

Efficacy of Three Bio-pesticides and Oxamyl Against Citrus Nematode (*Tylenchulus semipenetrans*) and on Productivity of Washington Navel Orange Trees

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A STUDY was conducted during two periods (March and September), for two successive growing seasons (2016 and 2017) to determine the impacts of three bio-pesticides products namely, abamectin (Tervigo®), azadirachtin (Achook®) and a mixture of *Trichoderma album* + *Bacillus megaterium* (Bio Zeid® + Bio Arc®), as well as oxamyl (Vydate®), on the number of citrus nematode populations (*Tylenchulus semipenetrans*), on 10-year-old Washington navel orange trees (*Citrus sinensis*, Osbeck) budded on sour orange rootstock grown in sandy soil. This work was carried out in private farm located at Hamour village, Boustan area, Behera governorate. The obtained results proved that all treatments reduced nematode populations during both seasons from 63.04 to 79.66% and from 69.53 to 89.87%, respectively. Meanwhile, the fruit yield during both seasons was increased significantly with all applied treatments, especially oxamyl treatment. The most of physical fruit properties were improved. Chemical fruit properties were markedly improved, especially with abamectin treatment which increased vitamin C. Moreover, oxamyl and azadirachtin significantly raised total solid soluble content in the fruit juice.

On the other hand, the leaf area was augmented through both seasons in all treatments. The leaf minerals composition also was increased with all applied treatments, except for calcium which significantly decreased with the mixture of *T. album* + *B. megaterium* during both seasons of study.

Keywords: *Tylenchulus semipenetrans*, Washington Navel orange, Fruit yield, fruit properties, Mineral composition and Bio-pesticides.

Introduction

Citrus especially oranges, play an important role in the agricultural production and economy. Orange crop in Egypt is considered as the most fruit production destined for exportation. Unfortunately, the citrus orchards are severally infested by numerous species of parasitic nematodes associated with the citrus rhizosphere system in all different soil texture types.

The most widespread genera of parasitic nematodes in citrus orchard is *Tylenchulus semipenetrans*. This serious pest cause significant economic losses. Most studies estimated yield losses of Navel orange trees according to *T. semipenetrans* to be in the range of 10 – 30% depending on the level of infestation. Moreover, *T. semipenetrans* nematode is a dominant

pathogenic species and the most important in most citrus regions in the Egyptian agricultural soils. It is well known that young trees appeared to poorly grow when planted into nematode – infested soil (Duncan and Cohn, 1990 and Verrdejo-Lucas and McKenry, 2004). In Egypt, the control of plant parasitic nematodes is recommended when nematode count reaches to 7000-9000 juveniles / kg soil sample.

The number of females infesting roots is the best indicator for seasonal activity of *Tylenchulus semipenetrans* (Sorribas et al., 2000) and also for evaluating the efficacy of nematicides (Hamid et al., 1988). The present study was conducted in order to evaluate the influence of three bio-pesticides namely, abamectin, azadirachtin and a mixture of two antagonistic microbes

(*Trichoderma album* + *Bacillus megaterium*), as well as oxamyl as standard nematicide against *T. semipenetrans* nematodes in relation to study their effects on the productivity of Washington navel orange trees.

Materials and Methods

The present investigation was conducted in a private orchard located at Hamour village, Boustan area, Behera governorate, Egypt during the 2016 and 2017 seasons on ten years old Washington Navel orange trees (*Citrus sinensis*, Osbeck) budded on sour orange rootstock grown in sandy soil. The main goal of this study was to evaluate the impact of some chemical and natural nematicides on yield, fruit quality, leaf area, and leaf mineral composition.

Mature twenty trees of Washington navel orange trees were used in this study, the selected trees were nearly uniform in vigor and size and spaced at 4 X 5 m apart (200 trees / Fed.), and received the same cultural practices usually adopted for this area according to the recommendation of Horticulture Research Institute, Ministry of Agriculture, Egypt. Surface drip irrigation system was used in the orchard. Trees received 6500 m³ of water per feddan per year distributed through the whole year. The fertigation program included 51 Kg nitrogen as ammonium sulphate and 67 kg ammonium nitrate, 62.5 kg potassium as potassium sulphate, 50 kg magnesium as magnesium sulphate and 32kg orthophosphoric acid per feddan per year distributed from February till October.

Besides, the trees were also sprayed with crystalon (19: 19: 19 N P K) only once, at bud sprouting in March at the rate of 4Kg / 600 Liters water. In addition, trees were sprayed with (250gm chelated iron 12%, 250gm chelated manganese 14%, 200gm chelated zinc 13% and 750 cm³ amino acid solution / 600 Liters of water), two times, at bud sprouting in March and during fruit growth, in August.

The applied treatments are presented as follows:

1. Control (untreated trees).
2. Vydate® 24% SL "Oxamyl", used at the rate of 3 liters / Feddan (15cm³ / tree).
3. Tervigo® 2% SC "Abamectin", used at the rate of 2.5 liters / feddan (12.5 cm³ / tree).
4. Achook® 0.15% EC " Azadirachtin", an insecticide used at the rate of 750 cm³ / feddan (3.75 cm³ / tree).

