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Research Article

Nematicidal effect of abamectin, boron, chitosan, hydrogen peroxide and *Bacillus thuringiensis* against citrus nematode on Valencia orange trees

MAM El-Saedy[1], Sandy E Hammad[2] and Sherin FA Awd Allah[2]*

[1]Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt
[2]Nematology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt

More Information

***Address for Correspondence:** Sherin Fadel Awd Allah, Nematology Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt, Tel: (+20) 11-5823-5078; Email: sherinfadel@gmail.com

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Abstract

The nematicidal efficacy of abamectin, boron, chitosan, hydrogen peroxide, *Bacillus thuringiensis* and oxamyl 24% SL against citrus nematode, *Tylenchulus semipenetrans* were examined on Valencia orange trees under field condition for two successive seasons (2017 and 2018). The experiment was conducted in a Valencia orange orchard infested with citrus nematode at Nubaria, El-Behera governorate, Egypt. The obtained results showed that all the tested treatments reduced nematode final population (P_f) and reproduction factor (R_f) compared with that obtained from the untreated trees. The highest percentages of P_f reductions (74.5-83.4 %) and (70%-82%) were recorded with oxamyl, boron, abamectin, chitosan and H_2O_2 in the 1st and the 2nd tested seasons, respectively. Whereas, *B. thuringiensis* had the least nematode P_f reduction with 60.7 and 55.8% in the 1st and 2nd seasons, respectively. Additionally, all treatments significantly improved orange yield (30.9-83.2% increase), physical fruit parameters and orange juice properties. The highest orange yield increase (83.2%) was recorded with boron treatment followed by oxamyl (70.3%). Also, boron increased total soluble solids (TSS) by 13.6%, volume of orange juice (36.4%) and vitamin C (19.7%) and decreased juice acidity (A) by (16.7%). It is concluded that abamectin, boron and the other tested compounds have potential as non-chemical control strategy tools in managing the citrus nematode. These bioagents reduced the amount of traditional chemical nematicides and are considered to be environmentally safe.

Introduction

Egypt is the sixth largest orange producer and the second largest exporter in the world. Oranges represent the largest cultivated area of all citrus varieties in Egypt. Several orange varieties are produced in Egypt but Valencia and Navel orange are the main export varieties whereas others are more for domestic consumption. Orange production, in Egypt, has increased in the last years to meet the demand from local and foreign markets [1]. Many pathogens including plant-parasitic nematodes infect citrus and affect their quality and quantity [2]. The most widespread plant-parasitic nematodes in citrus orchard are *Tylenchulus semipenetrans*, *Radopholus similis*, *Pratylenchus coffee* and *Meloidogyne* spp. which cause significant economic losses worldwide [3].

Citrus nematode, *T. semipenetrans* is one of the major nematode pests that can induce considerable losses in citrus yield worldwide [4] and in Egypt [5,6].

Management of citrus nematode is very important and need more efforts using different tactics, e.g., site selection, using non-infected nursery stocks, using at least one post plant control tactic, and careful management of other elements of the environment that may stress the trees. Nowadays, there has been tremendous increase in public alertness on environmental pollution and climate change associated with pesticide toxicity and residues. Therefore, alternative methods for managing citrus nematode are highly encouraged and recommended.

Abamectin is a natural fermentation product of *Streptomyces avermitilis*. It is a mix of avermactin B1a (80%) and avermactin B1b (20%), which represent a new generation of pesticides that exhibit good efficacy against phytonematodes [7-10]. Abamectin applied as seed treatment, soil application and as root dipping against phytonematodes, e.g., *Meloidogyne incognita*, *M. arenaria*, *M. javenica*, *T.*

semipenetrans and *Rotylenchulus reniformis.* It show good efficacy against nematodes and considered a new trend in the field of nematodes management [6,11-13].

Boron is one of the mineral nutrients which considered a micronutrient essential for plant growth as it plays a role in plant metabolism [14]. It also plays a major role in disease resistance and can improve crop yield and quality of cultivated crops [15]. El-Nagdi and Youssef, [16] and El-Saedy, et al. [17], reported that grape plants treated with boric acid improved grape yield and reduced root galls and egg masses numbers of root-knot nematode till harvest time.

