Research Article

Comparison of Eco-Friendly Control Strategies against Root-Knot Nematode in Relation to Growth and Fruit Yield of Pomegranate Orchards, with Special Reference to Structure of Plant-Parasitic Nematodes Community

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Abstract | Root-knot nematodes represent one of the most important plant pathogens in most Pomegranate cultivation regions. By studying the structure of the plant-parasitic nematodes community inhabiting the rhizosphere of Pomegranate fields, it was found that *Meloidogyne* is the most common genus and harmful one. So, the efficacy comparative of Chitosan, Marjoram emulsion oil, *Trichoderma asperellum*, and Vermicompost as alternative eco-friendly control agents for root-knot nematode *M. incognita* infected Pomegranate were evaluated under greenhouse and open field conditions. Results indicated the ability of tested strategies to suppress *M. incognita* after being applied three times. Under protected conditions the pest reduction percentages of *M. incognita* and increase percentages in shoot dry weight were associated with the use of Marjoram emulsion oil (82.5, 208%), Chitosan (75.3, 105.8%), and Vermicompost (72.1, 93.4%) after chemical nematicide (93.0, 424.3%). Whereas, under field conditions, the application of Chitosan achieved the greatest reduction percentage (82.2%), followed by Marjoram emulsion oil (71.2%), compared to the nematicide Nemaphos (83.5%). This effect has been shown significantly in the plant growth, fruit yield, and the quality of the extracted juice (pH, TSS, vitamin C, protein, carbohydrates, and fatty acids). Treatments of Marjoram emulsion oil, Chitosan, and Vermicompost recorded the best increase percentages after the chemical nematicide, compared to control. It could be concluded that, the use of tested eco-friendly agents in the control strategies resulted from an effective alternative strategy in the suppression of the root-knot nematode and other plant-parasitic nematodes.

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Introduction

Pomegranate (*Punica granatum*) is a fruit-bearing woody plant belonging to the family, Lythraceae, subfamily Punicoideae. Pomegranate is widely cultivated under different climatic and soil conditions (da Silva *et al*., 2013). Pomegranate is able to thrive in arid environments, so it is considered an economical commercial horticultural crop especially in the Middle East region, North and Tropical Africa, Central Asia, and Mediterranean basin (Chandra *et al*., 2010). In Egypt as a subtropical region with a climate typified by lengthy hot summers and low air humidity, the cultivated area reached 79.4 thousand acres in the 2021 season (CAPMAS, 2021). Several previous investigations have found that phytonematodes infest most Pomegranate orchards (Katooli *et al*., 2021; Poornima, 2020; Singh *et al*., 2019) where many parasitic genera were found, such as *Meloidogyne* sp., *Pratylenchus* sp., *Paratrichodorus* sp., *Helicotylenchus* sp., *Rotylenchulus* sp., *Tylenchorhynchus* sp., *Longidorus* sp., *Xiphinema* sp., and *Criconemella* sp. (Pradhan *et al*., 2020; El-Qurashi *et al*., 2019; Ibrahim *et al*., 2010). The root-knot nematode, *Meloidogyne* spp. management is more challenging than that of other pests due to a diverse group of hosts, short life span, and adaptability to a variety of environmental contexts (Mandal *et al*., 2021; Mitiku, 2018). For more than 50 years, the chemical nematicides have been effective as practices to control plant-parasitic nematodes (PPNs) diseases (Chen *et al*., 2020). Due to the detrimental effects of chemical pesticides on humans and the environment, clean agriculture is becoming more popular as an alternative strategy (Rani *et al*., 2021; Tudi *et al*., 2021). Therefore, it was necessary to create new safety and inexpensive economic strategies to reduce nematode damage, whether through direct influence or by increasing the plant's ability to compensate for the loss resulting from the damage (Desaeger *et al*., 2020; Oka *et al*., 2000; Stirling, 2018). The use of compounds such as Vermicompost, which depend on increasing plant health by raising the levels of some enzymes responsible for plant resistance to biological stresses resulting from plant pathogens such as amylase, lipase, and protease, is a promising approach in the integration of sustainable control strategies (Mandal *et al*., 2021; Chanu *et al*., 2018; Rasmann *et al*., 2012). On the other hand, bio-agents have proven their effectiveness as a tool for combating root-knot nematode (Tariq *et al*., 2021; Temitope *et al*., 2020). Several species of *Trichoderma* (Deuteromycetes, Dematiaceae) are commonly utilized as commercial formulations of biological control and plant growth promoters (Pocurull *et al*., 2020). The common saprophytic fungi, *Trichoderma asperellum* as a microbial inoculant is an effective eco-friendly alternative method of controlling plant pathogens (Temitope *et al*., 2020; dos Santos *et al*., 2021). In this aspect, essential oils such as Marjoram emulsion oil (MEO), *Majorana hortensis*, are emerging as an effective type of eco-friendly product with nematicidal properties (D'Addabbo and Avato, 2021; El-Ashry *et al*., 2021; Klein *et al*., 2020; Saroj *et al*., 2020). Especially if it's in a more stable form and transmittance, like emulsions (Boyko and Brygadyrenko, 2021; Sharar *et al*., 2017). Waste of bio-origin as by-products are currently gaining more attention due to their suppression of several plant pathogens, such as root-knot nematodes (Khan *et al*., 2021). Chitosan as a by-products of the shell of crabs through crustacean farming has been noted as a product to impact the plant's systemic resistance against root-knot nematodes (Giannakou *et al*., 2020; Radwan *et al*., 2012).