5. A mixture of Bio Zeid® 2.5% WP "contains 1 x 10⁷ cell / g of fungus, *Trichoderma album* and Bio Arc® 6% WP "contains 25 x 10⁶ cell / g of bacterium, *Bacillus megaterium* which used at the rate of 10 Kg / Feddan (50 g / tree).

The applied compounds were obtained from local markets at El-Nubarya zone. The treatments were applied under dripper at depth of 5 – 10 cm from soil surface. The applied treatments were carried out twice per season at March and September. Each treatment was replicated 4 times. The populations of citrus nematode (*Tylenchulus semipenetrans*), were recorded throughout soil samples of each replicate which were taken directly before application and after the treatments by a month for both seasons 2016 and 2017.

The soil samples were collected according to Barker (1985). Three sub-samples were collected from 10 to 30 cm depth of each replicate to form a composite sample of approximately 2 kg, which was then thoroughly mixed. The second stage juveniles (J2) of *Tylenchulus semipenetrans* were extracted from a 200g sub-sample soil of each replicate, using the sieving and Baermann plates' technique (Ayoub, 1980), and counted under a stereomicroscope. The reduction in the nematode population density expressed as a percentage was calculated at the termination of the experiment according to Henderson and Tilton's equation (1955) as follow:

$$\text{Reduction (\%)} = \left\{ 1 - \left(\frac{a}{b} \times \frac{c}{d} \right) \right\} \times 100$$

Where:

- a = Population density in treatment after application
- b = Population density in treatment before application
- c = Population density in check untreated (control) before application
- d = Population density in check untreated after application

leaf analysis :

Leaf samples of 20 – 30 full expanded mature leaves was taken from non – fruiting shoots of the previous spring growth flushes. Leaves were collected from all over the circumference of each tree. Leaf samples were taken at random on October, 22 of each season. Leaf samples were washed with tap water, rinsed three times in distilled water, oven dried at 65 – 70 C° to a constant weight and grounded to 20 mesh size. Leaf samples were

used for total nitrogen, phosphorus, potassium, calcium and magnesium determination. A sample of 0.3g of the ground dry material was digested with sulphuric acid and hydrogen peroxide according to Evenhuis and DeWaard (1980). In digested solution of each sample, total nitrogen, phosphorus were colorimetrically determined according to Evenhuis (1976) and Murphy and Riky (1962), respectively. Potassium was measured against a standard using carlzeis Jena flam photometer. Calcium and magnesium were determined by Vesenate method using eirochrom black T and ammonium purpurate indicator for calcium plus magnesium and calcium, respectively (Cheng and bray, 1951).

Leaf area :

The Leaf area of each experimental tree was measured using a planimeter by tracing the outline of eight (8) representative leaves.

Fruit number and Yield :

During October of 2016 and 2017 seasons, fruit number of each tree for both experimental seasons was recorded on October, 22. Average fruit weight of 10 mature fruits randomly selected was estimated for each tree. At harvest fruit yield of each experimental tree was then calculated as Kg per tree and ton per feddan.

Fruit quality:

At harvest time (on December, 12) of both seasons, average fruit length, diameter, fruit and pulp weights, rind weight, its thickness and juice volume of eight fruits, randomly taken from each tree, were determined. Total soluble solids content (TSS) in fruit juice was recorded by hand refractometer. Acidity as citric acid percent was determined by titration using 0.1 N sodium hydroxide. Vitamin C content in the juice was estimated by titration with 2,6 dichlorophenol endophenol dye (A.O.A.C., 1995).

All previous treatments were arranged in a Randomized Complete Blocks Design (RCBD) with four replicates for each treatment. The obtained data were statistically analyzed using analysis of variance technique. Means were compared using LSD method at 0.05 level of probability using Costat program (version 6.303, 2005). A combined analysis for both experimental seasons of fruit yield (as kg/tree) was carried out according to Gomez and Gomez (1984). Simple correlations were done according to Steel and Torrie (1980) between the number of nematode populations and both total fruit number/tree and

fruit yield as well as an average of leaf area after the application of nematicides.

Results and Discussion

The impact of some bio-pesticide products on the soil population of citrus nematode, *Tylenchulus semipenetrans*

Data presented in Table 1 illustrated the effect of three bio-pesticides products namely, abamectin (Tervigo®), azadirachtin (Achook®) and a mixture of *Trichoderma album* + *Bacillus megaterium* (Bio Zeid® + Bio Arc®), as well as oxamyl (Vydate®) on the presence of citrus nematode populations in the soil during two periods (March and September), for two successive seasons 2016 and 2017. The numbers of the populations were recorded before and after application by a month. Results in the first period (March, 2016) abamectin and oxamyl were the superior treatments which reduced the population by 79.66 and 79.61%, respectively, followed by the mixture of (*T. album* + *B. megaterium*) and azadirachtin by 78.79 and 74.94%, respectively. The same trend was observed in the second period (September, 2016) where abamectin proved its superiority and reduces the soil population by 78.12%, followed by oxamyl, the mixture of (*T. album* + *B. megaterium*) and azadirachtin with 72.78, 66.20 and 63.04% reduction, consecutively.