Similarly, chitin and its derivatives, chitosan and oligochitosan are the well-known biocontrol and environmental agents because of their nontoxic, biodegradable and biocompatible properties [18,19]. Chitosan has valuable nematistatic and nematicidal action used for disease management applications in agriculture. Many investigations confirmed the activity of chitosan against plant-parasitic nematodes [20-22].

Moreover, one of the novel trends in management of plant-parasitic nematodes is via hydrogen peroxide (H_2O_2) application. Ibrahim, et al. [23], studied the nematicidal properties of H_2O_2 at the concentrations of 100-2000 ppm against *M. incognita* and the potential for systemic resistance induction in sugar-beet. Results revealed that all tested concentrations were found to have nematicidal activity against nematode infection and improved plant growth parameters.

Also, the use of bio-management agents such as *Bacillus thuringiensis* is one of the untraditional microbes which have been reported to control different plant-parasitic nematodes [24-27].

Therefore, the present study was conducted in order to evaluate the efficacy of the five compounds; abamectin, boron, chitosan, H_2O_2 and *B. thuringiensis* as new alternative safe compounds for managing *T. semipenetrans* infesting orange trees soils and their effects on yield and fruit quality under field conditions.

Materials and Methods

The nematicidal effects of the five compounds namely, abamectin, boron, chitosan, H_2O_2, *Bacillus thuringiensis* and the chemical nematicide oxamyl 24% SL were examined against citrus nematode (*Tylenchulus semipenetrans,* Cobb) on orange trees (*Citrus sinensis* L.) cv. Valencia, under field conditions during 2017 and 2018 growing seasons in a private orchard infested with citrus nematode at Nubaria, El-Behera governorate, Egypt. Thirty five trees were selected randomly and assigned to a randomized complete blocks design (RCBD) with five replicates. The selected trees were nearly uniform in vigor and size and spaced at 4 × 5 m apart (200 trees/feddan). The applied treatments were carried out twice per season, at a depth of 10 cm from soil surface under dripper line. The 1st application was done at the begging of the experiment (in April) and the 2nd application after 3 months from the 1st application (in August). Fertilization, irrigation and other agricultural practices were applied according orange recommendations.

Orange trees were divided into 7 groups (treatments) of 5 trees each. The 1st group was treated with the commercial compound Tervigo® (Abamectin 2% SC) produced by Syngenta Agro Egypt, at the rate of 15 ml/ tree (recommended dose, 3 L/feddan). The 2nd group was treated with Maxboro B® (Boron), produced by Egyptian International Company for Agricultural and Industrial Development, at the rate of 10 g/ tree. The 3rd group was treated with Matador® (Chitosan 2.5 %, produced by Egy Chem, Pure Farma Company), at the rate of 20 ml/ tree (recommended dose, 4 L/feddan). The 4th group was treated with Huwa. San® (H_2O_2 50%), produced by Roam Chemie Company, NV Belgium, El-Ghonemy Group, at the rate of 20 ml / tree. The 5th group was treated with Agree® 50 WG contained *Bacillus thuringiensis* subsp. *aizawai*, strain GC-91 (spores and active toxins), produced by Certis Company, USA, at the rate of 20 g / tree. The 6th group was treated with Vydate® L (Oxamyl 24% SL) produced by DuPont™ Company, at the rate of 15 ml/tree (recommended dose, 3 L/feddan). The 7th group was left untreated to serve as a control treatment.

The population of *T. semipenetrans* in soil and roots were estimated during the 1st and 2nd growing seasons directly before application (P_i) and after treatments application (Pf) at the harvest time. Samples from each replicate (approximately 1 kg soil and 30 g of root/ replicate) were collected from a depth of 20-30 cm of the orange rhizosphere. About 250 g soil from each sample was processed by sieving and decanting methods [28] and roots from the same soil sample were gently washed free of soil and about 10 g per tree was cut into 2 cm long pieces, placed in Baermann plates with tap water and incubated under laboratory conditions for two days to extract nematode juveniles [29]. After that, the roots segments were blended for 3 minutes to extract females from roots [30]. The reproduction factor (R_f) was calculated for each treatment by dividing P_f/P_i [31]. Reduction percentages (R) of *T. semipenetrans* P_f in soil and roots were determined and calculated using the formula of Mulla, et al. [32], as follow:

$$R = \text{reduction }\% = 100 - [(C1/ T1) \times (T2/C2) \times 100].$$

Where: C1 = pre-treatment nematode density in control; C2=post-treatment nematode density in control habitat; T1 = pre-treatment nematode density in treatment habitat; T2 = post-treatment nematode density in treatment habitat.

