVA) according to Snedecor and Cochran (1967) using M-STAT program. Means values represented the various investigated treatments were compared by the Duncan's multiple range test Duncan (1955) at 0.05 level of significance.

**Biological study:** 

Only 300 seedlings infested with *P. citrella* divided into five treatments to apply the four tested bio-alternatives and the control. Ten leaves were collected randomly for each replicates. The tested seedlings were free of insecticide sprays previously with the same design for each year of study. Two of the most effective bio-alternatives used in the previous three experiments are selected and used in conducted a fourth experiment in a definite attempt to reduce the population of the studied pest. Percent population infestation of citrus leafminer was recorded before the application and after 24, 48 and 72 hos of spray.

Samples were taken every week until the newly infestation appears. The samples that had been collected were kept in polyethylene bags and brought to the laboratory so that they could be looked at with a stereomicroscope. Alive individuals (larvae and pupae) in each sample were counted and categorized. The reduction percentages of P. citrella larva and pupa stages were estimated according to Henderson and Tilton equation (1955).

Reduction% =  $[1 - (n. in Co. before treatment) \times (n. in T after treatment) / (n. in Co. after treatment) \times (n. in T before treatment)] <math>\times 100$ 

Where: n = Insect population, T = treated, Co = control

### **RESULTS AND DISCUSSION**

#### **Horticultural study:**

### 1) Vegetative growth measurements (First stage):

### A. Stem diameter and length:

Sour orange rootstock seedlings growth was determined by measuring the percentage of increment in both seedling stem length and diameter at the end of study. Obtained results indicate that, the percentage of increment in seedling stem diameter follows the same pattern as for stem length. Concerning the percentage of increase in stem length, **Table (1)** revealed that,

Spinosad at 0.5cm/L treatment cause expressive increase in the percentage of stem length increment as compared with control treatment. The highest percentage of increment in stem length was recorded (601.0 % - 647.0 %) during two successive seasons respectively. In the same line, the highest percentage of increment in stem diameter was recorded (380.0 % - 398.0 %) during two successive seasons respectively, when used lemongrass essential oil at 0.5cm/L. According to a study by Abdel-Moneim et al. (2019) the width of the green



onion stem rose when bio-product was applied, the green onion experiment's findings demonstrate that bio-product, which includes lemongrass, can have an impact on a plant's ability to grow as shown by the quantity of shoots, and stem diameter. In particular, the impact of applying the bio-products at varying dosages and intervals will vary on crop growth. This is in line with other research. A study conducted by Awais et al. (2009) revealed that a number of the chemicals found in bio-products stimulate plant growth. Comparably, Esmat et al. (2022) demonstrated that bio-products treatments raised the height, number of

branches on rose plants. Previous studies concluded that applications of lemongrass at the right concentration and frequency helped to increase plant growth as compared to the control (Han et al., 2013). Additionally, research by Corzo et al. (2007) indicated that, bio products contain a variety of organic chemicals that stimulate plant growth. These compounds are usually polyphenols and sulfur-based compounds like allicin, diallyl, disulphide, and other substances that are biologically active. As a result, different bio-product spray frequency and concentrations can promote plant growth in various ways.

**Table (1).** Impact of some bio-alternatives on percentage of increase in stem (length and diameter) of sour orange rootstock seedlings during two successive seasons.

T	Increase of stem length %	Increase of stem diameter %			
Treatments	1st season				
T <sub>1</sub> : Control (Synthetic insecticides)	229.0 D	200.0 D			
T2: Lemongrass essential oil at 0.5cm/L	560.0 B	380.0 A			
T <sub>3</sub> : Aquaprimo at 0.5cm/L	343.0 C	265.0 C			
T <sub>4</sub> : Spinosad at 0.5cm/L	601.0 A	354.0 B			
T <sub>5</sub> : Abamectin at 0.5cm/L	321.0 C	238.0 CD			
	2 <sup>nd</sup> s	season			
T <sub>1</sub> : Control (Synthetic insecticides)	276.0 E	222.0 C			
T2: Lemongrass essential oil at 0.5cm/L	588.0 B	398.0 A			
T <sub>3</sub> : Aquaprimo at 0.5cm/L	364.0 C	290.0 B			
T <sub>4</sub> : Spinosad at 0.5cm/L	647.0 A	372.0 AB			
T <sub>5</sub> : Abamectin at 0.5cm/L	305.0 D	268.0 BC			

At 5% level, there is no significant difference between means having the same letter.

