

## EFFECTS OF COMMERCIAL FORMULATIONS OF *BACILLUS THURINGIENSIS* AND *STREPTOMYCES AVERMITILIS* ON *TYLENCHULUS SEMIPENETRANS* AND ON NUTRITION STATUS, YIELD AND FRUIT QUALITY OF MANDARIN

W.M.A. El-Nagdi<sup>1</sup>, M.M.A. Youssef<sup>1</sup> and O.M. Hafez<sup>2</sup>

<sup>1</sup>Department of Plant Pathology, Nematology Laboratory

<sup>2</sup>Department of Pomology, National Research Centre, Dokki, P.O. Box 12622, Giza, Egypt

**Summary.** A study was conducted during two successive growing seasons (2007 and 2008) to assess the effects of a commercial formulation (Agerin®) containing an isolate of *Bacillus thuringiensis* and another, abamectin, containing fermentation products of *Streptomyces avermitilis*, on the citrus nematode, *Tylenchulus semipenetrans*, infecting 15-year-old Balady mandarin (*Citrus reticulata*) trees grafted on sour orange (*Citrus aurantium*) rootstock. The mandarin was grown in a sandy soil at a tree spacing of 3.5 m × 3.5 m, with drip irrigation and under field conditions. The application rates were 1, 2 and 3 kg/4200 m<sup>2</sup> for Agerin® and 5 l/tree of distilled water containing 200, 400 and 800 ppm for abamectin. All treatments suppressed nematode populations with the percentage of reduction being positively correlated with the rate of application. Also, the treatments markedly improved nutritional status, yield and fruit quality. The best nematode control was achieved with the highest rate of Agerin®, which reduced the reproduction rate of the nematode in both seasons (R = 0.16 and 0.15). Similar control was obtained with abamectin, with the highest rate of application resulting in the lowest reproduction rates (R = 0.21 and 0.15 in the two seasons), followed in effectiveness by the intermediate and the lowest rates. The best nutritional status of mandarin trees occurred when the treatments were applied at the highest rates. The treatments increased both macro (N, P, K, Ca and Mg) and micro (Fe, Zn and Mn) nutrient element contents in leaves. The yields increased by 52.9-69.2% and 84.6-115.4% following application of the products at the highest rates in both seasons. It is concluded that Agerin® and abamectin have potential as non-chemical control strategy tools in managing the citrus nematode. These two bioagents also have low associated production costs and are considered to be environmentally safe.

**Keywords:** Biological control, *Citrus aurantium*, *C. reticulata*, citrus nematode.

Citrus are the most important fruits produced in Egypt with the cultivated area reaching about 421,000 feddans (= 175,417 hectares) (Anonymous, 2008). This area represents about 36.2% of the total area devoted to fruits, producing about 40.5% of total fruit production. In 2008, a million tonnes were exported as fresh fruit and 200,000 tons were processed for juice, etc. Mandarin (*Citrus reticulata* Blanco) is one of the most important citrus fruits in Egypt, having significant value not only in the local market but also for export. Unfortunately, many nematode pests parasitize citrus, one of which is the citrus nematode *Tylenchulus semipenetrans* Cobb, the cause of the slow decline disease in Egypt (Oteifa and Tarjan, 1965) and several other parts of the world, causing yield losses ranging from 8.7 to 12.2% according to Cohn (1972) or from 8.7 to 14.2% according to Sasser (1989). In Egypt, Abd-Elgawad (1995) found significant negative correlation between navel orange yield and population density of the citrus nematode. Therefore, efforts are being made to increase yield through effective management of the citrus nematode.

This study was planned to evaluate *i*) the efficacy of the bioformulations, Agerin® and abamectin for the management of *T. semipenetrans* infesting mandarin trees, and *ii*) the effects on nutrition status, yield and

fruit quality under field conditions. Agerin® contains an isolate of *Bacillus thuringiensis* Berliner, one of the biocontrol agents considered to be an excellent alternative to chemical nematicides against plant-parasitic nematodes (Osman *et al.*, 1988; Ismail and Fadel, 1999; Radwan, 1999; Chen *et al.*, 2000; Radwan *et al.*, 2004; El-Sherif *et al.*, 2007; El-Nagdi and Youssef, 2009). Abamectin contains avermectins and is the fermentation product of the bacterium *Streptomyces avermitilis* (Burg *et al.*) Kim *et* Goodfellow, having potential for managing certain plant-parasitic nematodes on various crops (Burg *et al.*, 1979; Jansson and Rabatin, 1997; El-Nagdi, 2001; Youssef *et al.*, 2005; Rehman *et al.*, 2009).