Relative nematicide efficacy % (RNE) was calculated as follow:

$$RNA = [1-(\text{Treatment} - \text{Nematicide})/\text{Treatment}] \times 100.$$

Fruit quality and yield

At harvest time, at March, ten fruits from each tree were randomly selected to assess the physical and chemical characteristics of fruits and orange juice for both experimental seasons. Average fruit weight (g), yield/tree (kg/tree), fruit length (cm), fruit diameter (cm) and peel thickness (cm) were recorded. Juice volume, total soluble solids % (TSS), acidity % (A), TSS/A ratio and vitamin C content % were also recorded. TSS in fruit juice was recorded by using hand refractometer. Acidity was measured, as citric acid percent, by titration using 0.1 N sodium hydroxide. Vitamin C content % in orange juice was estimated by titration with 2, 6 dichlorophenol endophenol dye [33].

Statistical analysis

Analysis of variance (ANOVA) was carried out on the nematode final population (P_f), the reproduction factor (R_f) of citrus nematode, orange yield, physical parameters of orange fruits and chemical properties of orange juice by using the statistical analysis system (SAS) software computer program 6.03 Edition-6th [34]. Means of treatments were compared with the value of revised LSD at 5% level of probability.

Results and Discussion

Data presented in table 1 indicated that all the tested applied compound treatments and oxamyl reduced the nematode population (P_f) of *T. semipenetrans* infected Valencia orange trees in the 2017 and 2018 growing seasons under field conditions when compared with that obtained from the untreated trees. The highest effective treatments were oxamyl, boron, abamectin, chitosan and H_2O_2 which reduced nematode P_f by 74.5-83.4 % reduction in the 1st season. Similar results were obtained, in the 2nd season. Oxamyl, abamectin, boron and H_2O_2 treatments showed 72.7-82.0 % reduction followed by chitosan with 70 % reduction. Whereas, *B. thuringiensis* had the least nematode reduction P_f with 60.7% and 55.8% during the 1st and 2nd seasons, respectively.

According to relative nematicidal efficacy % (RNE), the obtained results indicated that abamectin and boron treatments had the highest RNE, in both seasons, with 83.9 and 83.3% reduction, in the 1st season and 93.9 and 84.7 % in the 2nd season followed by H_2O_2 and chitosan with 70.7 and 67.1 % in the 1st season and 67.8 and 62.1 % in the 2nd season, respectively. Meanwhile, *B. thuringiensis* gave the lowest RNE in both seasons with 41.6 and 40.1 % in the 1st and 2nd seasons, respectively (Table 1).

Many reports showed that abamectin was able to reduce plant-parasitic nematode populations on several crops [10,13, 27,35,36]. Our obtained result of the present work was confirmed by El-Nagdi, et al. [25]. They found that abamectin reduced the soil population of *T. semipenetrans* in mandarins up to 86% and 93% in the 1st and 2nd seasons, respectively. Abamectin is a new generation of nematicides that considered a new tool in the field of plant parasitic nematodes management. Abamectin have a unique mode of action in comparison with traditional nematicides. It is targeted the δ-amino-butyric acid in nematode receptors causing increments in the permeability of chloride ions which finally causing death [37]. The effect of abamectin against *T. semipenetrans* in our study may be attributed to the strong adsorption of abamectin on soil particles which help abamectin to stay in direct contact for more time with the nematode population [38-40]. Furthermore, abamectin causes immobility in second stage juveniles (J2) of root knot nematode and this may correlate with a reduction in oxygen uptake [27].

As well, boron element used nowadays as a control agent against phytonematodes and some studies clarified that boron plays a real role in reducing root-knot nematode densities in soil and roots [17].

Similarly, chitosan has been used to encourage the immunity of plants and to protect plants from phytonematodes and other pathogens. Aboud, et al. [20] reported that

Table 1: Effect of abamectin, boron, chitosan, H_2O_2, *B. thuringiensis* & oxamyl 24% SL applications on *Tylenchulus semipenetrans* infecting orange trees under field condition during 2017 and 2018 seasons.