### B. Leaf area, number of leaves/seedling, leaf and shoot dry matter percentage:

Data in Table (2) showed that, bioalternatives treatments improve sour orange rootstock seedlings growth parameters, in both seasons compared with control treatment. Lemongrass essential oil at 0.5cm/L (T<sub>2</sub>) significantly increased leaf area (cm<sup>2</sup>) and leaf dry matter (g) above the control (T<sub>1</sub>) by about (62.10% - 68.83 and for both seasons. 99.84% - 75.92) respectively. However, spinosad at 0.5cm/L (T<sub>4</sub>) showed the highest Number of leaves / seedling and shoots dry matter with about (112.12% - 99.34% and 100.00% - 115.85%)for both seasons respectively as compared with control treatment  $(T_1)$ . Khater, (2012)asserts that, essential oils may exhibit phyto-

toxicity when excessively applied and demonstrate limited efficacy against large herbivorous insects, such as lepidopterans and coleopterans. Alves et al., (2019) observed that, lemongrass oil at low concentrations of 1% and 0.5% has the potential to be employed as a bio-pesticide because it reduces *P. xylostella* populations while being harmless to plants. Lemongrass oil's volatile nature is one of the factors that influence its action. As a result, the action of lemongrass oil is linked to hazardous situations such as sunshine or severe temperatures. Furthermore, studies conducted by Dong et al., (2008) revealed that bio-products include a range of organic compounds that promote plant growth. Allicin, diallyl, disulphide, and other



physiologically active chemicals examples of sulfur-based compounds and polyphenols. Therefore, varied dosages and frequencies of bio-pesticide spraying might encourage plant development in different ways. In summary, the use of bioinsecticides impacted the number of leaves/plant and dry matter percentage with bio-pesticide concentration

frequency of applications affecting the number of leaves and dry matter percentage that develop. Applying bio- alternatives products with concentration of 0.05% at every 15 days seem to increase the number of leaves/seedling and leaf area of the citrus rootstock sour orange under greenhouse conditions.

c) Grafting success (%):

respectively. There is no evidence available Table (2). Impact of some bio-alternatives on vegetative growth of sour orange rootstock seedlings during two successive seasons.

Treatments	Leaf area (cm²)	No. of leaves/ seedling	Leaf dry matter (%)	Shoots dry matter (%)
		1st season		
T1: Control (Synthetic insecticides)	14.46 C	8.33 D	16.65 D	15.12 C
T2: Lemongrass essential oil at 0.5cm/L	23.44 A	16.00 AB	28.11 A	32.64 A
T <sub>3</sub> : Aquaprimo at 0.5cm/L	17.94 B	9.33 CD	20.11 C	19.78 B
T <sub>4</sub> : Spinosad at 0.5cm/L	22.92 A	17.67 A	25.90 B	30.14 AB
T <sub>5</sub> : Abamectin at 0.5cm/L	15.17 C	10.33 C	14.89 E	18.35 B
		2 <sup>nd</sup> season	III.	
T1: Control (Synthetic insecticides)	12.47 E	9.00 D	15.24 C	16.97 D
T2: Lemongrass essential oil at 0.5cm/L	24.92 A	17.67 AB	26.81 AB	34.60 AB
T <sub>3</sub> : Aquaprimo at 0.5cm/L	18.42 C	10.67 CD	19.86 B	20.56 C
T <sub>4</sub> : Spinosad at 0.5cm/L	21.42 B	18.00 A	29.31 A	36.63 A
T <sub>5</sub> : Abamectin at 0.5cm/L	14.51 D	11.67 C	13.87 DE	19.30 CD

At 5% level, there is no significant difference between means having the same letter.

For grafting success percentage, reported data in Table (3) reveal that, used bio-alternatives increased significantly the percentage of grafting success for sour orange rootstock seedlings for both seasons, and the highest significant percentage of success was recorded for lemongrass essential oil at 0.5cm/L (T<sub>2</sub>) (91.67% and 88.33%), followed by spinosad at 0.5cm/L (T<sub>4</sub>) (88.33% and 86.67%) for both seasons,

on the impact of lemongrass essential oil on the grafting success percentage increase in sour orange rootstock seedlings, as focus on its bioactivity, growth, and effects in other measurements. Where, grafting success would rely on increment in the vegetative growth by different measurements, methods and materials employed in a given study (Brahmi et al., 2022).

Table (3). Impact of some bio-alternatives on grafting success percentage of sour orange rootstock seedlings during two successive seasons.

Treatments	Grafting success (%)					
Treatments	1st Season	2 <sup>nd</sup> Season				
T1: Control (Synthetic insecticides)	60.00 D	58.33 C				
T2: Lemongrass essential oil at 0.5cm/L	91.67 A	88.33 A				
T <sub>3</sub> : Aquaprimo at 0.5cm/L	66.67 CD	68.33 B				
T <sub>4</sub> : Spinosad at 0.5cm/L	88.33 B	86.67AB				
T <sub>5</sub> : Abamectin at 0.5cm/L	70.00 C	66.67BC				

At 5% level, there is no significant difference between means having the same letter.

#### 2) Leaf chemical contents.

### Leaf pigments content (mg/100g F.W.):-

Data in Table (4) was used to analyze the photosynthesis of sour orange rootstock seedlings based on the levels of chlorophyll a, b, total, and carotenoids pigment. The seedlings showed significant changes in chlorophyll levels when treated with spinosad at 0.5cm/L.(T<sub>4</sub>) Chlorophyll a and total chlorophyll increased by approximately



118.89 - 105.88% and 120.43 - 121.48% in the  $1^{st}$  and  $2^{nd}$  seasons, respectively. However, chlorophyll b and total carotenoids were more influenced by lemongrass essential oil at 0.5 cm/L ( $T_2$ ), with increases of 146.15 - 60.87% and

127.91 – 62.74% in the 1<sup>st</sup> and 2<sup>nd</sup> seasons. Roses treated with bio-alternatives by spraying had higher levels of sugar, anthocyanin's, chlorophylls a & b, total chlorophylls and total carotenoids.