### MATERIALS AND METHODS

*Experimental plots and treatment design.* The experiment was conducted in a citrus orchard infested with the citrus nematode at Katta, Imbaba County, Giza governorate, Egypt, from February to December 2007 and 2008. Mandarin (*Citrus reticulata*) cv. Balady trees were grafted on sour orange (*C. aurantium* L.) rootstocks that were 15 years old. Trees were spaced 3.5 m × 3.5 m apart in an area of sandy loam soil (coarse sand 82%, fine sand 6.1%, silt 8.9%, clay 3%, pH 7.5 and Ec.1.36) with drip irrigation. Fertilization, irrigation and other agricultural practices were applied as recommended

<sup>1</sup> Corresponding author: wafaalnagdi@yahoo.com

(Abou-Aziz, 2003). Forty two uniform trees were selected in October 2006 randomly and assigned to a completely randomized design with six replicates per treatment (one tree/replicate). There were seven treatments, including untreated trees to serve as controls. Agerin<sup>®</sup>, containing spores and crystals (as powder) of strains C18 and C129 of an isolate of *Bacillus thuringiensis* sub sp. *aegypti*, was applied at rates of 6.25, 12.5 and 18.75 g/tree, which is equivalent to 1, 2 and 3 kg/feddan (1 feddan = 4,200 m<sup>2</sup>). Abamectin was applied at concentrations of 200, 400 and 800 ppm (0.2, 0.4 or 0.8 ml of abamectin added to 1000 ml distilled water) at the rate of 5 litres of the tested concentration per tree. Both bio-agent formulations were applied on February 15<sup>th</sup> in 2007 and 2008 under the canopy of each tree as soil drench using the same amount of water and the tree were soon irrigated.

*Observations on nematode populations.* Soil samples, including roots, were collected on March 15 and May 15 and again at harvest on December 15, in 2007 and 2008 to estimate the effects of the treatments on nematode populations. Samples from each replicate were collected within 150 cm of the trunk beneath the canopy in the mandarin rhizosphere to a depth of 30-40 cm. Each sample was composed of four cores (5 cm diameter), 250 g soil per core, giving a total soil weight of 1 kg, which included about 28 g of root per tree (about 7 g root per core). An aliquot of 250 g soil from each sample (replicate) was processed by sieving and decanting methods (Barker, 1985). Roots from the same soil sample were gently washed free of soil and an aliquot of 5 g per replicate (tree) was cut into 2-cm-long pieces, placed in Petri dishes with distilled water and incubated under laboratory conditions (25 ± 5 °C) for a week to extract to *T. semipenetrans* J<sub>2</sub>. Also, an aliquot of 2 g roots per replicate (tree) was blended at 3 × 10<sup>3</sup> rpm for 3 minutes to extract females and eggs from roots (Southey, 1970). Percentage reductions of the *T. semipenetrans* populations in soil and roots were determined according to the formula of Handerson and Tilton (Puntener, 1981):

$$\text{Nematode reduction (\%)} = [1 - (\text{PTA}/\text{PTB} \times \text{PCB}/\text{PCA})] \times 100$$

where PTA = Population in the treated mandarin after application, PTB = Population in the treated mandarin before application, PCB = Population in the check mandarin before application, PCA = Population in the check mandarin after application.

The reproduction rate of the nematode was also calculated according to the formula suggested by Oostenbrink (1966):

$$R = \text{Reproduction rate of nematode} = Pf/Pi$$

where Pf = final nematode population density and Pi =

initial nematode population density.

In all cases, the total nematode population considered was that in 250 g soil including that in the 7 g of roots occurring in the same amount of soil.

*Observations on plant status and yield.* At harvest, estimates were made of leaf area, nutrition status, yield, yield components and characteristics of fruit quality of mandarin.

For leaf area, 30 leaves per tree were collected by selecting the third and fourth leaf from non-fruiting shoots of spring flush in the last week of September. Leaf area was measured with a planimeter, according to Nauliyal *et al.* (1990).

The leaf samples were also used to determine the percentage of macro elements (N, P, K, Ca, Mg) as per cent of dry weight according to Evenhuis and De-Waard (1980) and leaf content of microelements (Fe, Mn, Zn, Cu) in ppm of dry weight according to Jacson and Ulrich (1959).

In December 2007 and 2008, the number of fruits per tree, fruit weight (mean of 15 fruits per tree) and yield per tree were recorded.

To assess fruit quality, twenty fruits were randomly selected per tree, from which the chemical characteristics of the fruit juice [i.e., total soluble solids percentage (TSS %), total acidity (TA %) and ascorbic acid (VC)] were determined (A.O.A.C., 1995).

*Statistical analysis.* Data on initial populations were analyzed following arcsin transformation (Snedecor and Cochran, 1980). All data were subjected to analysis of variance and means compared according to Duncan's Multiple Range Test at P ≤ 0.05. Factorial analysis was done to assess the significance of the different factors and their interactions (Duncan, 1955).

## RESULTS

*Effects on nematode population.* In 2007 and 2008, Agerin<sup>®</sup> and abamectin at different doses were both effective in suppressing (P ≤ 0.05) *T. semipenetrans* populations in soil and roots of mandarin trees, compared to the untreated check, one and two months after application and at harvest (Table I).

