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Citrus Leafminer Management: Jasmonic Acid versus Efficient Pesticides

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Abstract

The citrus leafminer (*Phyllocnistis citrella* Stainton) is a significant pest for *Citrus* spp. worldwide. Hence, the effectiveness of jasmonic acid (JA) was compared to three pesticides, abamectin, thiamethoxam, and acetamiprid, against *P. citrella* infesting mandarin (*Citrus reticulata* L.) and lime (*C. aurantifolia* L.) seedlings. Mortality rate was significantly different due to JA and other pesticides treatments. Moreover, on the 3rd day after treatment, JA demonstrated the highest reduction percentage of leafminer (77.08 and 33.33%) on mandarin and lime, respectively. By the 10th day after treatment, JA and abamectin displayed 100% reduction in both plant species. Furthermore, the foliar application of JA enhanced the most vegetative characteristics in the treated seedlings, including growth rate (shoot length/root length), fresh and dry weights of shoot and root as well as the number of leaves/seedling. Moreover, soluble protein content was increased significantly under JA treatment in the two *Citrus* spp. Jasmonic acid showed a good biological activity, which gives a practical reason to recommend it to be integrated in pest management programs as an alternative product for controlling *P. citrella*.

Keywords *Phyllocnistis citrella* · Lime · Mandarin · Biological management · Chlorophyll · Protein

Introduction

Citrus spp. (Fam.: Rutaceae) is an economically important fruit crop worldwide. It is cultivated in an area of 9.8 million hectares with a global production exceeded 156 million tons in 2014 (FAO 2014). 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 the diseases and pest infestations, which cause severe yield losses. The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), a tiny moth pest of citrus (Arshad et al. 2018; Fletcher et al. 2018), is considered one of the major biotic constraints. *P. citrella* can affect growth and yield of nursery and newly planted trees; however, the effect on mature trees is less serious (Goane et al. 2015). Pesticides are considered an essential, effective, and efficient control tool for leafminer. Among commonly used pesticides, abamectin is well-known for its controlling of citrus leafminer. However, other pesticides such as thiamethoxam and acetamiprid are worthy to be comparably involved in the study. They are a relatively new class of systemic insecticides with a relatedness to neonicotinoids which makes them act as the agonists to the nicotinic acetylcholine receptor (nAChRs) in a way different of 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.

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Plant responses to many biotic and abiotic stresses are orchestrated locally and systemically by signaling molecules known as the jasmonates. Jasmonic acid has progressively become known as a defense and fertility hormone. It is recognized as a key signaling molecule which can stimulate gene expression of secondary metabolite biosynthetic pathways (Xu et al. 2005). Moreover, JA cooperates in many cellular activities and many processes relating to development, stress, disease, pest and wounding responses (Rosahl and Feussner 2004; Memelink 2009).

Since the 1960s, JA has been discovered as secondary metabolite from jasmine flower oil (Demole et al. 1962). The biological functions of JA have received a great attention of researchers in the last two decades (El-Wakeil 2003; El-Wakeil et al. 2010). The main objectives of the current research are (1) to investigate the effect of *P. citrella* on some morphophysiological traits in mandarin and lime seedlings, (2) to assess the efficiency of JA as a foliar application for controlling the citrus leafminer in comparison with some synthetic pesticides.

Materials and Methods

Plant Materials and Growth Conditions

One-and-a-half-year-old seedlings of lime (*Citrus aurantifolia* L.) and mandarin (*Citrus reticulata* L.) were used to compare the effectiveness of JA and three synthetic pesticides on leafminer infestation. Seedlings were maintained in a greenhouse of the research farm of Pomology Dept., Faculty of Agriculture, Assiut University during May to July 2017. Culture pots were placed at ~ 60 cm from each other, each pot was filled by 4.5 kg of 1:1 sandy/clay soil. Seedlings were irrigated every other day with Hogland solution (Hogland and Arnon 1950).

Tested Compounds

Three pesticides were selected as used for foliar applications: 0.3 g/L abamectin 1.8% EC, 0.25 g L^{-1} thiamethoxam 25% WG, and 0.5 g L^{-1} acetamiprid 20% SL, obtained from the Central Agricultural Pesticide Laboratory (CAPL), Dokki, Giza, Egypt. In addition, 100 µM Jasmonic acid (99%, Sigma-Aldrich Co.) was used for comparison. A water spray was included as a control. Each treatment comprised 5 seedlings and was replicated 3 times. The JA and the selected pesticides were sprayed using a hand sprayer equipped (5 L) with a flat-fan nozzle.