**Table (4).** Impact of some bio-alternatives on leaf pigments contents of sour orange rootstock seedlings during two successive seasons.

Treatments	Chlorophyll a (mg/100g F.W.)	Chlorophyll b (mg/100g F.W.)	Total chlorophylls (mg/100g F.W.)	Carotenoids (mg/100g F.W.)						
	1st season									
T1: Control (Synthetic insecticides)	0.90 CD	0.39 CD	1.53 D	0.46 C						
T2: Lemongrass essential oil at 0.5cm/L	1.76 AB	0.96 A	2.98 AB	0.74 A						
T <sub>3</sub> : Aquaprimo at 0.5cm/L	1.24 B	0.50 C	1.94 B	0.61B						
T <sub>4</sub> : Spinosad at 0.5cm/L	1.97 A	0.83 B	3.15 A	0.68 AB						
T <sub>5</sub> : Abamectin at 0.5cm/L	1.02 C	0.43 C	1.64 C	0.52 BC						
	2 <sup>nd</sup> season									
T <sub>1</sub> : Control (Synthetic insecticides)	0.93 DE	0.43 CD	1.49 C	0.51 C						
T2: Lemongrass essential oil at 0.5cm/L	1.87 B	0.98 A	3.17 AB	0.83 A						
T <sub>3</sub> : Aquaprimo at 0.5cm/L	1.37 C	0.52 C	2.04 B	0.69 B						
T <sub>4</sub> : Spinosad at 0.5cm/L	2.05 A	0.88 B	3.30 A	0.79 A						
T <sub>5</sub> : Abamectin at 0.5cm/L	1.10 D	0.44 CD	1.75 BC	0.65 B						

At 5% level, there is no significant difference between means having the same letter.

### 3) Economical evaluation:-

Data in **Table (5)** showed that, Lemongrass essential oil and Spinosad effectively get the highest net income (LE 595 and 550) as well as (LE 565 and 535) through the two studied seasons respectively. On the other hand, control treatment declared the least net income while Aquaprimo and Abamectin bio- alternatives get middle results.

**Table (5).** Impact of some bio-alternatives on net income of grafted seedlings during two successive seasons.

7.	Chemicals	Labors	Chemicals+	Constant	Total cost	Grafted	Seedlings	Net income			
Treatments	(LE)	(LE)	Labors (LE)	cost (LE)	(LE)	seedlings	price (LE)	(LE)			
	1 <sup>st</sup> season										
T <sub>1</sub> : Control (Synthetic insecticides)	8 <del></del> (8	85	85	120	205	36	540	335			
T2: Lemongrass essential oil at 0.5cm/L	25	85	110	120	230	55	825	595			
T <sub>3</sub> : Aquaprimo at 0.5cm/L	25	85	110	120	230	40	600	370			
T <sub>4</sub> : Spinosad at 0.5cm/L	40	85	125	120	245	53	795	550			
T <sub>5</sub> : Abamectin at 0.5cm/L	25	85	110	120	230	42	630	400			
			2012/2011	21	<sup>nd</sup> season	5465.40					
T <sub>1</sub> : Control (Synthetic insecticides)		85	85	120	205	35	525	320			
T2: Lemongrass essential oil at 0.5cm/L	25	85	110	120	230	53	795	565			
T <sub>3</sub> : Aquaprimo at 0.5cm/L	25	85	110	120	230	41	615	385			
T <sub>4</sub> : Spinosad at 0.5cm/L	40	85	125	120	245	52	780	535			
T <sub>5</sub> : Abamectin at 0.5cm/L	25	85	110	120	230	40	600	370			

### 4) Vegetative growth measurements (Second stage):

### Number of leaves/transplant, leaf and shoots dry matter percentage:

Presented data in **Table (6)** showed that lemongrass essential oil at 0.5cm/L (T<sub>2</sub>) had the highest significant of No. of leaves/ transplant, leaf dry matter (g) and shoots dry matter (g), while control (T<sub>1</sub>) showed the lowest significant value for both seasons. **Table (6)** reveals the superiority effect of lemongrass essential oil at 0.5cm/L (T<sub>2</sub>) on number of leaves/transplant (14.83 and

15.00). Leaf dry matter percentage (31.70 and 29.50 %) as well as shoots dry matter percentage (45.00 and 44.70 %) throughout the two studied seasons respectively. However, Spinosad at 0.5cm/L get less effect than lemongrass essential oil at 0.5cm/L but more than control treatment on No. of leaves (9.00 and 10.33) or leaf dry matter percentage (20.35 and 18.90%) or shoots dry matter percentage (25.70 and 23.95 %) respectively with significant variations. Specifically, according to a study by Abdel-Moneim et al., (2019), the effects



of applying the bio-products at different dosages and intervals will vary on crop development. According to a study by Esmat et al., (2022), rose plants treated with bio-products grew taller and had more branches. According to earlier research, when lemongrass was applied at the proper frequency and concentration, it increased plant growth in comparison to the control (Han et al., 2013). In conclusion, the number

of leaves per plant and the percentage of dry matter were affected by the use of bio-insecticides, with the concentration and frequency of applications of the bio-products having an effect on the number of developing leaves and the percentage of dry matter. Furthermore, studies by Nguyen et al., (2023) showed that, bio-products include a range of organic compounds that promote plant growth.