Second stage juveniles in soil and roots and females and eggs on roots of mandarin were significantly (P ≤ 0.05) reduced by all treatments in both seasons. The number of nematodes was sometimes negatively correlated with the tested doses but not in all cases. For example at harvest of 2007, J<sub>2</sub> in the soil were significantly reduced, but there was no negative correlation between the tested doses and the numbers of J<sub>2</sub>. In the roots, there was a negative correlation between rates of Agerin<sup>®</sup> and numbers of J<sub>2</sub>. Similar results were found with the numbers of females or eggs. In 2008, all nematode stages were significantly (P ≤ 0.05) reduced and, in

**Table I.** Effect of the commercial products of Agerin® and abamectin at different doses on the population densities of *Tylenchulus semipenetrans* infesting mandarin trees under field conditions, before, one and two months after treating and at harvest, in the 2007 and 2008 growing seasons.

Treatment	Rate	Initial <sup>#</sup>				After one month				After two months				At harvest			
		Eggs in roots (2 g)	Females in roots (2 g)	J <sub>2</sub> in roots (5 g)	J <sub>2</sub> in Soil (250 g)	Eggs in roots (2 g)	Females in roots (2 g)	J <sub>2</sub> in roots (5 g)	J <sub>2</sub> in Soil (250 g)	Eggs in roots (2 g)	Females in roots (2 g)	J <sub>2</sub> in roots (5 g)	J <sub>2</sub> in Soil (250g)	Eggs in roots (2 g)	Females in roots (2 g)	J <sub>2</sub> in roots (5 g)	J <sub>2</sub> in Soil (250 g)
<b>Season 2007</b>																	
Agerin® (kg/fed.)	1	1165a	315a	1075a	569a	572 c	46 d	293 c	590 a	178 b	236 b	245b	105 b	374 bc	114 b	781bc	64 b
	2	1510a	745a	1640a	288a	942 b	181 cd	470 c	297 c	361c	46 c	99 b	80 b	168 c	104 b	405 cd	144 b
	3	1390a	1470a	3219a	257a	1008b	484 bc	551 c	326bc	285b	83 bc	272 b	118 b	382 bc	58 b	220 d	112 b
Abamectin (ppm)	200	1040a	1195a	2025a	473a	534 c	127 cd	1243b	573 a	398b	98 bc	470 b	352ab	956 a	124 b	1122b	209 b
	400	3650a	1100a	3255a	1540a	1320b	603 b	685 c	226 c	202b	84 bc	342 b	428a	777 ab	268 b	519cd	437 b
	800	798a	1110a	1829a	292a	393 c	106 cd	402 c	324bc	268b	78 bc	226 b	259ab	159 c	66 b	966 b	130 b
Untreated (control)	0.0	1371a	800a	1739a	563a	4964a	1116 a	2049a	690 a	700 a	528 a	2068a	165 a	700 ab	507 a	2516a	448 a
<b>Season 2008</b>																	
Agerin® (kg/fed.)	1	2791a	593a	2102a	515a	644cd	180cd	368cd	450ab	165 b	80 b	122b	64b	695c	35 c	321c	97 bc
	2	1230a	1416a	2431a	576a	845bc	268c	460bcd	216b	860 b	143 b	456b	283ab	233bc	138 bc	369c	94 bc
	3	1648a	668a	1112a	220a	919bc	247 c	387cd	210b	338 b	128 b	374b	140b	139c	80 c	c 479	82 c
Abamectin (ppm)	200	1607a	1471a	1742a	1039a	818bc	249 c	776b	690a	418 b	124 b	142b	589a	647b	211 b	571 b	276bc
	400	1166a	1280a	1558a	457a	1610b	480 b	555bc	503ab	240 b	97 b	201b	262ab	298c	92 c	1175bc	435 a
	800	1245a	1488a	2801a	480a	305d	55 d	140d	432ab	190 b	165 b	253b	475 a	143c	71 c	600 bc	102bc
Untreated (control)	0.0	1040a	995a	1308a	638a	5022a	1230 a	1778a	400ab	1160a	434 a	1899a	327a	3030a	415 a	2849 a	430 a

Data are means of 6 replicates.

<sup>#</sup>Initial population densities are not significantly different after Arcsin transformation (Snedecor and Cochran, 1980).

Means followed by the same letter(s) in each column are not significantly different according to Duncan's multiple range test (P≤0.05).

some cases, there were negative correlations between the tested doses and the numbers of the nematode stages (Table I).

The reproduction rate of the nematode ( $R = Pf/Pi$ ) decreased with the increased rates of applications of the two formulations (Figs 1 and 2).

When the data were subjected to factorial analysis, the results for overall effects of Agerin® and abamectin indicate that in most cases Agerin® was superior to abamectin in reducing the  $J_2$  nematode soil populations at 2 months in both seasons and in roots at harvest (Table II). The pattern of nematode reproduction at harvest was similar to that during the growing season (Figs 1 and 2). Also, the two tested materials at the dif-

ferent doses or as overall effects significantly ( $P \leq 0.05$ ) reduced the total numbers of nematodes and the percentages of nematode reduction were, especially at harvest, positively correlated with the tested doses in both seasons (Table III). However, percentage reductions in the second season were higher than those in the first season.