Infestation Parameters

The number of alive larvae of *P. citrella* was recorded before treatment (15 larvae were for Mandarin and 21 for lime) and after spray application (on the 1st, 3rd, 5th, 7th, and 10th day). Percentage of population reduction was calculated according to Henderson and Tilton (1955). However, negative control involved in the equation as followed:

$$\text{Reduction } \% = \left(1 - \frac{n \text{ in Co before treatment } * \ n \text{ in T after treatment}}{n \text{ in Co after treatment } * \ n \text{ in T before treatment}}\right) \times 100$$

where n = Insect population, T = treated, Co = control.

Vegetative Parameters

At the end of the trial (8 weeks after treatments), several vegetative parameters were measured to assess plant growth, including shoot and root lengths (cm), fresh and dry weights of shoot and root (g), and number of leaves per seedling.

Physiological Parameters

The photosynthetic pigments via, chlorophyll a, chlorophyll b and carotenoids were estimated using the spectrophotometric method according to Lichtenthaler (1987). The photosynthetic pigments were extracted from a definite fresh weight leaf sample (50 mg) in 5 ml ethyl alcohol (95%) in a test tube at 60 °C. Then the extraction was completed to 10 ml with 95% ethyl alcohol and absorbance readings were determined with a spectrophotometer (Unico UV-2100). Chlorophylls and carotenoids concentrations were calculated as mg/g FW at 663, 644 and 452 nm using the following equations:

$$\text{Chl.a} = \left(13.36 * A_{663}\right) - \left(5.19 * A_{644}\right)$$

$$\text{Chl.b} = \left(27.49 * A_{644}\right) - \left(8.12 * A_{663}\right)$$

$$\text{Carot.} = \left[\left(1000 * A_{452}\right) - (2.13 * \text{Chl.a}) - (97.6 * \text{Chl.b})\right] / 209$$

Some primary metabolites were measured including soluble metabolites, soluble proteins and total free amino acids (TFAA) after the methods described by Lowry et al. (1951) and Lee and Takahashi (1966). Additionally, proline content was assessed according to the procedure adopted by Bates et al. (1973). The content of each metabolite in the studied plant was expressed in mg g^{-1} dry weight. For Secondary metabolites, the methanolic extraction was used according to Khan et al. (2014) and Salama and Al Rabiah (2015) to determine total soluble phenols (Free and cell wall-bound phenolics) and flavonoid content. Total soluble phenols were determined using Folin-Ciocalteu reagent according to Sampietro et al. (2009). Total flavonoid content determination was carried out against aluminum chloride according to the method described by Sampietro et al. (2009). Quercetin was used as a standard flavonoid for calibration curve.

Statistical Analysis

The reduction experiment was set up in a randomized complete block design with three replicates. Statistical analyses were performed by the GLM procedure using SAS software v. 9.0 (The SAS Institute Inc., Cary, NC, USA 2003). The plant parameters experiment was set up in a randomized complete block design (RCBD) with three replications for each variety and each treatment. One-way analysis of variance (ANOVA) was carried out to differentiate among treatments within each species using Proc Mixed of SAS package version 9.2 (SAS 2008) and means were compared by Duncan's multiple range test at 5% level of probability (Steel and Torrie 1980). Differences between citrus species within each treatment was performed using *T* test.

Results

The effect of three pesticides i.e., abamectin, thiamethoxam and acetamiprid in comparing with JA was evaluated based on several morphological and physiological parameters and on reducing the leafminer infestation of two citrus species seedlings (*C. aurantifolia* and *C. reticulata*).

Reduction of Leafminer Infestation

The leafminer reduction percentage of infestation on mandarin and lime using selected novel pesticides in comparison with JA are presented in Table 1. On the 1th day after treatment, neither the selected pesticides nor JA demonstrated any reduction. However, on the 3rd day after treatment, JA had the highest percentage of reduction on infestation on mandarin (77.08%) and lime (33.33%). On the 5th day after treatment, the same trend was observed on both species. Furthermore, on the 7th day after treatment, JA and abamectin displayed a 100% reduction in infestation in both species. Neither Thiamethoxam nor Acetamiprid achieved a 100% reduction in infestation by the 10th day following treatment.