**Table (6).** Impact of some bio-alternatives on vegetative growth of Washington navel orange transplants during two successive seasons.

Treatments	No. of leaves / transplant	Leaf dry matter (%) 1st season	Shoots dry matter (%)		
T1: Control (Synthetic insecticides)	9.00 B	20.35 C	25.70 C		
T2: Lemongrass essential oil at 0.5cm/L	14.83 A	31.70 A	45.00 A		
T <sub>3</sub> : Spinosad at 0.5cm/L	13.33 AB	26.30 B	33.80 B		
		2 <sup>nd</sup> season			
T <sub>1</sub> : Control (Synthetic insecticides)	10.33 C	18.90 B	23.95 C		
T <sub>2</sub> : Lemongrass essential oil at 0.5cm/L	15.00 A	29.50 A	44.70 A		
T <sub>3</sub> : Spinosad at 0.5cm/L	11.67 B	27.60 AB	35.50 B		

At 5% level, there is no significant difference between means having the same letter.

### 5) Leaf chemical contents: Leaf pigments content (mg/100g F.W.):-

presented **Table** Data in demonstrate that plant pigment concentration varies within different studied treatments; however, the highest significant value of leaf chlorophyll a and total carotenoids content was recorded for lemongrass essential oil at 0.5cm/L (T<sub>2</sub>) in 2021 season (1.98 and 0.74 mg/100g F.W.) and in 2022 season (2.04 and 0.80 mg/100g F.W.). Furthermore, the highest significant

value of leaf chlorophyll b content was recorded for spinosad at 0.5cm/L (T<sub>3</sub>) through the two studied season (0.84 and 0.88 mg/100g F.W.) as well as, total chlorophylls during 2<sup>nd</sup> season only (3.23 mg/100g F.W.). On the other hand, the lowest significant value of all parameters was observed for control (T<sub>1</sub>) in both seasons. Roses sprayed with bio-alternatives had increased amounts of anthocyanins, sugar, total chlorophylls, total carotenoids, and chlorophylls a and b.

**Table (7).** Impact of some bio-alternatives on leaf pigment contents of Washington navel orange transplants during two successive seasons.

1.07	Chlorophyll a	Chlorophyll b	Total chlorophylls	Carotenoids					
Treatments	(mg/100g F.W.)	(mg/100g F.W.)	(mg/100g F.W.)	(mg100g F.W.)					
	1 <u>st</u> season								
T1: Control (Synthetic insecticides)	0.93 C	0.49 C	1.71 C	0.49 C					
T2: Lemongrass essential oil at 0.5cm/L	1.98 A	0.70 B	3.18 A	0.74 A					
T <sub>3</sub> : Spinosad at 0.5cm/L	1.55 B	0.84 A	2.87 B	0.61 B					
		2nd s	season						
T1: Control (Synthetic insecticides)	0.86 C	0.44 C	1.67 C	0.53 C					
T2: Lemongrass essential oil at 0.5cm/L	2.04 A	0.68 B	2.96 B	0.80 A					
T <sub>3</sub> : Spinosad at 0.5cm/L	1.71 B	0.88 A	3.23 A	0.65 B					

At 5% level, there is no significant difference between means having the same letter.

### 6) Leaf content of minerals and total carbohydrates:-

According to the analysis of the data in **Table (8)**, there was no statistical significant variation in nitrogen percentage between the

treatments; this was verified throughout the two seasons. While, significant differences were found between the treatments in terms of phosphorous (P), potassium (K) and total carbohydrates in (leaves and shoots). The



Spinosad at 0.5cm/L (T<sub>3</sub>) had the highest significant value of all mentioned parameters of which were confirmed during the study seasons. Additionally, data in Table (8) exhibited that, Spinosad bioalternatives disclosed higher nitrogen (N) and phosphorus (P) in 2021 season while Lemongrass oil scored higher N and P content in leaves of the seedlings without significant variation in 2022 season of study. Furthermore, Spinosad caused higher and significant leaf content of potassium (K) through the two studied season. Spinosad also induced higher total carbohydrates in shoots (33.95 and 32.70 g/100g D.W.) in

1<sup>st</sup> and 2<sup>nd</sup> seasons as well as higher total carbohydrates in leaves through 2<sup>nd</sup> season (24.08 g/100g D.W.). Bio-alternatives like Lemongrass essential oil and Spinosad can increase leaf mineral content and influence total carbohydrates by improving nutrient availability and enhancing plant physiology. Because, bio-alternatives directly boost macro-and micronutrients like nitrogen, phosphorus and iron in leaves, while, indirectly improve nutrient supply and uptake can increase promote growth, and boost overall plant quality (Nguyen et al., 2023; Hassan et al., 2023 and Abdel-Moneim et al., 2019).

**Table (8).** Impact of some bio-alternatives on leaf mineral contents and total carbohydrates of Washington navel orange transplants during two successive seasons.