The obtained results of the present work was confirmed by Davis and Wilhite (1985) who found that oxamyl and fenamiphos were effective for managing citrus nematode (*Tylenchulus semipenetrans*) in the soil of grapefruit for two successive seasons. Meanwhile, oxamyl failed to improve the yield of grapefruit during both seasons.

Recently, abamectin which is a new registered nematicide in Egypt had recorded a highly nematicidal performance against *Tylenchulus semipenetrans*. Certain reports showed that abamectin was able to reduce certain genera of plant parasitic nematodes such as *Meloidogyne*, *Ditylenchus*, *Radopholus*, *Hoplolaimus* and *Tylenchorhynchus* on several crops (Blackburn et al., 1996, Jansson & Rabatin, 1998, Becker, 1999, Khalil et al., 2012, El-Nagdi et al., 2015 and Saad et al., 2017). Also, it was found that abamectin reduced the soil population of *T. semipenetrans* in mandarins up to 86% during the first season, while in the second season minimized the soil population up to 93% (El-Nagdi et al., 2010). The effect of abamectin against the citrus

nematodes (*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 (Huang *et al.*, 2014 and Lopez-Perez *et al.*, 2011). Furthermore, abamectin causes immobility in second stage juveniles (J_2) of root knot nematode and this may correlate with a reduction in oxygen uptake (Nordmeyer and Dickson, 1989).

On the other hand, in the second season (2017), the intervals during the two periods (March & September), showed that all applied treatments minimized the soil population significantly. In the first period (March, 2017), the highest reductions were observed with oxamyl, azadirachtin and abamectin with 89.87, 86.26 and 84.96%, respectively. The mixture of (*T. album* + *B. megaterium*) was relatively the least effective treatment with 75.07% reduction. In the second period (September, 2017), abamectin (87.06%) and oxamyl (82.44%) had possessed the best effectiveness in controlling juveniles of *Tylenchulus semipenetrans* in the soil. However, azadirachtin and the mixture of (*T. album* + *B. megaterium*), were the least treatments which came in the next category with relative reductions by 72.74 and 69.53%, respectively.

According to this study the activity of azadirachtin as a nematicide was moderate. The water extract of neem leaves recorded the superior mortality in the soil final population of *T. semipenetrans*, significantly than *Datura alba* and *Calotropis procera* on *Citrus jambhiri* (Ahmed *et al.*, 2004). The neem seed extract contains azadirachtin which is complex tetranortriterpenoid. Azadirachtin which is the main component of neem is responsible for both antifeedant and toxic effects in plant nematode as in insects (Mordue & Nisbet, 2000). Also, the active constituents of neem which absorbed through plant root can manage plant parasitic nematodes (Gill & Lewis, 1971 and Nisbet *et al.*, 1993).

In certain reports, the nematicidal mechanisms of neem were suggested the involvement of phenolic compounds absorbed systemically by the roots might have induced tolerance against nematodes (Mohan, 2011). The decomposition of neem formulation in soil release ammonia, formaldehyde, phenols and fatty acids which is very toxic to plant parasitic

nematodes (Khan *et al.*, 1974). Furthermore, the direct toxicity of neem formulations is due to the active constituents such as nimbin, salanine, thionemone, azadirachtin and nimbidine (Khan *et al.*, 1974 and Devakumar *et al.*, 1985). Moreover, it was suggested that the efficacy of neem constitutes on plant parasitic nematodes could be refer to chemoreceptors (Amphids and Phasmids), which are responsible for recognizing the host plant and work as repellent compound (Khalil, 2013). Also, the relative low effect of Azadirachtin in our study may be also due to the rapid degradation of neem by the environmental factors such as UV radiation in sunlight, heat, air moisture, acidity and enzymes present in foliar surfaces (Khalil, 2013).

In respect to antagonistic microorganisms such as *Bacillus* sp. and *Trichoderma* sp. it was proved to be effective against the plant parasitic nematodes in certain investigations. It was found that *Bacillus subtilis* succeed to reduce *Tylenchulus semipenetrans* on mandarin trees. The recorded reduction in soil population of *T. semipenetrans* was at range from 59.7 up to 74.4%. Meanwhile, the Fruit yield (kg/tree) was increased with *B. subtilis* by 31.4% (Hammam *et al.*, 2016). Meanwhile, El-Mohamedy *et al.* (2016) reported that some isolates of *Trichoderma viride*, *T. harzianum* and *Bacillus subtilis* were effective against the *Tylenchulus semipenetrans* on Sour orange seedlings under greenhouse conditions.