*Effects on leaf area.* Agerin® and abamectin both increased leaf area of mandarin trees (Table IV) relative to the untreated check, with increases being positively correlated with the increases in application rate of the two formulations. A significant increase of leaf area was observed with the largest application rate of Agerin® (9.4

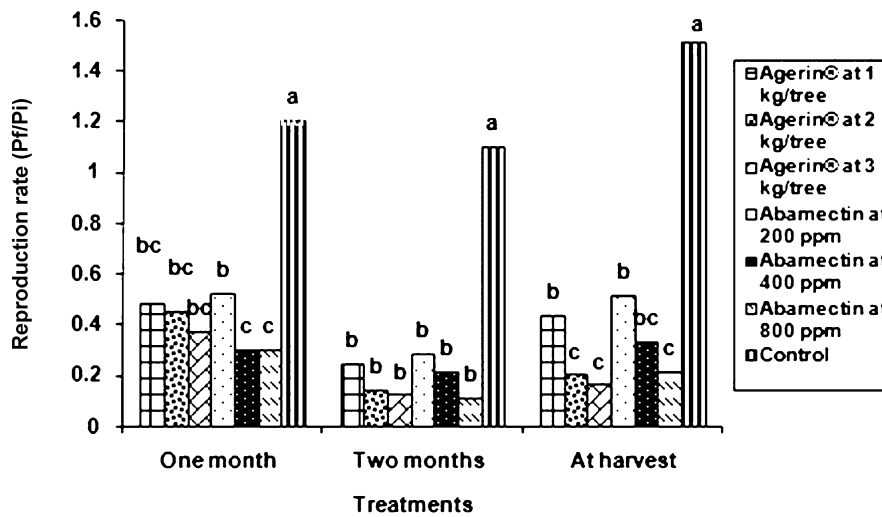


Fig. 1. Reproduction rate of *Tylenchulus semipenetrans* on mandarin trees as affected by different rates of Agerin® and abamectin in the 2007 season. (Pf = Final nematode population in the soil and roots; Pi = initial nematode population in the soil and roots).

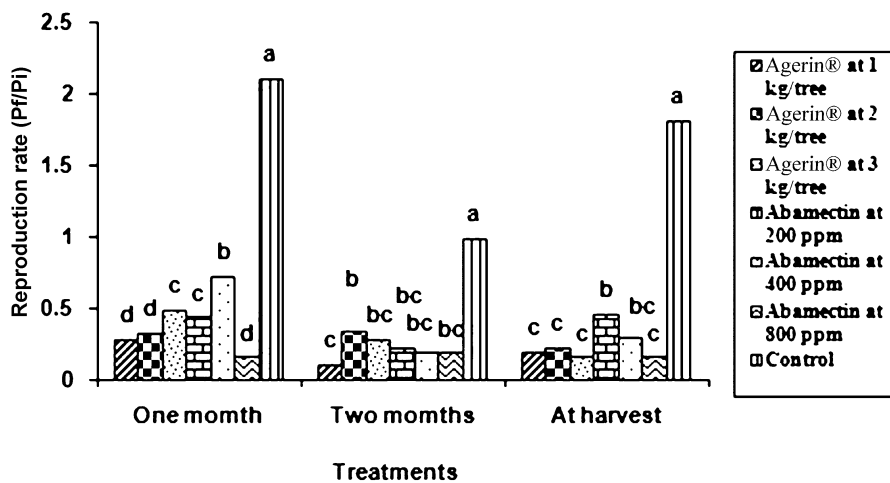


Fig. 2. Reproduction rate of *T. semipenetrans* on mandarin trees as affected by different doses of Agerin® and abamectin in the 2008 season. (Pf = Final nematode population in the soil and roots; Pi = initial nematode population in the soil and root).

**Table II.** Comparative overall effects of Agerin® and abamectin on the dynamics of *T. semipenetrans* infesting mandarin trees, one and two months after treating and at harvest, in the 2007 and 2008 growing seasons.

Treatment	One month				Two months				At harvest			
	Eggs	Female	Juveniles (J <sub>2</sub> )		Eggs	Female	Juveniles (J <sub>2</sub> )		Eggs	Female	Juveniles (J <sub>2</sub> )	
			Roots	Soil			Roots	Soil			Roots	Soil
<b>Season 2007</b>												
Agerin®	841b	237b	438b	404b	275b	122b	205b	101b	308b	92b	469c	107b
Abamectin	749b	279b	777b	374b	389b	86b	346b	346a	631a	153b	869b	259b
Untreated (control)	4964a	1116 a	2049a	690 a	700 a	528 a	2068a	165 a	700 a	507 a	2516a	448 a
<b>Season 2008</b>												
Agerin®	803b	232b	405b	292b	454b	117b	317b	162b	356b	84b	390c	91b
Abamectin	911b	338b	490b	542a	283b	129b	284b	442a	363b	125b	782b	271b
Untreated (control)	5022a	1230a	1778a	400a	1160a	434a	1899a	327a	3030a	415a	2849a	430a