Therefore, the current study aims to compare some eco-friendly strategies in the control of root-knot nematode, *Meloidogyne incognita,* infecting Pomegranate orchards, to chemical control, under greenhouse and open field conditions.

Materials and Methods

The nematicidal effects of some eco-friendly control agents: Marjoram emulsion oil, three commercial bio-agents, Vermicompost with *Trichoderma asperellum*, and Chitosan oligosaccharide, were evaluated compared to the chemical nematicide Nemaphos against the root-knot nematode and the subsequent impact on plant growth parameters of Pomegranate plants in greenhouse and field experiments. The chemical composition was used to determine each treatment's induced resistance (IR).

Root-knot nematode preparation

Root-knot nematode, *Meloidogyne incognita* eggs were isolated from egg-masses on the roots of the tomato plant according to Hussey and Barker (1976). The eggs were transferred to Petri dishes (100mm x15mm) and incubated at room temperature (28±2°C) for three days. The hatched second-stage juveniles (J2) were acquired by using the Baermann plate technique

(Seinhorst, 1962). A calculated suspension containing 1000 active juveniles was used as an inoculum.

Preparation of tested treatments

Vermicompost®: A native commercial product of Vermicompost contains some enzymes (such as Amylase, Lipase, Glycolytic digesting enzymes, and Protease enzymes), in addition to earthworms; tiger worms (*Eisenia fetida* Savigny,1826), African night crawler (*Eudrilus eugeniae*) and loaded on some organic matter (animal and plant wastes). The product provided by Miegos Company, Egypt and applied at the recommended rate (14 kg /tree).

Agre-Viva®: A commercial bio-product composition of *Trichoderma asperellum* contained 10% nitrogen, 10% phosphorus, and 10% potassium provided by View Green Company, Egypt, and used at the recommended rate (1L / Feddan).

BioHope®: A commercial bio-product named chitosan (extracted from the shell of crabs) was obtained from Tagrow Company, Egypt, and used at the recommended rate (400g / Feddan).

Marjoram emulsion oil (MEO): Marjoram oil was obtained from Harraz Company for the food industry and natural products, Egypt. The emulsion essential oil was created by combining one volume of MEO (2.5%) with two volumes of surfactant [polyethylene glycol dioleate (non-ionic surfactant) + Toximol (ionic surfactant)] and water. At room temperature, the mixture was vortexed numerous times and subjected to stability testing and specifications. After two days the Stability was tested according to Muschiolik's protocol (Muschiolik *et al*., 2006). The transparency of formulation was determined by Date and Nagarsenker's protocol (Date and Nagarsenker, 2008).

Chemical nematicide

A commercial product of organophosphate systemic nematicide; Nemaphos 40 % EC, common name: Thionazin, chemical composition: Ethyl- 3methyl -4-(methyl thiophenyl)-methylethyl was applied at the rate of 3L / Fadden.

In vitro experiment

Using five rates, the inhibitory effect of Marjoram emulsion oil (MEO) on *M. incognita* second-stage juveniles (J2) were examined (1000, 2000, 3000, 4000, and 5000ppm). Second-stage juveniles were extracted, numbered, and concentrated in suspension until they reached 100 J2 in 1 ml of distilled water, according to Demeure and Freckman's (1981) methods. One ml of juvenile suspension was deposited in screw-capped test tubes containing 5 ml of various rates of tested materials and incubated at 25±2°C for 3 days, then, the number of active and inactive juveniles were counted using a nematode counting slide at 24, 48, and 72 hours (Hussey, 1973). Each treatment was repeated five times, with distilled water serving as the control. The revised mortality percentages were calculated using the Schneider-Orelli formula (Schneider and Orelli, 1947):

$$\text{Corrected mortality \%} = \left(\frac{\text{Number of dead juveniles in treatmentl} - \text{Number ofdead juveniles in control}}{[100 - \text{mortality percentage in the negative control}] \times \text{Number ofdead juveniles in control}}\right) \text{x}100$$

Pots experiment

The nematicidal characteristics of MEO at 5000 ppm and Vermicompost, *Trichoderma asperellum*, and Chitosan, were compared against the root-knot nematode, *M. incognita,* and the influence on Pomegranate growth parameters.

One seedling of Pomegranate cv. Manfaluty (6 month-old) was planted in 20 cm diam Thirty pots containing a sterilized mixture of clay and sandy soil (1:1 w/w), each pot contained 3k, in addition, five pots were filled with 750 g of Vermicompost and 2250 g of soil per pot, which were combined in a 1:1 ratio (sterile soil with sand), and irrigated immediately.