Effect of three bio-pesticides and oxamyl on fruit yield of Washington navel orange trees:

The obtained results as shown in Table 2 indicated that oxamyl (Vydate®) treatment, in both seasons, resulted in the highest significant increasing effect on number of fruit/tree, average fruit weight and fruit yield, where the values are 162.25, 321.88 (gm.) and 10.97 ton/ feddan, respectively at the 1st season, while the corresponding values are 201.00, 278.13 (g) and 11.74 ton/feddan at the 2nd season. The remaining treatments had increasing significant influences when comparing with the untreated control trees.

As an overall average of both experimental seasons, the yield as kg/tree of navel oranges receiving the different nematicides treatments could be arranged in the following descending order: oxamyl, azadirachtin, abamectin, the mixture of *T. album* + *B. megaterium*, and then the untreated control (Table 2).

TABLE 1. Effect of three bio-pesticides and oxamyl on the numbers of citrus nematode populations in the soil of Washington Navel orange trees during 2016 and 2017 seasons

Treatments	First season 2016					Second season 2017						
	March		September		March		September		September			
	Means Before treatment (200g soil)	Means After treatment (30 days)	*R (%)	Means Before treatment (200g soil)	Means After treatment (30 days)	R (%)	Means Before treatment (200g soil)	Means After treatment (30 days)	R (%)	Means Before treatment (200g soil)	Means After treatment (30 days)	R (%)
Untreated check	1473.00 ^c	3556.33 ^a	---	1550.67 ^a	2998.33 ^a	---	2170.00 ^c	6334.00 ^a	---	1930.33 ^b	3921.33 ^a	---
Oxamyl	7080.00 ^a	3486 ^a	79.61	1735.67 ^a	908.67 ^{bc}	72.78	5606.67 ^a	1670.67 ^b	89.87	668.67 ^c	239.67 ^c	82.44
Abamectin	5958.00 ^a	2925.33 ^a	79.66	1293.00 ^a	544.00 ^c	78.12	3084.00 ^{bc}	1364.00 ^b	84.96	1176.00 ^c	310.67 ^c	87.06
Azadirachtin	5864.00 ^a	3583.67 ^a	74.94	1498.33 ^a	1065.00 ^b	63.04	4446.67 ^{ab}	1797.33 ^b	86.26	1110.67 ^c	618.00 ^c	72.74
Mixture of (<i>T. album</i> + <i>B. megaterium</i>)	3694.00 ^b	1911.33 ^a	78.79	1754.67 ^a	1140.67 ^b	66.20	2671.67 ^{bc}	1958.67 ^b	75.07	2632.00 ^a	1636.67 ^b	69.53
LSD _{0.05}	2005.28	2615.78	---	900.59	477.18	---	1909.08	1127.33	---	552.69	573.59	---

Means within each column with the same letter (s) are not significantly different at level P = 0.05

* R (%): Reduction percentage

TABLE 2. Effect of three bio-pesticides and oxamyl on Number of fruits per tree, Average fruit weight (g), Fruit yield expressed as kg/ tree and Ton/ feddan of Washington Navel orange trees during 2016 and 2017 seasons

Treatments	First season 2016				Second season 2017				Overall average of both seasons Fruit yield (kg/ tree)
	Number of fruits/tree	Average fruit weight (g)	Fruit yield (kg/ tree)	Fruit yield (Ton/ feddan)	Number of fruits/tree	Average fruit weight (g)	Fruit yield (kg/ tree)	Fruit yield (ton/feddan)	
Untreated check	88.75 c	231.25 c	20.52 c	4.31 c	95.5 c	250.00 b	23.88 c	5.02 c	21.69 c
Oxamyl	162.25 a	321.88 a	52.23 a	10.97 a	201.00 a	278.13 a	55.90 a	11.74 a	51.87 a
Abamectin	112.75 bc	293.75 b	33.12 b	6.96 b	185.00 ab	250.00 b	46.25 ab	9.71 ab	41.45 b
Azadirachtin	177.75 a	287.75 b	51.73 a	10.86 a	169.75 ab	256.25 b	43.50 b	9.14 b	47.70 a
Mixture of (<i>T. album</i> + <i>B. megaterium</i>)	128.25 b	284.38 b	36.47 b	7.66 b	152.00b	281.25 a	42.75 b	8.98 b	39.06 b
LSD _{0.05}	27.81	21.18	7.00	1.48	34.40	21.42	10.35	2.17	6.07

Means within each column with the same letter (s) are not significantly different at level P = 0.05

The present results are in harmony with Verdejo-lucas and McKenry (2004) who found that *Tylenchulus semipenetrans* nematode caused yield losses was in range of 10-30% depending on the level of infestation. Likewise, Walker and Morey (1999) reported that granular cadusafos nematicide has shown superior efficacy against the citrus nematode and reduced citrus nematode populations. Moreover, Deepa et al. (2011) used *Pseudomonas florescens* that are commercially available at 20 gm./tree it was the most the most effective treatment to reduce *T. semipenetrans* populations of infected lemon trees (79.49%) and increased fruit yield three folds compared to untreated control. this bio agent nematicide recorded the highest fruit weight over control.