Morphological Characteristics

Vegetative data represented in Table 2 show the growth rate during the growing period under conditions of applying JA and the other three pesticides. In lime, the growth rate (shoot length/root length) showed its highest values when it was exposed to JA, followed by acetamiprid; these values were significantly different than the other treatments. On the other hand, the mandarin seedlings gave the highest growth rate with the application of acetamiprid followed by abamectin and JA. (Table 2).

Table 1 Effect of selected novel pesticides and jasmonic acid on the reduction percentage of citrus leafminer on mandarin and lime seedlings

Days of treatment	Compounds	Reduction percentage (%)	
		On mandarin	On lime
0	Control	0.00	0.00
1	Control	0.00	0.00
	Abamectin 1.8% EC	0.00	0.00
	Thiamethoxam 25% WG	0.00	0.00
	Acetamiprid 20% SL	0.00	0.00
	Jasmonic acid	0.00	0.00
3	Control	0.00	0.00
	Abamectin 1.8% EC	8.33	0.00
	Thiamethoxam 25% WG	26.05	0.00
	Acetamiprid 20% SL	15.38	0.00
	Jasmonic Acid	77.08	33.33
5	Control	0.00	0.00
	Abamectin 1.8% EC	54.17	33.33
	Thiamethoxam 25% WG	35.29	0.00
	Acetamiprid 20% SL	47.12	20.00
	Jasmonic acid	83.63	77.08
7	Control	0.00	0.00
	Abamectin 1.8% EC	100	100
	Thiamethoxam 25% WG	67.65	0.00
	Acetamiprid 20% SL	69.78	60.00
	Jasmonic acid	100	100
10	Control	0.00	0.00
	Abamectin 1.8% EC	100	100
	Thiamethoxam 25% WG	67.65	50.00
	Acetamiprid 20% SL	78.85	60.00
	Jasmonic acid	100	100
LSD 0.05	Time	0.93	0.889
	Compounds	0.849	0.811
	Time × Compounds	2.077	1.985

LSD 0.05 least statistical difference at 5% probability

Concerning number of leaves/seedling, a positive significant effect was observed in lime seedlings by thiamethoxam treatment (36.33) followed by JA application (32.33), higher than that of the other treatments. On the other hand, the application of acetamiprid and JA recorded the highest number of leaves/seedling (45.67and 44.33, respectively) in mandarin.

The application of JA positively affected shoot fresh and dry biomass of mandarin seedlings, meanwhile, JA came second in root fresh and dry weights. However, the trend changed in lime, where Acetamiprid showed the highest

Table 2 Effect of selected novel pesticides and Jasmonic acid on growth rate, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and leave number per seedling of mandarin and lime seedlings under leafminer infestation

Species	Treatments	Traits					
		GR	SFW (mg)	SDW (mg)	RFW (mg)	RDW (mg)	LN
Mandarin	Control	$2.14 \pm 0.12^{c,B}$	$33.67 \pm 2.03^{a,A}$	$14.01 \pm 1.06^{a,A}$	$16.33 \pm 0.33^{cd,A}$	$9.42 \pm 0.76^{bc,A}$	$39.33 \pm 1.76^{bc,A}$
	Abamectin	$2.69 \pm 0.11^{b,B}$	$29.00 \pm 4.16^{a,A}$	$13.56 \pm 1.56^{a,A}$	$22.67 \pm 0.67^{a,A}$	$13.44 \pm 1.29^{a,A}$	$34.00 \pm 0.58^{c,A}$
	Thiamethoxam	$2.03 \pm 0.18^{c,B}$	$31.67 \pm 2.19^{a,A}$	$13.20 \pm 1.21^{a,A}$	$18.67 \pm 0.88^{bc,A}$	$8.58 \pm 1.27^{bc,A}$	$39.67 \pm 0.33^{abc,A}$
	Acetamiprid	$3.83 \pm 0.25^{a,B}$	$31.33 \pm 0.67^{a,A}$	$12.96 \pm 0.80^{a,A}$	$14.67 \pm 0.33^{d,A}$	$8.14 \pm 0.41^{c,A}$	$45.67 \pm 2.85^{a,A}$
	Jasmonic acid	$2.25 \pm 0.22^{c,B}$	$35.00 \pm 0.58^{a,A}$	$13.86 \pm 0.85^{a,A}$	$20.33 \pm 1.45^{ab,A}$	$11.9 \pm 0.94^{ab,A}$	$44.33 \pm 1.86^{ab,A}$
Lime	Control	$3.62 \pm 0.16^{b,A}$	$18.97 \pm 0.03^{b,B}$	$8.53 \pm 0.05^{b,B}$	$4.67 \pm 0.33^{bc,B}$	$2.66 \pm 0.14^{a,B}$	$28.67 \pm 0.67^{bc,B}$
	Abamectin	$4.59 \pm 0.09^{a,A}$	$15.47 \pm 0.28^{d,B}$	$7.51 \pm 0.11^{d,B}$	$4.33 \pm 0.33^{bc,B}$	$2.68 \pm 0.33^{a,B}$	$25.33 \pm 0.33^{c<b}$
	Thiamethoxam	$4.73 \pm 0.16^{a,A}$	$17.85 \pm 0.13^{c,B}$	$7.95 \pm 0.08^{c,B}$	$3.67 \pm 0.33^{c,B}$	$2.33 \pm 0.40^{a,B}$	$36.33 \pm 1.86^{a,A}$
	Acetamiprid	$4.62 \pm 0.13^{a,A}$	$20.76 \pm 0.06^{a,B}$	$9.79 \pm 0.04^{a,B}$	$6.00 \pm 0.00^{a,B}$	$3.41 \pm 0.13^{a,B}$	$28.00 \pm 1.15b^{c,B}$
	Jasmonic acid	$5.05 \pm 0.31^{a,A}$	$19.15 \pm 0.1^{b,B}$	$8.67 \pm 0.02^{b,B}$	$5.33 \pm 0.33^{ab,B}$	$3.12 \pm 0.43^{a,B}$	$32.33 \pm 2.33^{ab,B}$