**Table III.** % reduction of population densities of *T. semipenetrans* on mandarin trees as affected by the different doses of Agerin® and abamectin, one and two months after treating and at harvest, compared to the population of the nematode before treating, in the 2007 and 2008 growing seasons. (Pi and Pf are per 250 g soil)

Treatment	Rate	Initial* Nematodes (Pi)	One month		Two months		At harvest	
			Final nematodes (Pf)	% Nematode reduction	Final nematodes (Pf)	% Nematode reduction	Final nematodes (Pf)	% Nematode reduction
<b>Season 2007</b>								
Agerin® (kg/fed.)	1	3124a <sup>#</sup>	1501bc	44	764c	69	1333c	72
	2	4183a	1890b	48	586c	82	821d	87
	3	4946a	2369b	44	758c	80	772d	90
Abamectin (ppm)	200	4733a	2477b	39	1318b	64	2411b	66
	400	4029a	2834b	18	831c	73	1321c	78
	800	9545a	1225c	85	1056b	86	2001b	86
Untreated (control)	0.0	4473a	3855a		3461a		6761a	
<b>Season 2008</b>								
Agerin® (kg/fed.)	1	6001a	1642c	87	431d	93	1148bc	89
	2	3645a	1789c	77	1842b	47	780c	88
	3	5653a	1763c	85	980c	82	834c	92
Abamectin (ppm)	200	5859a	2533b	80	1273b	77	2650b	74
	400	4461a	3148b	67	800c	81	1300bc	83
	800	6014a	932d	93	1083b	81	916c	91
Untreated (control)	0.0	3981a	8430a		3820a		6997a	

\*Pi = Initial nematode population in soil including roots.

Pf = Final nematode population in soil including roots at each sampling time.

<sup>#</sup>Figures of initial populations are not significantly different after arcsin transformation (Snedecor and Cochran, 1980).

and 9.0 cm<sup>2</sup>) and abamectin (8.6 and 9.0 cm<sup>2</sup>), in both seasons, respectively, with no significant differences among them, followed by the intermediate and smallest rates of the treatments. The smallest leaf area of mandarin trees was recorded in untreated trees (5.8 and 5.8 cm<sup>2</sup>) in the two seasons. However, there were no statistical differences in leaf area among the control, intermediate, or lowest application rates for the two formulations. The largest (and significant) average leaf area of the three rates was observed on trees treated with

Agerin® (7.8 and 7.7 cm<sup>2</sup> in 2007 and 2008, respectively). The average increases of leaf area of trees treated with abamectin were not significant when compared with the control in 2008.

*Effects on nutrition status.* Macro element content in the leaves of mandarin (Tables IV and V) was significantly increased by all treatments in both seasons except for Ca and P in the 2007 season. The highest N content of leaves resulted from the highest rate of

**Table IV.** Leaf area and leaf N and P contents of mandarin trees as affected by Agerin® and abamectin, in September of the 2007 and 2008 growing seasons, in a field infested with *T. semipenetrans*.

Treatment	Rate	Leaf area (cm <sup>2</sup> )		N (%)		P (%)	
		Per rate	Mean	Per rate	Mean	Per rate	Mean
<b>Season 2007</b>							
Agerin® (kg/fed.)	1	6.8 bc		1.9 d		0.12 f	
	2	7.2 bc	7.8 a	2.3 b	2.18 a	0.14c	0.14b
	3	9.4 a		2.3 a		0.15b	
Abamectin (ppm)	200	6.3 c		1.9 e		0.12e	
	400	6.5 c	7.1 ab	2.2 c	2.14 b	0.13d	0.14a
	800	8.6 ab		2.3 b		0.16a	
Untreated (control)	0.0	5.8 c	5.8 c	1.4 f	1.37 c	0.12e	0.12c
<b>Season 2008</b>							
Agerin® (kg/fed.)	1	6.9 ab		2.1 e		0.12e	
	2	7.3 ab	7.7 a	2.3 c	2.35 a	0.13c	0.13b
	3	9.0 a		2.7 a		0.13b	
Abamectin (ppm)	200	6.3 b		2.2 d		0.12d	
	400	6.6 b	7.3 ab	2.3 b	2.29 b	0.13c	0.13a
	800	9.0 a		2.4 b		0.14a	
Untreated (control)	0.0	5.8 b	5.8 b	2.0 f	2.05 c	0.11f	0.11c

Agerin®, followed by the highest concentration of abamectin. Phosphorus (P) (Table IV), potassium (K) (Table V) and magnesium (Mg) (Table V) contents showed gradual increases with increasing doses of both products in the two seasons. Calcium (Ca) content (Table V) increased slightly in both growth seasons.