In Egypt, El-Nagdi et al. (2010) found that the best nematicide control was achieved with the highest rate of Agerin® (containing an isolate of *Bacillus thuringiensis*), in balady mandarin orchard. This treatment reduced reproduction rate of the nematode in both seasons. They added that both Agerin® 0.5% WP of abamectin (containing fermentation products of *Streptomyces avermitilis* as bio-agents are significantly increased fruit yield expressed as fruit number or weight compared to untreated control. It was concluded that these two bio-agents also have low associated production costs and are considered to be environmentally safe. Noteworthy, Timmer and French (1979) using grapefruit trees (*Citrus paradisi*) reported that soil application of DBCP (1,2-dibromo-3-chloropropane) reduced nematode populations and rust mite damage increased yield and fruit size. Otherwise, Duncan (1989) as well as Davis and Wilhite (1985) observed that both fenamiphos and oxamyl weren't showed any impacts on either fruit yield or size of both grapefruit and some citrus trees.

The correlation analysis between the number of nematode populations due to the infestation of soil samples collected after 30 days from the bio-pesticides and oxamyl application in either March or September might support such notation. Negative and significant correlation coefficients, especially in the second season were observed between number of soil population and both total fruit number / tree and fruit yield during both seasons of study (Table 3). This relation was negative but insignificant after 30 days from application in either March or September in the first season.

Effect of three bio-pesticides and oxamyl on some Egypt. J. Hort. Vol. 45, No. 2 (2018)

physical fruit properties

The effects of three bio-pesticide products and oxamyl were evaluated on physical fruit properties that included (fruit length, diameter, pulp weight, rind and fruit weights, peel thickness and fruit juice volume) are presented in Table (4). In general, the gained data indicated that all treatments during both seasons significantly increased the studied physical parameters of fruit in compared with untreated check. The only exception was noticed in rind weight which didn't significantly affected by the evaluated treatments. The treatment of oxamyl, during both seasons, was significantly superior in all fruit physical properties comparing with all other treatments including untreated check. Conversely, rind thickness property showed that oxamyl treatment had significantly the lowest values during the both seasons of 2016 and 2017 (Table 4).

Results of the present study are in general agreement with those reported by Wheaton et al. (1985) who stated that soil applied with oxamyl increased the average fruit size (length and diameter) and weights of Valencia orange fruit. In addition, El-Nagdi et al. (2010) found that the *Bacillus thuringiensis* (Agerin®) and abamectin (Vertemic®), markedly improved fruit quality of mandarin trees. Also, they found that these treatments suppressed the population of *T. semipenetrans* and improved nutritional status.

Many investigators, such as Timer and French (1979), Garabedian and Van Gundy (1983), Philis (1995 and 1997) revealed that soil applied with chemical nematicides reduced citrus nematode populations (*T. semipenetrans*) on citrus and grapefruit trees, as well as significantly increased fruit yield, fruit size, and improved other external fruit properties compared to the untreated control during two seasons. On the contrary, Abdelaal et al. (2010) reported that certain microbial bio-fertilizers as nematicides didn't significantly had an effect on fruit peel thickness of Washington navel oranges.

The impact of three bio-pesticides and oxamyl on some chemical fruit properties

The efficacy of applied treatments (abamectin, azadirachtin, the microbial mixture and oxamyl) on total soluble solids (TSS %), acidity (%) and vitamin C (mg/100ml juice) are shown in Table 5. The obtained results revealed that azadirachtin, oxamyl and abamectin during the both seasons had enhanced the TSS (%) with estimated values of 13.45, 13.38 and 12.90 %, respectively, at the

first season. While, the corresponding values were 14.00, 13.98 and 13.50% in the second season, respectively. However, abamectin was the only treatment that recorded increasing effect in acidity (1.02 and 1.08%) and vitamin C (44.78 and 47.82 mg/100ml juice) in first and second seasons, respectively. Meanwhile, the microbial mixture (*T. album* + *B. megaterium*) had also increased the vitamin C by 44.37 and 45.44 mg/100ml juice during first and second seasons, consecutively.

On the other hand, azadirachtin treatment markedly reduced the juice acidity percentage during both seasons (2016 and 2017) as shown in Table 5. Our results are similar to previous studies of Wheaton et al. (1985) who reported that average brix percentages (TSS %) in the fruit juice of both Valencia and Halmin oranges, were increased linearly with the increment of Aldicarb doses. They attributed the increases in juice brix to an added benefit of Aldicarb Treatment. Moreover, El-Ngdi et al. (2010) found that both *Bacillus thuringiensis* (Agerin®) and abamectin (Vertemic®), significantly increased TSS% and

ascorbic acid (V.C) content in the fruit juice of mandarin trees. Meanwhile, Abdelaal et al. (2010) observed that microbial strains of *Pseudomonas florescence* 843 and *Azospirillum brasilense* W 24 at the rate of 300 or 500 ml/ tree exhibited significant increases in total soluble solid (TSS) and fruit juice acidity (%), particularly in Washington Navel orange trees during the first season. Moreover, the content of vitamin C in fruit juice was promoted with *P. florescence* significantly. Conversely, it was mentioned that the nematicide aldicarb did not affect juice acidity or ratio of Florida citrus (Wheaton et al., 1985).