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)	Total carbohydrates in leaves (g/100g D.W.)	Total carbohydrates in shoots (g/100g D.W.)					
	1 <sup>st</sup> season									
T1:Control (Synthetic insecticides)	2.10 A	0.10 B	0.75 C	15.46 B	21.33 B					
T <sub>2</sub> :Lemongrass essential oil at 0.5cm/L	2.37 A	0.13 AB	0.91 B	23.03 A	31.70 A					
T <sub>3</sub> :Spinosad at 0.5cm/L	2.49 A	0.14 A	1.18 A	22.28 A	33.95 A					
ong term and a second	2 <sup>nd</sup> season									
T1: Control (Synthetic insecticides)	2.04 A	0.09 B	0.73 C	15.51 B	19.75 B					
T2: Lemongrass essential oil at 0.5cm/L	2.47 A	0.15 A	0.89 B	21.18 AB	29.60 A					
T <sub>3</sub> : Spinosad at 0.5cm/L	2.31 A	0.12 AB	1.21 A	24.08 A	32.70 A					

At 5% level, there is no significant difference between means having the same letter.

### **Biological study:**

### First stage:

### 1<sup>st</sup> application:

Data showed in **Table (9)** indicate that after a week of application, Spinosad recorded the highest reduction percentage in larval stage of *P. citrella* on seedling leaves with an average of 95%, followed by Lemon grass oil (93%), Aquaprimo and Abamectin recording (90% for each). The same arrangement for the pupal stage was obtained with a reduction percent of 89, 84, 82 and 79%, respectively. The reduction percentage of tested bio-resources (Spinosad, Lemon grass oil, Aquaprimo and Abamectin) gradually decreased along the experiment to

record 34, 40, 31 and 25 (for larval stage) and 29, 31, 25 and 18% (for pupal stage) after four weeks of application. Finally, the mean reductions of the tested compounds against larval stage were 64.75, 63.5, 58.75 and 54 for Lemon grass oil, Spinosad, Aguaprimo and Abamectin, respectively with significant differences between the tested treatments with each other. On the same side the mean reduction percentage against pupal stage recording 57.25, 53.75, 50.75 and 44.5 for Lemon grass oil, Spinosad, Aquaprimo and Abamectin with significant difference between each other. The differences between Lemon grass oil and Spinosad were statistically insignificant.



Table (9). Mean reduction percentages of *P. citrella* as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the first season

Data		% reduction (after week)										
Treatments Date	1st post t	reatment	reatment	1aeriaa								
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae	larvae	pupae		
Spinosad	95	89	74	59	51	38	34	29	63.5 a	53.75 ab		
Aquaprimo	90	84	68	62	46	32	31	25	58.75 b	50.75 b		
Lemon grass Oil	93	82	72	66	54	50	40	31	64.75 a	57.25 a		
Abamectin	90	79	62	51	39	30	25	18	54 c	44.5 c		
F value								144.25	19.48			
			L.S	D.					1.26	3.80		

Data compiled in **Table (10)** show that results of the first experiment during the second season take the same trend as that of the first season, where the tested treatments gave high reduction percentage in the tested insect stages (larva and pupa) after a week of application. The tested compounds were arranged in descending order as follows Spinosad (97%), Lemon grass oil (95%), Aquaprimo (92%) and Abamectin (91%) for larval stage. But, the arrangement in case of pupal stage began with Spinosad (92%), Aquaprimo (88%), Lemon grass oil (87%)

and Abamectin (83%). Also, the reduction percentages gradually decreased with time till the end of the experiment. The mean % reduction of the tested insecticides against larval stage significantly recorded 67.75, 67.75, 58.5 and 57.75 for Lemon grass oil, Spinosad, Aquaprimo and Abamectin, respectively. On the same side, corresponding mean reduction percentages against pupal stage significantly showed 56.75, 57.75, 50.25 and 49.75%, respectively.

Table (10). Mean reduction percentages of *P. citrella* as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the second season

Date		% reduction (after week)											
	1st post t	1st post treatment		2nd post treatment		3rd post treatment		reatment	- Mean % reduction				
Treatments	Larva	pupa	Larva	Pupa	larva	Pupa	larva	Pupa	Larva	pupa			
Spinosad	97	92	80	62	54	45	40	32	67.75 a	57.75 a			
Aquaprimo	92	88	71	59	41	30	30	24	58.5 b	50.25b			
Lemon grass Oil	95	87	79	70	50	41	47	29	67.75a	56.75 a			
Abamectin	91	83	68	60	42	34	30	22	57.75 b	49.75 b			
f value									20.65	4.26			
2			L.	S.D.					3.77	6.29			

### 2<sup>nd</sup> application:

Data tabulated in Table (11) indicate that after a week of application, Spinosad achieved the highest reduction percentage of 97% for larval stage of *P. citrella* on seedling leaves, followed by Lemon grass oil (95), Aquaprimo (93) and Abamectin (89%). Whereas, the reduction percentages of pupal stage was as follows 93% for Spinosad, 88% for Aquaprimo, 84% for Lemon grass oil and 80% for Abamectin. The reduction percentage of the tested insecticides (Spinosad, Lemon grass oil,

Aquaprimo and Abamectin) gradually decreased along the investigation period to record 45, 40, 37 and 28% (for larval stage) and 33, 28, 29 and 20% (for pupal stage) after four weeks of application. Generally, the mean reduction percentage of the tested insecticides against P. citrella larvae during the second application were significantly arranged in descending order as Spinosad, Lemon grass oil, Aquaprimo and Abamectin (70.75,68.25, 63.25 and 51.75%, respectively). Also, the same arrangement was significantly obtained for pupal stage



(60, 55.75, 55.5 and 43.25) for Spinosad,

Lemon grass oil, Aquaprimo and Abamectin, respectively.