Treatment	Pi	P_f	*R*	R_f	*RNE*
		1st season			
Abamectin	10658	9254 c	*79.7*	0.9 cd	*83.9*
Boron	11428	9324 c	*80.9*	0.8 cd	*83.3*
Chitosan	10624	11572 c	*74.5*	1.2 c	*67.1*
H_2O_2	10708	10989 c	*76.0*	1.1 c	*70.7*
Bt	11144	18692 b	*60.7*	1.7 b	*41.6*
Oxamyl	10952	7768 c	*83.4*	0.7 d	-
Untreated	10724	45772 a	*0.00*	4.3 a	-
		2nd season			
Abamectin	11038	7654 d	*80.3*	0.7 cd	*93.9*
Boron	11768	8484 cd	*79.5*	0.7 cd	*84.7*
Chitosan	10964	11572 c	*70.0*	1.2 bc	*62.1*
H_2O_2	11068	10609 cd	*72.7*	1.0 cd	*67.8*
Bt	11544	17932 b	*55.8*	1.6 b	*40.1*
Oxamyl	11352	7188 d	*82.0*	0.6 d	-
Untreated	10004	35169 a	*0.00*	3.8 a	-

Data are average of 5 replicates; values within each column, in each season, followed by the same letter(s) are not significantly different at $p = 0.05$; *Bt*: *Bacillus thuringiensis*; P_i: nematode initial population of J_2/ kg soil + J_2; and females/10 g root fresh weight; P_f: nematode final population of J_2/kg soil + J_2 and females/10 g root fresh weight; *R*: reduction % was calculated using Mulla formula; ($R = 100 - [(C1/ T1) \times (T2/C2) \times 100]$); R_f= nematode reproduction factor = (P_f/P_i), *RNE*: Relative Nematicidal Efficacy = [1-(Treatment – Nematicide) / Treatment] ×100.

chitosan reduced phytonematode invasion and affected the morphophysiological and population parameters of *M. incognita*. The efficacy of chitosan treatment possibly refers to encourage systemic acquired resistance in plants and may serve as a natural nematicide [21].

Likewise, the bioagent, *B. thuringiensis* is one of the untraditional microbes used against different genera of plant nematodes especially root-knot nematodes. *B. thuringiensis* produce parasporal crystalline proteinaceous inclusions or δ - endotoxins which are toxic to parasitic nematodes [24,25,27,41-43]. Our results confirmed the nematicidal potential of *B. thuringiensis* against *T.* semipenetrans and are in agreement with results of El-Nagdi, et al. [25], who found that the best phytonematde control was achieved with using the highest rate of *B. thuringiensis*, in balady mandarin orchard than the low rate.

Similar to the present results, many previous studies recorded the killing ability of hydrogen peroxide (H_2O_2) produced by some microorganisms or applied exogenously on nematodes [44-46]. It considered a stress signal in plants, mediating adaptive responses to various stresses. Exposure to various abiotic and biotic stresses results in the accumulation of H_2O_2 [47]. The effect of H_2O_2 against citrus nematodes in the current study may be refer to that H_2O_2 can induce genes expression that involved in antioxidant defense [48,49].

The effect of abamectin, boron, chitosan, H_2O_2, *B. thuringiensis* and oxamyl 24% SL applications on Valencia orange fruit yield was shown in table 2. Results showed that all the tested treatments increased orange yield by 30.9-83.2 %. The highest yield increase (83.2 %) was recorded with boron treatment application followed by oxamyl (70.3%) then abamectin and *B. thuringiensis* with 45.4 and 42.5 % increase, respectively. The lowest yield increase of 30.9 and 35% were recorded with H_2O_2 and chitosan treatment applications, respectively.

Moreover, data in table 2 showed that all treatments increased weight, length and diameter of fruits by 20.1-37.6, 26.8-44.6 and 25% - 40.4%, respectively. The highest fruit weight increase percentages of 34% and 37.6% were recorded with boron and oxamyl treatments followed by the other treatment applications with 20.1% - 28.6% increase. Also, oxamyl were recorded the highest increase of fruit length and diameter by 44.6% and 40.4%, consecutively. Whereas, oxamyl, abamectin and *B. thuringiensis* increased orange fruit peel thickness by 100% - 150% increase.