Effect of three bio-pesticides and oxamyl against citrus nematode on leaf area and some leaf mineral composition of Washington navel orange trees:

Data concerning the influence of oxamyl, abamectin, azadirachtin and a mixture of *T. album* + *B. megaterium*, on the leaf area (cm²) shown in Table 6. The different applied treatments had increasing effects on the aforementioned parameter.

TABLE 3. Correlation, regression coefficient for treatment between the numbers of nematode population density in soil samples collected after 30 days from three bio-pesticides and oxamyl application for fruit number per tree and fruit yield (kg/tree) in Washington Navel orange trees during 2016 and 2017 seasons

The number of nematode population densities	Relations	First season (2016)		Second season (2017)	
		Total fruit number/ tree	Fruit yield (kg/ tree)	Total fruit number/ tree	Fruit yield (kg/ tree)
After 30 days of application (March)	# r	-0.16 ns	-0.15 ns	-0.66**	-0.63**
	§ b	-0.006	-0.002	-0.019	-0.005
	@ Equation	$\hat{y}=153.06-0.006 X$	$\hat{y}=43.50-0.002X$	$\hat{y}=206.12-0.019 X$	$\hat{y}=56.05-0.005 X$
After 30 days of application (September)	R	-0.35 ns	-0.49 ns	-0.70**	-0.72**
	B	-0.018	-0.008*	0.027	0.009
	Equation	$\hat{y}=158-0.018 X$	$\hat{y}=49.43-0.008 X$	$\hat{y}= 197.50-0.027 X$	$\hat{y}= 52.32-0.009 X$
LSR	0.05	0.44	0.44	0.44	0.44
	0.01	0.56	0.56	0.56	0.56

r = Correlation coefficient, § b = Regression coefficient, @ $\hat{y}= a + b X$

TABLE 4. Effect of three bio-pesticides and oxamyl on some physical fruit properties of Washington Navel orange trees during 2016 and 2017 seasons

Treatments	First season 2016						Second season 2017					
	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Pulp weight (g)	Rind weight (g)	Juice volume (ml)	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	Pulp weight (g)	Rind weight (g)	Juice volume (ml)
Untreated check	7.85 d	7.29 c	178.13 c	53.15 a	231.26 c	0.43 c	8.06 b	7.56 c	195.63 cd	58.13 a	253.76 b	68.20 c
Oxamyl	8.89 a	8.21 a	268.75 a	53.13 a	321.88 a	0.45 c	8.47 a	8.09 a	225.00 a	53.13 a	278.13 a	105.95 a
Abamectin	8.74ab	7.93 b	232.50 b	63.13 a	295.63 b	0.64 a	8.24 ab	7.41 c	186.88 d	63.13 a	250.01 b	92.20 b
Azadirachtin	8.59 bc	7.96 b	225.00 b	62.75 a	287.75 b	0.55 b	8.38 a	7.53 c	203.13 c	53.13 a	256.26 b	85.30 b
Mixture of (<i>T. album</i> + <i>B. megaterium</i>)	8.44 c	7.94 b	223.44 b	60.94 a	284.38 b	0.55 b	8.26 ab	7.78 b	217.19 b	64.06 a	281.25 a	92.50 b
LSD _{0.05}	0.20	0.16	17.16	10.53	21.18	0.07	0.25	0.17	13.08	17.08	21.42	8.12

Means within each column with the same letter (s) are not significantly different at level P = 0.05

TABLE 5. Effect of three bio-pesticides and oxamyl application on some chemical fruit properties of Washington Navel orange trees during 2016 and 2017 seasons

Treatments	First season 2016			Second season 2017		
	Total soluble solids (TSS) (%)	Acidity (%)	Vitamin C (mg/100ml juice)	Total soluble solids (TSS) (%)	Acidity (%)	Vitamin C (mg/100ml juice)
Untreated check	12.63 c	0.85 bc	38.60 c	13.50 b	0.89 c	37.71 d
Oxamyl	13.38 a	0.88 bc	39.35 c	13.98 a	0.99 b	42.24 c
Abamectin	12.90 b	1.02 a	44.78 a	13.50 b	1.08 a	47.82 a
Azadirachtin	13.45 a	0.77 c	41.48 b	14.00 a	0.77 d	44.37 b
Mixture of (<i>T. album</i> + <i>B. megaterium</i>)	12.88 bc	0.92 b	44.37 a	12.98 c	0.91 c	45.44 b
LSD _{0.05}	0.25	0.12	1.17	0.41	0.07	2.06

Means within each column with the same letter (s) are not significantly different at level P = 0.05