With reference to micro elements, Agerin® increased Fe and Cu content in leaves more, compared to the untreated control, than did abamectin. In contrast, Mn and Zn leaf contents were greater in trees treated with abamectin than with Agerin® in both seasons (Table VI).

**Table V.** K, Ca and Mg contents of leaves (in dry weight) of mandarin trees as affected by Agerin® and abamectin in September of the 2007 and 2008 seasons, in a field infested with *T. semipenetrans*.

Treatment	Rate	K (%)		Ca (%)		Mg (%)	
		Per rate	Mean	Per rate	Mean	Per rate	Mean
<b>Season 2007</b>							
Agerin® (kg/fed.)	1	0.82d		1.84a		0.39f	
	2	1.01a	0.94a	1.87a	1.93a	0.41e	0.42b
	3	1.01a		2.09a		0.45b	
Abamectin (ppm)	200	0.74e		1.69a		0.42d	
	400	0.86c	0.84b	2.18a	2.03a	0.44c	0.44a
	800	0.92b		2.23a		0.46a	
Untreated (control)	0.0	0.60f	0.60c	1.84a	1.84a	0.38g	0.38c
<b>Season 2008</b>							
Agerin® (kg/fed.)	1	0.74e		1.97d		0.38e	
	2	0.96a	0.89a	2.06c	2.05a	0.41d	0.422b
	3	0.96a		2.11b		0.47b	
Abamectin (ppm)	200	0.79d		1.94e		0.39f	
	400	0.82c	0.82b	1.94e	2.00b	0.44c	0.45a
	800	0.84b		2.13a		0.51a	
Untreated (control)	0.0	0.62f	0.62c	1.79f	1.79c	0.37g	0.37c

**Table VI.** Micro-elements (ppm) content of mandarin leaves (in dry weight) as affected by Agerin® and abamectin in September of the 2007 and 2008 seasons, in a field infested with *T. semipenetans*.

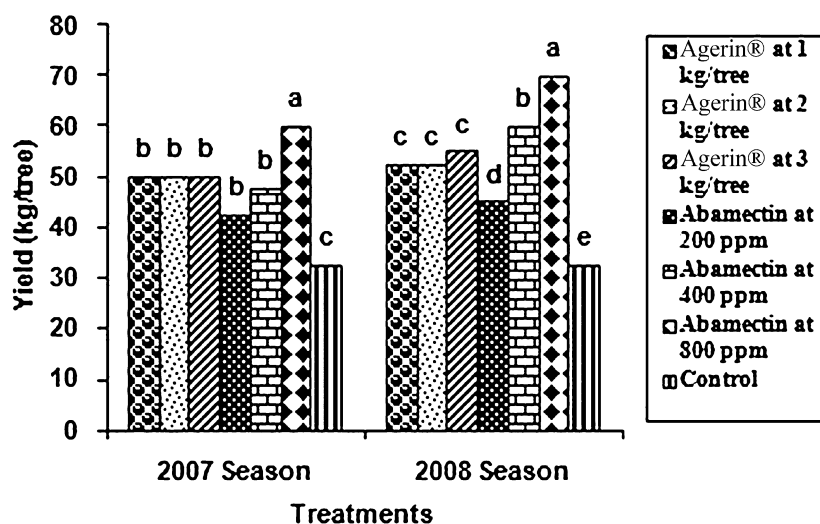
Treatment	Rate	Fe		Zn		Mn		Cu	
		Per rate	Mean	Per rate	Means	Per rate	Means	Per rate	Mean
<b>Season 2007</b>									
Agerin® (kg/fed.)	1	336.0c		18.5e		36.5f		6.0c	
	2	378.0b	371.5a	19.5d	19.8b	37.5e	37.8b	6.5b	6.5a
	3	400.5a		21.5b		39.5d		7.0a	
Abamectin (ppm)	200	338.5c		17.5e		41.0c		5.0e	
	400	363.5b	358.2b	20.5c	20.0a	43.0b	43.8a	5.5d	5.5b
	800	372.5b		22.0a		47.5a		6.0c	
Untreated (control)	0.0	334.0c	334.0c	16.0g	16.0c	35.5g	35.5c	5.0e	5.0c
<b>Season 2008</b>									
Agerin® (kg/fed.)	1	294.5a		19.0c		36.0f		5.0b	
	2	375.5a	355.2a	20.0c	20.3b	37.0e	36.5b	5.0b	5.5a
	3	395.5a		22.0b		37.5d		6.5a	
Abamectin (ppm)	200	309.0a		21.5b		39.0c		4.5c	
	400	326.5a	325.2b	22.0b	22.0a	42.5b	44.8a	5.0b	5.3b
	800	340.0a		24.5a		53.0a		6.5a	
Untreated (control)	0.0	255.5a	255.5c	18.0d	18.0c	32.5g	32.5c	4.0c	4.0c

*Effects on yield quantity and quality.* The yield (kg/tree) of mandarin increased with increasing doses of both products in the two seasons, except for Agerin® in the first season, when there were no differences among its rates (Fig. 3). The largest concentration of abamectin resulted in the greatest yield/tree, with average increases over the control of 84.6 and 115.4 %, in 2007 and 2008, respectively.