Table (11). Mean reduction percentages of *P. citrella* as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the first season

date/treatment	1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean % reduction	
	larva	Pupa	Larva	Pupa	Larva	Pupa	larva	Pupa	Larva	Pupa
Spinosad	97	93	79	70	62	45	45	33	70.75 a	60.25 a
Aquaprimo	93	88	72	65	51	40	37	29	63.25ab	55.5a
Lemon grass Oil	95	84	80	60	58	51	40	28	68.25a	55.75a
Abamectin	89	80	59	44	31	29	28	20	51.75c	43.25b
	F value								26.66	8.86
			L.S			5.03	7.55			

Data cleared in Table (12) indicate, after week of spraying, that Spinosad greatly reduced larvae of the tested pest recording the highest reduction percentage on seedling leaves (98%) followed in descending order by Lemon grass oil 997%), Aquaprimo (93%) and Abamectin (89%). The reduction percentage of pupal stage descendingly arranged as Spinosad (94%), Lemon grass oil (90%), Aquaprimo (88%) and Abamectin (81%). The reduction percentage of tested insecticides decreased gradually along the experiment to record 45, 37, 50 and 29 (for larval stage) and 35, 29, 33 and 20 (for

pupal stage) for Spinosad, Aquaprimo, Abamectin. Lemon grass oil and respectively. The mean reduction percentage of the tested insecticides against P. citrella larvae were significantly arranged as Lemon grass oil, Spinosad, Aquaprimo Abamectin with reduction percentages of 73.5, 71.5, 63.25 and 55.75, respectively. But in case of pupal stage, the efficiency of the used compounds were significantly arranged as Spinosad, Lemon grass oil, Aguaprimo and Abamectin with a reduction percentages of 63.5, 63, 55.5 and 48.25, respectively.

Table (12). Mean percentages of *P. citrella* reduction as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the second season

Date/ treatment	1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean % reduction	
	Larva	Pupa	Larva	Pupa	Larva	Pupa	larva	Pupa	Larva	pupa
Spinosad	98	94	85	75	58	50	45	35	71.5 a	63.5a
Aquaprimo	93	88	72	65	51	40	37	29	63.25 b	55.5 b
Lemon grass Oil	97	90	86	73	61	56	50	33	73.5 a	63.00 a
Abamectin	89	81	61	55	44	37	29	20	55.75 c	48.25 c
	F value								99.56	19.47
	L.S.D.									

### 3<sup>rd</sup> application:

Data investigated in **Table (13)** reveal that the reduction percentage of *P. citrella* caused by Spinosad, Lemon grass oil, Abamectin and Aquaprimo showed 96, 95, 90 and 89 in case of larval stage. On the other side, the used compounds against pupal stage efficiently arranged as Lemon grass oil, Spinosad, Abamectin and Aquaprimo which recorded 90, 86, 83 and 82% reduction. The efficacy of tested insecticides (Spinosad, Lemon grass oil,

Aquaprimo and Abamectin) gradually decreased along the time to record 46, 43, 32 and 27% (for larval stage) and 26, 30, 17 and 22% (for pupal stage) after four weeks, respectively. In general, the mean reduction percentages of the tested insecticides against P. citrella larval stage during the third experiment significantly were 71.5, 70.25, 55.5 and 54.25 for Lemon grass oil, Spinosad, Aquaprimo and Abamectin, respectively. But, in case of pupal stage the reduction percentages significantly were



58.5, 57, 49.5 and 45.5 for Lemon grass oil,

Spinosad, Abamectin and Aquaprimo, respectively.

Table (13). Mean percentages of *P. citrella* reduction as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the first season

Date/treatment	1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean No.	
	Larva	Pupa	Larva	Pupa	Larva	pupa	larva	Pupa	1arva	pupa
Spinosad	95	90	82	72	63	40	46	26	71.5 a	57 a
Aquaprimo	89	82	62	57	39	26	32	17	55.5 b	45.5 b
Lemon grass Oil	96	86	82	66	60	52	43	30	70.25 a	58.5 a
Abamectin	90	83	64	61	36	32	27	22	54.25 b	49.5 ab
f value L.S.D.								57.24	9.14	
									3.77	6.29

Data tabulated in Table (14) reveal that the reduction percentage in case of larval stage of P. citrella of Spinosad, Lemon grass oil, Aquaprimo and Abamectin recorded 96, 94, 90 and 89%, respectively. The same trend was observed in case of pupal stage which showed reduction percentages of 91, 90, 83 and 80% for Spinosad, Lemon grass oil, Aquaprimo and Abamectin, respectively. The efficacy of tested insecticides (Lemon grass oil, Spinosad, Aquaprimo and Abamectin) gradually decreased along the experimental time to record 49, 46, 40 and 29% (for larval

stage) and 30, 29, 20 and 24% (for pupal stage) after 4 weeks of application, respectively. The efficiency of the tested insecticides against *P. citrella* larval stage significantly arranged as Spinosad, Lemon grass oil, Aquaprimo and Abamectin with mean reduction percentages of 72.25, 72, 61.75 and 54.25%), respectively. But, in case of pupal stage the arrangement significantly was Lemon grass oil, Spinosad, Aquaprimo and Abamectin with mean reduction percentages of 60.25, 59.75, 48.75 and 46.75), respectively.