The obtained results are in harmony with EL-Nagdi et al (2010) who found that both Agerin® and Vertemic® increased the leaf area of mandarin trees relative to untreated check, with increases being positively correlated with the increases in application rate of the formulations. They added that the smallest leaf area of mandarin trees was recorded in untreated trees. They also attributed the increment of the leaf area due to applied these bio-products to stimulate, especially carbohydrates and proteins, and enhancement of the formation and movement of natural hormones, which are vital to improved cell division, especially in the meristem tissues. This explanation agrees with those reported by Nomier (2000), Hafez (2001) and Mahmoud and Hafez (2004). Similarly, Philis (1997) found that heavily nematode infested trees exhibit symptoms of poor growth, reduced fruit size, twig die-back and small leaves. Nakhla et al (1998) reported that inorganic-n(ammonium nitrate 33.5% and calcium nitrate 15.5%) significantly ($P \leq 0.05$) increased fruiting intensity and total sugars content as well as significantly ($P \leq 0.01$) decrease populations of the nematode in both seasons. Also, concluded that application of organic-N 75% plus inorganic-N 25% was the most effective combination treatments in this respect.

On the other hand, obtained results in Table 6 indicated that the abamectin treatment, in both seasons, gave significantly the highest percentages in both leaf nitrogen and phosphorus content compared with the untreated control trees. The percentages of nitrogen and phosphorus values were 2.69, 0.59 at the first season and 3.32 and 0.60 at the second one, respectively. Similarly, significantly the highest percentages of both leaf calcium and magnesium content were obtained by oxamyl nematocide application compared to the untreated control where the values were 5.37 and 0.87 at the 1st season and 5.32 and 0.92 at the 2nd one, respectively. In general, the control treatment, in both seasons, gave significantly the lowest values in all leaf macro nutrients content studied herein during both years of study (Table 6). Generally, the assessed treatments had a positive effect on leaf minerals of Washington navel orange trees.

Concerning leaf potassium content in both seasons, all evaluated treatments generally increased leaf potassium percentages compared with untreated control (Table 6). Both abamectin and *T. album* + *B. megaterium* during both seasons recorded the highest leaf potassium content significantly in compared with the rest treatments including the control where the values were 1.60 and 1.56% at 1st season and 1.54 and 1.66% at the 2nd one, for abamectin and the mixture of *T. album* + *B. megaterium*,

respectively. Nevertheless, oxamyl also markedly exhibited increment in potassium percentage than the untreated treatment. The significantly lowest potassium percentages were obtained from the untreated control trees where the values were 0.75 and 0.85%, in the first and the second seasons, consecutively.

In accordance with these results are those reported by Wheaton et al. (1985) who stated that application of oxamyl to citrus trees grown in presence of citrus nematode increased leaf phosphorus, calcium and magnesium contents, but leaf nitrogen wasn't affected by oxamyl treatment. Noteworthy, it was reported that macro nutrients content of mandarin leaves was significantly increased with applied treatments of *B. thuringiensis* and abamectin (El-Nagdi et al., 2010). Also, they found that the highest content of macro nutrients (N, Ca and Mg) are correlated with the highest applied rates of *B. thuringiensis* and abamectin. Furthermore, the leaf phosphorus and potassium contents showed gradual augmentation with increasing doses of products during both seasons. On the contrary, in the same year, Abdelaal et al. (2010) concluded that both nitrogen and phosphorus percentages were significantly increased due to application of microbial bio-fertilizer (as nematocide) with the two examined strains (*Azospirillum brasilense* and *Pseudomonas florescence*) compared by control. They explained that strain of *A. brasilense* gave high nitrogen percent than the strain of *P. florescence* which is a free living nitrogen fixing bacteria. Additionally, the phosphorus and potassium content were also increased due to application of bio-fertilizer with the two examined strains. Atwaia and El-Dosouky (1997) and Abdalla et al. (1998) reported that the improvement in nutrient status followed by the treatment with these two bio-products (Agerin® and Vertemic®) might be to faster absorption of the nutrient via citrus roots.

The relation between the numbers of soil nematode populations and average of leaf area was statistically examined by calculating the correlation coefficient between these two variables as shown in Table 7. The correlation coefficient between the number of citrus nematode populations and the leaf area in Washington navel Orange trees, in both seasons was negative and insignificant, i.e. with increasing number of nematode population density, average leaf area was reduced. The only exceptional case was after 30 days from the application of products in March during the second season, the correlation coefficient was negative and significant (Table 7)

TABLE 6 Effect of three bio-pesticides and oxamyl on leaf area, leaf nitrogen, phosphorus, potassium, calcium and magnesium percentages of Washington Navel orange trees during 2016 and 2017 seasons