In both seasons, all treatments significantly increased the single fruit weight of mandarin trees (Table VII). The greatest fruit weights were found with the highest

and intermediate concentrations of abamectin: 167 and 163 g in 2007 and 169 and 168.5 g in 2008.

All treated trees produced more fruits/tree than untreated trees. The best performance was obtained with the highest concentration of abamectin, 359 and 414.2 fruits/tree in the first and second growing seasons, respectively, followed by Agerin®, although there were no statistical differences among these results. The other concentrations of abamectin came next but no significant differences were found between the two formulations (Table VII). The fewest fruits/tree were found in

**Fig. 3.** Yield of mandarin (kg/tree) as affected by different rates of Agerin® and abamectin, in a mandarin grove infested with *T. semipenetans* in the 2007 and 2008 seasons.

**Table VII.** Weight of a single mandarin fruit and numbers of mandarin fruits per tree as affected by Agerin® and abamectin in the 2007 and 2008 seasons, in a field infested with *T. semipenetrans*.

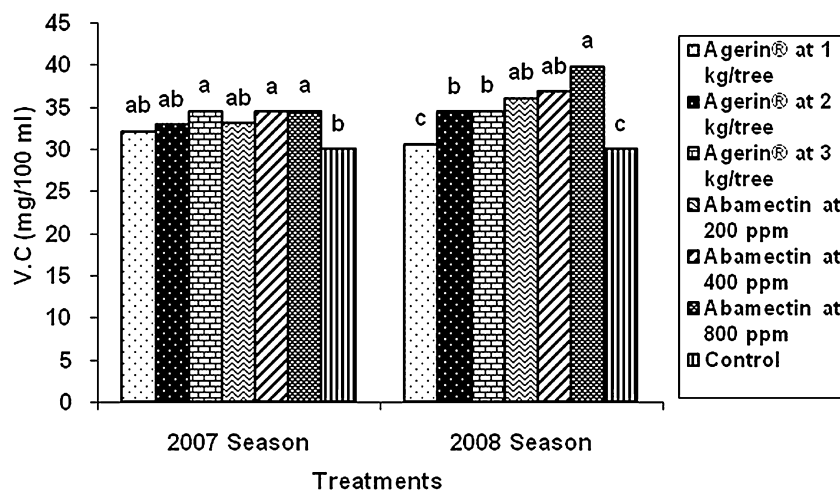
Treatment	Rate	Number of fruits /tree		Fruit weight (g)	
		Per rate	Mean	Per rate	Mean
<b>Season 2007</b>					
Agerin® (kg/fed.)	1	321.5b	318.5a	155.5b	157.8a
	2	317.5b		157.5b	
	3	316.5b		158.0ab	
Abamectin (ppm)	200	280.0c	310.2b	151.8b	160.6a
	400	291.4c		163.0a	
	800	359.5a		167.0a	
Untreated (control)	0.0	272.5c	272.5c	119.5c	119.5b
<b>Season 2008</b>					
Agerin® (kg/fed.)	1	334.8b	332.6a	156.8ab	160.4a
	2	327.5b		160.3ab	
	3	335.4b		164.0ab	
Abamectin (ppm)	200	290.3c	333.6a	155.0ab	164.2a
	400	296.7c		168.5a	
	800	414.2a		169.0a	
Untreated (control)	0.0	259.0d	259.0b	125.0b	125.0b

untreated trees, 272.5 and 259 in 2007 and 2008, respectively.

All treatments significantly increased TSS% in fruit juice of mandarin in both seasons (Table VIII), which ranged from 9.4 to 10.9% in 2007 and from 9.7 to 11.1% in 2008 compared with 8.2 and 8.4% in the untreated trees, respectively. The greatest TSS percentages (10.9 and 10.6%) were obtained with the highest and the intermediate rates of abamectin in first season and the highest rates of Agerin® (11.1%) and abamectin (10.9) in the second season, without significant differ-

ences between them. These results were followed by the lowest concentration of abamectin (10.1%) and the largest and intermediate rates of Agerin® (10.0 and 9.9%) in 2007. Fruit quality was also improved by the intermediate and lowest concentrations of abamectin and the intermediate rate of Agerin® in 2008. The lowest rate of Agerin® came next, 9.4 and 9.7% in 2007 and 2008, respectively. Significantly lower values of TSS% (8.2 and 8.4%) were found in the controls in both seasons.

The highest and the intermediate doses of both com-



**Fig. 4.** Yield of mandarin (kg/tree) as affected by different rates of Agerin® and abamectin, in a mandarin grove infested with *T. semipenetrans* in the 2007 and 2008 seasons.



**Table VIII.** Fruit quality of mandarin as affected by Agerin® and abamectin in 2007 and 2008, in a field infested with *T. semipenetrans*.