Table (14). Mean percentages of *P. citrella* reduction as a result of applying different tested pesticides on seedling leaves at Giza Governorate during the second season

Date/ treatment	1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean % reduction	
	larva	pupa	Larva	Pupa	Larva	pupa	larva	Pupa	larva	pupa
Spinosad	96	91	84	73	63	46	46	29	72.25 a	59.75 a
Aquaprimo	90	83	70	59	47	32	40	21	61.75 b	48.75b
Lemon grass Oil	94	90	82	68	63	53	49	30	72 a	60.25 a
Abamectin	89	80	59	48	40	35	29	24	54.25 b	46.75 b
F value									12.65	6.21
L.S.D.									7.55	8.81

### Second stage: 1<sup>st</sup> application:-

Results of previous experiments proved that both Lemon grass oil and Spinosad were the most potent compounds against larvae and pupae of *P. citrella*, so they were selected for the final experiment, which were prepared to spray seedlings. Data tabulated in **Table (15)** reveal that the reduction percentage of *P. citrella* for Spinosad and Lemon grass oil recorded 97 and 96 in case of larval stage, but in case of pupal stage the two compounds equally

recorded the same reduction percentage of 88. The efficacy of the two selected insecticides (Spinosad and Lemon grass oil) gradually decreased along the experimental time to record 50, 44% for larval stage and (34, 32%) for pupal stage after four weeks of application, respectively. The potency of the two selected insecticides against *P. citrella* larval stage insignificantly arranged as Spinosad and Lemon grass oil with mean reduction percentages of 71.75 and 69.25, respectively. But, in case of pupal stage the reverse took place where Lemon grass oil



slightly and insignificantly surpassed

Spinosad that recorded mean reduction percentages of 57.25 and 56%, respectively.

Table (15). Mean percentages of *P. citrella* reduction as a result of applying two different tested pesticides on seedling leaves at Giza Governorate during the first season

Date 1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean % reduction		
Treatments	larva	pupa	Larva	Pupa	Larva	pupa	Larva	Pupa	Larva	pupa
Spinosad	97	88	75	62	65	40	50	34	71.75	56
Lemon grass Oil	96	88	80	60	57	49	44	32	69.25	57.25
F value									2.08	0.19
L.S.D.								0.20	0.25	

Data investigated in **Table (16)** reveal that reduction percentages in larval stage of *P. citrella* of tested insecticides Spinosad and Lemon grass oil were 98 and 97 after a week of application, respectively. But, in case of pupal stage the reverse was obtained where the reduction percentages were 91 and 90 for Lemon grass oil and Spinosad, respectively. The efficacy of two selected insecticides (Spinosad and Lemon grass oil) gradually decreased along the experiment to

reach 50 and 39% in the fourth week for larval stage and 28 and 34% for pupal stage, respectively. The mean reduction percentages of the two selected insecticides against *P. citrella* larval stage insignificantly were 73 and 72 for Lemon grass oil and Spinosad, respectively. Whereas, in case of stage the efficacy insignificantly arranged as Spinosad and Lemon grass oil with reduction percentages of 61.25 and 58.75, respectively.

Table (16). Mean percentages of *P. citrella* reduction as a result of applying two different tested pesticides on seedling leaves at Giza Governorate during the second season

Date	1st post tr	1st post treatment		2nd post treatment		3rd post treatment		4th post treatment		Mean % reduction	
Treatment	larva	Pupa	Larva	pupa	Larva	pupa	larva	Pupa	Larva	pupa	
Spinosad	98	90	86	78	65	42	39	34	72	61	
Lemon grass Oil	97	91	85	65	60	51	50	28	73	58.75	
F value								0.33	0.61		
			T	SD							

The obtained data are in harmony with those obtained by many researchers all over the world such as Mafi and Ohbayashi (2006) who stated that the corrected mortalities of the first instar larvae of P. citrella exposed to various insecticides had a wide range of mortality (23-100%) 3 d after treatment, where mortality after 5 d reached to 100%, except for nicotinoide group. Among nicotinoide group. the effects imidacloprid, thiamethoxam and acetamiprid were significantly lower than that obtained with clothianidin and dinotefuran. The citrus leaf miner control with abamectin alone significantly reduce incidence of citrus canker on fruits in two seasons when compared with unsprayed control (Stein et