However, at the harvest time, in both seasons, all the tested treatments significantly increased the orange juice volume and its chemical properties, e.g., total soluble salts (TSS), TSS/A (acidity) ratio and Vitamin C and decreased juice acidity (A) compared to the untreated trees fruits (Table 3).

Data also showed that oxamyl, boron and abamectin treatment applications increased juice volume by 47.3, 36.4% and 29.4% compared with the untreated tree fruits, respectively. Similarly, TSS was increased with boron and chitosan treatments by 13.6% and 11.2%, respectively followed by *B. thuringiensis* with 6.6% increase. TSS/A ratio was also increased with all treatment applications. The highest TSS/A increase of 35.3% and 26.3% was recorded with boron and chitosan treatments, respectively followed by the other tested treatments with 13.2% - 19% increase. On the other hand, acidity (A) of orange juice was decreased with all treatments by 8% - 16.7%. The highest decrease of 16.7 % was recorded with boron treatment compared with untreated trees fruits (Table 3). Data indicated also that Vitamin C contents % was increased with boron and chitosan treatments with 19.7 and 15.7% compared with the untreated fruits, consecutively.

Results of the present study are in the same trend with those reported by El-Nagdi, et al. [25], D'Errico, et al. [50], and El-Tanany, et al. [6]. Our results are in agreement with those given by El-Nagdi, et al, [25], who found that the *B. thuringiensis* (Agerin®) and abamectin (Vertemic®) significantly suppressed the population of *T. semipenetrans* and improved nutritional status. They also found that these treatments increased fruit yield expressed as fruit number or weight compared to untreated control and markedly improved fruit quality of mandarin trees. It was concluded that these two bio-agents also have low associated production costs and are considered to be environmentally safe. Also, D'Errico, et al. [50] reported that abamectin effective *in vivo* for controlling *M. incognita*-infected tomatoes and they also found that no visual phytotoxicity symptoms were detected for the products.

Similarly, our results regarding growth parameters and juice properties are in conformity with those obtained by

Table 2: Effect of abamectin, boron, chitosan, H_2O_2, *B. thuringiensis* & oxamyl 24% SL applications on average of orange yield and physical characteristics of fruits and increase % during 2017 and 2018 seasons.

Treatment	Yield/tree (kg)	I	Fruit							
			Weight (g)	I	Length (cm)	I	Diameter (cm)	I	Peel thickness (cm)	I
Abamectin	71.1 c	*45.4*	178.8 bc	*28.6*	7.4 bc	*32.1*	6.7 b	*28.8*	0.4 ab	*100.0*
Boron	89.6 a	*83.2*	191.3 a	*37.6*	7.4 bc	*32.1*	6.7 b	*28.8*	0.3 bc	-
Chitosan	66.0 d	*35.0*	167.5 c	*20.5*	7.4 bc	*32.1*	6.6 b	*26.9*	0.3 bc	-
H_2O_2	64.0 d	*30.9*	167.0 c	*20.1*	7.1 c	*26.8*	6.5 b	*25.0*	0.3 bc	-
Bt	69.7 c	*42.5*	170.3 c	*22.5*	7.5 b	*33.9*	6.7 b	*28.8*	0.4 ab	*100.0*
Oxamyl	83.3 b	*70.3*	186.3 ab	*34.0*	8.1 a	*44.6*	7.3 a	*40.4*	0.5 a	*150.0*
Untreated	48.9 e	-	139.0 d	-	5.6 d	-	5.2 c	-	0.2 c	-

Means within each column with the same letter(s) are not significantly different at p = 0.05, *Bt*: *Bacillus thuringiensis*; *I:* Increase; %: [(Treatment-Control)/Control] ×100.

Table 3: Effect of abamectin, boron, chitosan, H_2O_2, *B. thuringiensis* & oxamyl 24% SL applications on average of orange juice volume, its chemical properties and change % during 2017 and 2018 seasons.