Values are means ± standard error ($n = 3$), different lowercase letters in columns within the same species indicate significant difference, different uppercase letters indicate significant differences between cultivars within the same treatment

GR growth rate (shoot length/root length), *SFW* shoot fresh weight, *SDW* shoot dry weight, *RFW* root fresh weight, *RDW* root dry weight, *LN* leaf number per seedling

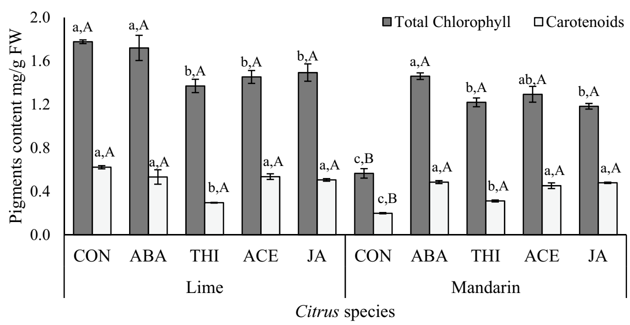

Fig. 1 Averages of total chlorophyll and carotenoids contents in lime and mandarin species under different treatments of pesticides and jasmonic acid. *CON* control, *ABA* Abamectin, *THI* Thiamethoxam, *ACE* Acetamiprid and *JA* Jasmonic acid. Different lowercase letters on the same colored bars within the same species indicate significant differences ($n = 3$), different uppercase letters indicate significant differences between the two species within the same treatment (*t* test, $\alpha = 0.05$)

values for shoot and root fresh and dry weights followed by JA (Table 2).

Physiological Characteristics

Total Pigments

The highest value of total pigments in lime seedlings were in the untreated plants (Fig. 1). Differently, the total pigment content reached its highest values in mandarin seedlings when they treated with abamectin, followed by acetamiprid and both of thiamethoxam and JA under leafminer infestation. In this regard, the chlorophyll a and b in untreated mandarin seedlings seemed to be significantly affected by infestation and showed a reduction of approximately half of that of the treated seedlings. Regarding carotenoids content, JA, acetamiprid and abamectin treatments showed a significant increase in mandarin seedling leaves. However, carotenoids took a different route in lime seedlings; where its highest content was in untreated plants, while the lowest was in the thiamethoxam-treated seedlings.

Osmolytes

Soluble Proteins

Mandarin seedlings treated with JA had a significantly greater amount of soluble proteins (54.83 mg g^{-1} DW) than seedlings in the other treatment groups. However, no significant differences were observed among the different treatments in lime (Fig. 2).