al., 2007). Also, Shinde et al. (2017) found that similar trend of effect on percent leaves infestation of citrus leaf miner at 3 and 7 days after treatment. Minimum sprayings of different insecticide were required for keeping the infestation of citrus leaf miner below economic injury level. Amongst different insecticides tested against citrus leafminer in orchard, Thiamethoxam 25 WG (at 0.06%) was significantly superior and followed by Spinosad 45 SC (at 0.03%), Imidacloprid 17.8 SL (at 0.06%), Diflubenzuron 0.08%), 25 WP (at Acetamiprid 20 SP (at 0.04%), and Diafenthiuron 50 EC (at 0.05%). Phyllocnistis citrella mortality was higher when the combination treatment (abamectin



with plant extracts) was applied as compared to the extracts alone. The combination of abamectin with Azaderachta indica extracts showed greater mortality of P. citrella as compared to other plant extracts but the mortality was not satisfactory, i.e., 50% in ratio 1:2. By mixing abamectin with A. indica at the 2:1 ratio, a higher P. citrella mortality (> 60%) was observed (Arshad et al., 2019). On another side, the maximum mortalities of nymphs and adults of mealybug Nipaecoccus nipae after 24 hrs., of exposure, were recorded by the highest concentration of 10000ppm essential oil of lemongrass (86.67±0.00 and 90.00±3.30%, respectively) while, after 48 hrs., the high mortalities of lemongrass essential oil was (93.33±3.33 and 96.67±3.33 %) with the same concentration. Lemongrass essential highest mortalities oil achieved the

(99.00±0.58 and 99.00±0.58%) against nymphs and adults of mealybug N. nipae after 72 hrs. However, the essential oil of lemongrass approximately showed 100% mortality of the *N. nipae* nymphs and adults at the three tested concentrations 1000, 5000, 10000ppm after 72 hrs. The minimum mortality value of lemongrass essential oil was exhibited by the lowest concentration (1000ppm) after 24 hrs and recorded the same mortality percentages of 60.33±5.77 and 60.00±5.77% for nymphs and adults, respectively. The results showed that essential lemongrass oil presented considerably high insecticidal toxicity to the nymphs and adults of the mealybug (N. nipae) with more than 90% mortality at the three examined concentrations (1000, 5000 and 10000ppm) (Hassan et al., 2023).

### **CONCLUSION**

This study investigated the effect of four bio-alternatives and tested their insecticidal on nymphs and an adult of citrus leafminer (*Phyllocnistis citrella* Stainton) under green-house. The compositional analysis of Lemongrass essential oil revealed that general and neral, the major components of Lemongrass essential, had toxic and lethal effects on citrus leafminer and can be used as an alternative to synthetic chemical insecticides. This study showed the

potential of Lemongrass essential oil as a bio-alternatives approach to manage citrus leafminer and in order to reduce the environmental damage caused by pesticides, more studies should be carried out on lemongrass essential oil. Our findings revealed that in spray bioassays, Lemongrass essential oil at 0.5 cm/L exhibited a toxic effect against citrus leafminer nymphs and adults.

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# الملخص العربي المدائل الحيوية لتحسين نمو الشتلات ومكافحة حشرة صانعة الأنفاق في مشاتل الموالح

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تُعد حشرة صانعة الأنفاق في مشاتل الموالح من الأفات الرئيسية والخطرة التي تُلحق أضرارًا بالغة بالأوراق الجديدة. في هذا الصدد، أجريت تجربة تحت الصوب في مشتل الموالح التابع لمعهد بحوث البساتين بالجيزة، مصر. خلال موسمين متنالين (2022-2021)، حيث تم فحص تأثير أربعة بدائل حيوية وهي: زيت حشيشة الليمون العطري، وأكوابريمو، وسبينوساد، وأبامكتين بتركيزات 0.5 سم/لتر، بشكل فردي ضد الإصابة بحشرة صانعة الأنفاق بهدف تعزيز نمو وربحية الشتلات وزيادة نسبة نجاح التطعيم بشكل ملحوظ لأصل النارنج في المشاتل مقارنة بالشتلات الغير معامله باستخدام تصميم القطاعات كاملة العشوائية. سُجِّلت نسبة الإصابة بحشرة صانعة الأنفاق قبل الرش وبعد ٢٤ و ٤٨ و ٢٧ ساعة. أشارت النتائج إلى زيادة ملحوظة في القياسات المدروسه، وخاصة مساحة الورقة، و عدد الأوراق/الشتلة، ونسبة المادة الجافة للأوراق والأفرع، ونسبة الزيادة في كلٍّ من ساق الشتلة البذريه (الطول والسمك)، ونسبة نجاح التطعيم، ومحتوى الأوراق من الصبغات وكذلك محتوى الأوراق من العبناصر الكبرى، كمقارنة بالشتلات الغير معامله. علاوة على ذلك،أوضحت الدراسه بلوغ ذروة الإصابة بحشرة صانعة الأنفاق من بداية مايو إلى نهاية سبتمبر. وقد تم الحصول على أفضل نتيجة عند استخدام زيت حشيشة الليمون العطري للقضاء على الإصابة بحشرة صانعة بتركيز 0.5 سم/لتر في تلك الدراسه. وعليه يُنصح باستخدام زيت حشيشة الليمون العطري للقضاء على الإصابة بحشرة صانعة الأنفاق في شتلات الموالح.