Treatment	Rate	TA (%)		TSS (%)	
		Per rate	Mean	Per rate	Mean
<b>Season 2007</b>					
Agerin® (kg/fed.)	1	0.83bc		9.4c	
	2	1.07a	1.0a	9.9b	9.80b
	3	1.10a		10.0b	
Abamectin (ppm)	200	0.90bc		10.1b	
	400	0.99ab	0.96ab	10.6a	10.53a
	800	1.00a		10.9a	
Untreated (control)	0.0	0.87c	0.87b	8.2d	8.20c
<b>Season 2008</b>					
Agerin® (kg/fed.)	1	0.77c		9.7c	
	2	0.97b	0.98c	10.1b	10.3a
	3	1.21a		11.1a	
Abamectin (ppm)	200	0.96b		9.8b	
	400	1.04b	1.06a	10.1b	10.3a
	800	1.21a		10.9a	
Untreated (control)	0.0	1.04b	1.04b	8.4d	8.4b

TSS% = Percentage of total soluble solids.

TA% = Percentage of total acidity.

pounds in the first season and the highest doses of the two compounds in the second season gave the greatest values of TA% (Table VIII). The lowest rate of Agerin® produced a TA% value significantly lower than that of the control in the 2008 season.

Both products resulted in gradually increasing ascorbic acid (VC) content (mg/100 ml) with increasing application rates (Fig. 4). The greatest VC contents were found in mandarin fruits treated with the highest and intermediate concentrations of abamectin and the highest rate of Agerin® (34.5, 34.4 and 34.5 mg/100 ml), respectively, in the 2007 season, while the highest rate of abamectin gave the best result (39.8 mg/100 ml) in 2008. Low values were found in the control treatments (30.0 and 30.6 mg/100 ml in 2007 and 2008) in both seasons.

## DISCUSSION

The commercial formulations of Agerin® and abamectin were effective in suppressing the citrus nematode population density associated with mandarin trees, and Agerin® lowered the citrus nematode population in both soil and roots. These results agree with those obtained by Ignoffo and Dropkin (1977), Ismail and Fadel (1999) and El-Nagdi and Youssef (2009). It may be assumed that the exotoxins from the Bt strain are responsible for the observed nematicidal activity. Beta exotoxins from Bt strains have been shown to pos-

sess nematicidal effect against certain plant parasitic nematodes (Prasad *et al.*, 1972; Ignoffo and Dropkin, 1977). Delta exotoxins released into the soil upon lysis of bacterium crystals are known to provide the active principle acting against *Meloidogyne incognita* (Kofoid *et al.* White) Chitw. (Tohamy *et al.*, 1995). Another Bt commercial product ("Protecto") suppressed the citrus nematode more than horse manure or marigold dry leaf powder (El-Sherif *et al.*, 2006). Sampson and Gooday (1998) found that *B. thuringiensis* subsp. *israelensis* IP576 and subsp. *aizawai* HD133 both secreted exochitinase enzymes when grown on a medium containing chitin. Also, Soliman (2008) stated that the wild type of chitinolytic bacterium Bt and three mutants (nos 24, 10 and 32, obtained by UV irradiation) suppressed hatching of *M. incognita* egg masses and caused a high degree of percentage mortality of juveniles.

Abamectin was also effective in controlling *T. semipenetrans* in mandarins. This may be due to abamectin in nematodes functioning as antagonists or stimulators of  $\gamma$ -amino butyric acid released from pre-synaptic inhibitory terminals (Tumer and Schaeffer, 1989). Nordmeyer and Dickson (1989) observed that, after 24 hr of treatment with avermectins, the  $J_2$  of *M. javanica* (Treub) Chitw. showed no movement. This was correlated with a reduction in oxygen uptake by the  $J_2$ . They suggested that the mortality of nematodes was probably due to the additive effects of lack of oxygen and the toxicity of the bio-products. These properties of this bio-product would prevent the movement of the nema-

todes towards the host plants.

The enhancement of mandarin growth in terms of leaf area by Agerin® and abamectin may be attributed to possible effects of these products in stimulating the biosynthesis of organic materials, especially carbohydrates and proteins, and enhancement of the formation and movement of natural hormones, which are vital to improved cell division, especially in the meristematic tissues (Nijjar, 1985). Our findings agree with those obtained by Nomier (2000), Hafez (2001) and Mohamed and Hafez (2004).

Improvement in nutrient status following the treatment with these two products may be due to faster absorption of the nutrients via roots (Ahmed *et al.*, 1997; Atawia and El-Desouky, 1997; Abdalla *et al.*, 1998). The improvement that occurred in mandarin leaf area and nutritional status surely reflected the effects of these formulations in improving yield, number of fruits, fruit weight (El-Nagdi and Youssef, 2009) and quality (Nijjar, 1985), thus agreeing with Mansour (1998)

In summary, both Agerin® and abamectin appear promising for the management of *T. semipenetrans* in citrus. Furthermore, both commercial products have low associated production costs and are considered environmentally safe and not hazardous to humans.

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