Amino acids

As shown in Fig. 2, the maximum content of total free amino acids (TFAA) in mandarin was under acetamiprid treatment (26.33 mg g^{-1} DW). Meanwhile, JA caused the maximum mean of TFAA in lime (32.06 mg g^{-1} DW) followed by equal values of acetamiprid and control (29.23 mg g^{-1} DW).

Proline Content

The free proline content of mandarin seedlings significantly ranged from 0.42 to 0.56 mg g^{-1} DW under abamectin and

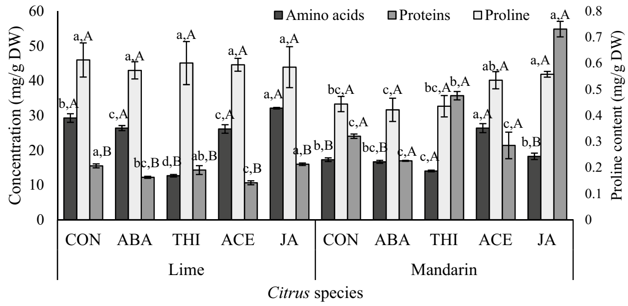

Fig. 2 Averages of amino acids and total proteins concentration (primary Y axis) and proline content (secondary Y axis) in lime and mandarin species under different treatments of pesticides and jasmonic acid. *CON* control, *ABA* Abamectin, *THI* Thiamethoxam, *ACE* Acetamiprid and *JA* Jasmonic acid. Different lowercase letters on the same colored bars within the same species indicate significant differences ($n=3$), different uppercase letters indicate significant differences between the two species within the same treatment (*t* test, $\alpha=0.05$)

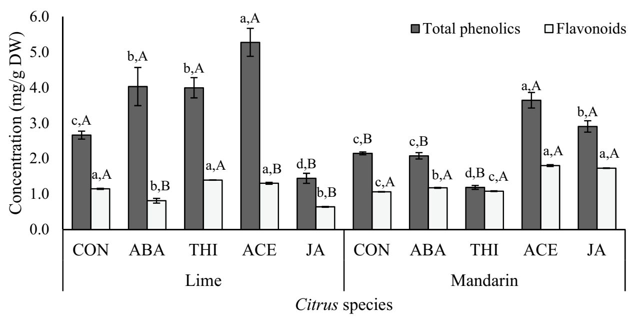

Fig. 3 Averages of total phenolics and flavonoids concentrations in lime and mandarin species under different treatments of pesticides and jasmonic acid. *CON* control, *ABA* Abamectin, *THI* Thiamethoxam, *ACE* Acetamiprid and *JA* Jasmonic acid. Different lowercase letters on the same colored bars within the same species indicate significant differences ($n=3$), different uppercase letters indicate significant differences between the two species within the same treatment (*t* test, $\alpha=0.05$)

JA treatments, respectively. In contrast, proline content in lime seedlings was similar among all treatments (Fig. 2).

Antioxidants

Total flavonoid contents in mandarin seedlings increased due to acetamiprid and JA (1.8 and 1.73 mg g^{-1} DW, respectively). Also, total phenolics showed the same response with a maximum mean of 3.64 mg g^{-1} DW under acetamiprid followed by 2.9 mg g^{-1} DW due to JA. On the other hand, thiamethoxam significantly decreased the total phenolics to 1.2 mg g^{-1} DW (Fig. 3). In lime, the total antioxidants made an arch under the insecticide treatments with the maximum total phenolics under acetamiprid with 5. 28 mg g^{-1} DW (Fig. 3).

The two citrus species used in the present study showed significant differences between each other, as resulted by *T*-test, in all morphological traits under all treatments with exception of leaf number under THI. In contrast, physiological traits differed in showing variation between the two species depending on trait and treatment. In this regard, both amino acid and protein content showed significant differences between species. In addition, the two species were significantly different under all treatments in total phenolics and flavonoids except under ACE and under control and THI, respectively. However, significant differences were observed between species in total chlorophyll and carotenoids only under control. Moreover, no significant differences were detected in proline across all treatments.

Discussion

The most perceived trends in the study were; first, that the most well-defined reduction percentage was noticed in mandarin rather than a lime, in which the later was more interactive with treatments specially JA. Second, JA was the most potent compound among the tested compounds which exhibited its effects from the 3rd day after treatment. Third, abamectin was the most powerful pesticide among the selected pesticides followed by thiamethoxam and acetamiprid.