Treatments	First season (2016)					Second season (2017)						
	Leaf area (cm ²)	Nitrogen (N %)	Phosphorus (P %)	Potassium (K %)	Calcium (Ca %)	Magnesium (Mg %)	Leaf area (cm ²)	Nitrogen (N %)	Phosphorus (P %)	Potassium (K %)	Calcium (Ca %)	Magnesium (Mg %)
Untreated check	25.34 c	1.04 d	0.31 c	0.75 c	4.18 b	0.66 c	30.91 c	1.01 c	0.43 c	0.85 c	3.88 c	0.69 b
Oxamyl	28.29 c	1.70 c	0.54 ab	1.45 a	5.37 a	0.87 a	36.03 b	2.24 b	0.61 a	1.16 b	5.32 a	0.92 a
Abamectin	36.73 ab	2.69 a	0.59 a	1.60 a	5.00 a	0.74 bc	37.14 ab	3.32 a	0.60 a	1.54 a	4.39 bc	0.85 a
Azadirachtin	34.10 b	2.31 ab	0.53 b	1.05 b	4.22 b	0.79 ab	37.11 ab	2.23 b	0.50 b	1.26 b	4.71 ab	0.88 a
Mixture of (<i>T. album</i> + <i>B. megaterium</i>)	38.90 a	2.00 bc	0.36 c	1.56 a	4.05 b	0.82 ab	40.68 a	2.14 b	0.52 b	1.66 a	3.96 bc	0.92 a
L.S.D _{0.05}	3.00	0.42	0.05	0.22	0.76	0.11	4.12	0.29	0.06	0.15	0.76	0.10

Means within each column with the same letter (s) are not significantly different at level P = 0.05

TABLE 7. Correlation, regression coefficient for treatment of the number of nematode population density in soil samples collected after 30 days from bio-pesticides and oxamyl application for leaf area in Washington navel orange trees during 2016 and 2017 seasons

The number of nematode population densities	Relations	First season (2016)	Second season (2017)
		Average of leaf area (cm ²)	Average of leaf area (cm ²)
After 30 days of application (March)	[#] r	-0.13 ns	-0.48*
	^{\$} b	-0.001	-0.002
After 30 days of application (September)	[@] Equation	$\hat{y}=34.50-0.001X$	$\hat{y}=40.28-0.002X$
	R	-0.42 ns	-0.39 ns
	B	-0.003	0.002 ns
	Equation	$\hat{y}=36.51-0.003X$	$\hat{y}=38.84-0.002X$
LSR	0.05	0.44	0.44
	0.01	0.56	0.56

[#] r = Correlation coefficient, ^{\$} b = Regression coefficient, [@] $\hat{y}=a + b X$

Conclusion

Overall, it could be concluded 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 total soluble solids, as well as increased leaf macro nutrient content. While, abamectin increased the juice content of acidity and vitamin C. Nevertheless, the combination between *T. album* and *B. megaterium* increased leaf area.

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فاعلية ثلاثة مبيدات حيوية و مبيد الأوكساميل ضد نيماتودا الموالح (تيلنكيولس سيميبترانس) وعلى إنتاجية اشجار البرتقال ابو صرة واشنظن

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**المعمل المركزي للمبيدات – محطة بحوث الصباحية – مركز البحوث الزراعية - الاسكندرية - مصر.

تم إجراء هذه الدراسة خلال فترتين (مارس و ابريل) و لمدته موسمين متتاليين ٢٠١٦ و ٢٠١٧ وذلك لتقييم تأثير ثلاثة مبيدات حيوية وهي الأباكتين (ترفيجو)، الأزادراختين (الأشوك) و مخلوط التريكو درما اليم + باسيلس ميجاتريوم (بايو زيد + بايو ارك) بالإضافة الى المبيد النيماتودي الأوكساميل (فايديت) ضد نيماتودا الموالح (تيلنكيولس سيميبترانس) على اشجار البرتقال ابو صرة (عمر ١٠ سنوات) في التربة الرملية. تم تنفيذ هذا البحث في مزرعة خاصة في قرية الهامور – بمنطقة البستان – محافظة البحيرة. و قد بينت النتائج المتحصل عليها ان كل المعاملات المستخدمة قد ادت لتخفيض تعداد عشيرة النيماتودا خلال موسمي الدراسة و ذلك بنسبة ٦٣,٠٤ لـ ٧٩,٦٦٪ و من ٦٩,٥٣ لـ ٨٩,٨٧٪ على التوالي.

و في نفس الوقت فإن محصول الثمار قد ازداد معنويا خلال كلا الموسمين مع كل المعاملات المستخدمة وخاصة مبيد الأوكساميل. كذلك فإن معظم الخصائص الفيزيائية للثمار قد تحسنت. أيضا الخصائص الكيميائية للثمار قد تحسنت بشكل ملحوظ وخاصة مع المعاملة بمركب الأباكتين حيث ادى لزيادة فيتامين سي. علاوة على ذلك فإن الأوكساميل و الأزادراختين ادى لزيادة معنوية في المحتوى من المواد الصلبة الذاتية الكلية في عصير الثمار.

و من ناحية اخرى فإن المساحة الورقية قد ازدادت مع كل المعاملات المستخدمة خلال كلا الموسمين. ايضا بينت النتائج و جود زيادة ملحوظة في التركيب المعدني للأوراق مع كل المعاملات المختبرة، ماعدا الكالسيوم الذى انخفض معنويا عند استخدام مخلوط التريكو درما اليم + باسيلس ميجاتريوم وذلك خلال كلا موسمي الدراسة.