Treatment	Juice volume (ml)	*I*	TSS %	*I*	Acidity % (A)	*D*	TSS/A	*I*	Vitamin C (mg/100 ml)	*I*
Abamectin	92.5 ab	*29.4*	10.4 bcd	-	1.3 b	*9.4*	8.3 c	*15.7*	45.1 bc	-
Boron	97.5 ab	*36.4*	11.1 a	*13.6*	1.1 c	*16.7*	9.7 a	*35.3*	50.4 a	*19.7*
Chitosan	83.0 bc	-	10.9 ab	*11.2*	1.2 bc	*12.4*	9.0 ab	*26.3*	48.7 ab	*15.7*
H_2O_2	81.3 bc	-	10.1 cd	-	1.2 bc	*10.9*	8.2 c	*15.3*	44.1 bc	-
Bt	85.8 bc	-	10.5 bc	*6.6*	1.2 bc	*10.9*	8.5 bc	*19.0*	45.3 abc	-
Oxamyl	105.3 a	*47.3*	10.3 cd	-	1.3 b	*8.0*	8.1 c	*13.2*	46.9 abc	-
Control	71.5 c	-	9.8 d	-	1.4 a	-	7.1 d	-	42.1 c	-

Means within each column with the same letter (s) are not significantly different at level. p = 0.05, *Bt*: *Bacillus thuringiensis*; TSS: Total Soluble Solids; A: Acidity; *I*: Increase; %: [(Treatment-Control)/Control] ×100; *D*: Decrease; %: [(Control-Treatment)/Control] ×100.

Radwan, [51], Ibrahim, et al. [52], Khan, et al. [53], Khalil, et al. [54], Khalil, [9], Saad, et al. [8] and Muzhandu, et al. [39] and El-Tanany, et al. [6]. They reported that the detected improvement in trees nutrient status followed by *B. thuringiensis* and abamectin treatment application might be explained by faster absorption of nutrients via the existence of new normal roots. Meanwhile, El-Nagdi, et al. [25], found that both *B. thuringiensis* and abamectin significantly increased TSS and ascorbic acid content of mandarin juice. They also found that *B. thuringiensis* significantly increased shoot system indices. Moreover, abamectin enhanced the shoot and root length and fresh weight of tomato plants.

El-Tanany, et al. [6] reported that soil application with oxamyl increased all fruit physical properties of Valencia orange fruits. Conversely, peel thickness had the lowest values with oxamyl treatment. They also found that oxamyl and/or abamectin were the efficient treatments to reduce the citrus nematode population densities in soil and significantly enhanced fruit yield expressed as weights or numbers. Moreover, oxamyl treatment improved all physical fruit properties and fruit juice content of TSS, as well as increased leaf macro nutrient content. While, abamectin increased juice acidity and vitamin C.

In accordance with our data, some researchers indicated that application of mineral fertilizer decreasing the need for chemical control in some cases. Partially, applying fertilizer can offset nematode-induced damage by stimulating plant development [55]. Likewise, Santana-Gomes, et al. [56] reported that phytonematodes are among the pathogens that can be affected by plant nutrition. The balanced application between macro and micronutrients is the best way of ensuring that the crop is able to tolerate the damage caused by phytopathogens.

Boron is one of the essential micronutrient required for normal growth of most crops. It is necessary for growth of pollen tubes during pollination and increases pollen grain germination and pollen tube elongation which increase the percentages of the fruit set and consequently the yield [57]. Moreover, Edward Raja, [58] found that micronutrients (e.g. zinc and boron) that used in citrus fruit crop is important for growth, yield and quality. The enhancement of growth following the treatment with boron may be attributed to possible effects in plays an important role in movement of natural hormones and encouragement of both cell division and cell enlargement [17].

Our finding is in agreement with earlier observations made by many scientists who confirmed that chitosan enhanced the growth parameters of plants. Chitosan has been used to increase the plant product, to stimulate the immunity of plants and to protect plants from microorganisms infections. A positive effect of chitosan was observed on the growth of roots, shoots, and leaves of several crop plants [21,59-61]. Khalil and Badawy, [21] showed that chitosan treatment improved the growth of tomato seedling compared with the check plants.

Generally, results demonstrated that abamectin, boron and the other tested compounds have a significant nematicidal activity against citrus nematode. In addition, these applications significantly increased fruit yield and markedly improved fruit quality of orange trees compared to untreated trees control. Moreover, all the applied compounds considered promising environmentally safe alternatives to manage citrus nematode and reducing the amount of traditional chemical nematicides.

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