In agreement to our findings, Knapp et al. (1995) and Jacas et al. (1997), reported that the differences in susceptibility between various citrus species seemed to be associated with the pattern of flushing of the trees. In our results, most of vegetative growth parameters were affected by leafminer infestation. For instance, the wounded leaves in mandarin seedlings crumpled and became chlorotic and then necrotic as a result of the leafminer infestation. Additionally, the number of leaves decreased in untreated plants due to leafminer effect. These results are in agreement with those of Pena et al. (1996), Knapp et al. (1993) and Patil (2013).

In the present study, JA had a significant effect on leafminer reduction rather than the other tested pesticides. However, there are lack of previous data on the effects of JA on citrus leafminer in mandarin and lime. There were certain studies on the effects of JA on different species of leafminer and other pests in other plant species. In this regard, Black et al. (2003) found that significantly fewer agromyzid leafminer adults emerged from celery plants sprayed with JA compared to control plants. In another study, Fouad and Khalil (2016) revealed that JA at 1.141 µM showed a good reduction (36.24 and 45.05% in two successive seasons) of the tomato leafminer, *Tuta absoluta* (Meyrick), on tomato plants under field conditions. Furthermore, results of the previous study on tomato showed that foliage spraying of chemical elicitors such as JA or methyl JA represents a practical way to manipulate induced resistance. Many other studies confirmed the effect of JA on different insect pests (Inbar et al. 1998; Thaler 1999a, b; Omer et al. 2000, 2001).

Maserti et al. (2011) reported that methyl jasmonate (MeJA) is playing a key role as a defense mechanism of citrus to insect/arthropod attack. Significant variations were observed on mite infestation in citrus leaves after the treatment of MeJA, reflected by a difference of 43 protein spots due to the treatment compared to untreated plants.

However, other studies indicated that plant volatiles induced by JA repelled aphids from plants in field trials which indicated that JA induced pest resistance in wheat and may involve in resistance mechanism of wheat against insect herbivores (El-Wakeil et al. 2010; Slesak et al. 2001). After facing abiotic and/or biotic stresses, specific mechanisms are stimulated including; ion channels and kinase cascades (Fraire-Velázquez et al. 2011), accumulation of reactive oxygen species (ROS) (Laloi et al. 2004) and some phytohormones like JA (Spoel and Dong 2008). In addition, a reprogramming of the genetic mechanism results occurred in sufficient defense reactions to elevate plant tolerance to reduce the stress caused damages (Fujita et al. 2006). This information is in accordance with our results, as JA treatment enhanced the most vegetative growth and physiological parameters in mandarin seedlings. This might be due to the phytohormone nature of JA that allows plants to face attack by insects (Chamberlainet al. 2000).

Jasmonic acid is an essential signaling molecule which coordinates many cellular activities. In the presence of JA or its bioactive derivatives, some proteins that serve in switch on/off signaling are degraded, making some transcription factors free to involve in expression of specific sets of JA-responsive genes, thereby promoting physiological activity (Memelink 2009).

In the present study, an efficient role of JA was observed on some primary metabolites in *Citrus aurantifolia*. Soluble proteins and total free amino acids were negatively affected by the leafminer equally in the presence or absence of insecticides. However, these metabolites were improved under JA treatment. On the contrary, proline and phenolic compounds including flavonoids were reduced under JA treatment. These results correspond well with data reported by Weidhase et al. (1987) who found that JA application induced accumulation of several proteins, which involved in the degradation of rubisco protein. In the present study, *Citrus reticulata* showed different responses that, most of investigated metabolites were increased under JA. Gundlach et al. (1992) found that JA induced accumulation of secondary metabolites and de novo transcription of defense-related genes, such as phenylalanine ammonia lyase (PAL), in plant cell cultures. This reaction is catalyzed by PAL and is commonly regarded as a key step in the biosynthesis of phenolic compounds (Rösler et al. 1997). These findings demonstrated the integral role of JA in a general signal transduction system regulating inducible defense responses in plants which endorsed the results of the present study.

In conclusion, the biological activity of JA observed in our study gives a realistic reason to integrate it in the pest management programs as an alternative product for controlling *P. citrella*. However, further investigation should be conducted to demonstrate its effect on natural enemies, honeybees, and humans. Finally, these control strategies are important and need to be included in making pest management related decisions. Implementation of IPM (Integrated Pest Management) or ecological based pest management relies on the knowledge that stability in biological systems relies on feedback between organisms.

Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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