The Pomegranate seedlings were infected with 1000 newly hatched juveniles of *M. incognita* for each plant after one week. Each application was repeated five times, with the application MEO at 5000 ppm rates being applied three times after inoculation (first, third, and fifth weeks). Treatment by *T. asperellum* a rate 3.3 ml /pot and chitosan applied at 1.2g / pot as the same applications. Majorana emulsion oil was applied three times, with nematicide Nemaphos 40% EC (6 ml/100 ml water). All treatments were applied as a soil drench. Five inoculated pots were left without adding any materials as a negative control, in addition to another five replicated healthy seedlings without inoculation with nematodes as a positive control. All pots were arranged in the greenhouse at 27±5°C in a randomized block design. After fifty days of inoculation, the plants were harvested. Juveniles of *M. incognita* were extracted from soil by sieving and modified Baermann technique (Seinhorst, 1962). Roots were stained by acid fuchsin (Bybd *et al*., 1983) and examined under a stereomicroscope for counting developmental stages, females, galls, and egg-masses. Root galling or egg masses numbers were rated on a scale of 0-5 where 0= no galls or egg masses, 1= 1-2 galls or egg masses, 2= 3-10 galls or egg masses, 3= 11-30 galls or egg masses, 4= 31-100 galls or egg masses, 5= more than 100 galls or egg masses per root system (Taylor and Sasser, 1978).

Open field experiment

Before the beginning of the experiment, the nematode samples were taken from the infected soil of pomegranate orchards and used as a zero-day. The open-field experiment was carried out in three locations: South Tahrer, Wadi Al-Natroun, and Nubariya of El-Beheira Governorate, with three associated villages as follows:

1. South Tahrer: Umm saber, Omar Shaheen, and Omar Makram.
2. Wadi Al-Natroun: Banaa Salama, Alhamra, and Alwadaa Alfarigh.
3. Nubariya: Elshaaraw, Naguib Mahfouz, and Abu Bakr Alsidiyq.

The investigation was conducted during the season of 2021, from the beginning of the natural growing season to an assessment of the nematodes community, (as shown in Table 3). Ten samples (250g) were monthly taken from the start of the Pomegranate tree's activity (February) until the fruit yield was harvested (July), from each of the above-mentioned areas.

The investigated trees were 5 years old and spaced (4x3m) apart, and irrigated with a drip irrigation system. On soil naturally infested with *M. incognita*, the same treatments employed in the pot experiment were repeated. In February and March of 2021. Each treatment is made up of five duplicates, each with ten trees. All applications were made to the soil as a soil drench. Except for Nemaphos, all therapies were applied three times monthly, and samples were obtained two, four, and six months later.

The root-knot nematode was extracted from soil by Cobb's sieving method (Cobb, 1918) and modified Baermann funnel method (Baermann, 1917). According to Byrd *et al.* (1983) roots were colored with acid fuchsine in lactic acid and microscopically analyzed for counting root-knot nematode developmental stages and females. The reproduction factor (Rf) is calculated for each treatment by dividing the final population (Pf) by the initial population (Pi).

Chemical analysis of juice

For the study, fifteen mature Pomegranate fruits from each treatment were split into three repetitions. There are 5 fruits in each refined. An electric juicer was used to remove the Pomegranate seeds from the juice solution and store the juice so that, the following properties could be analyzed:

pH: A pH meter was used to determine the hydrogen ion concentration in the juice solution (Mousavi et.al., 2011).

Total soluble solids (TSS) of pomegranate juice: The technique defended by Preece and Moersfelder (2016) was used to determine total soluble solids (TSS) in the juice.

Vitamin C: Vitamin was estimated in the prepared Pomegranate juice according to El-Sharaa and Mussa (2019).

Crude protein and total carbohydrate: To estimate the crude proteins, Bradford's method (Bradford, 1976) was used. And Hodge and Hofreiter's methods (Hodge and Hofreiter, 1962) were used to determine the total carbohydrate content.

Fatty acid isolation and extraction: Using processes for synthesizing fatty acid methyl esters (FAME) from lipids and Pomegranate juice, the fatty acids in the extracted Pomegranate juice were identified using gas chromatography technique (ISO 12966, 2015).

Data analysis

All of the experiments were examined separately. Duncan's Multiple Range Test (DMRT) was used to compare the treatment means (Gomez and Gomez, 1984). WASP1 was used for the analysis, with the crucial difference set at P= 0.05 and the results interpreted.

Results and Discussion

In vitro: Evaluation of Marjoram emulsion oil viability of root-knot nematode, M. incognita

As shown in Figure 1, the inhibitory effect of five concentrations (1000, 2000, 3000, 4000, and

5000 ppm) of Marjoram emulsion oil (MEO) on the mortality of *Meloidogyne incognita* juveniles was concentration-dependent, i.e. the nematode's mortality increased as the concentration and exposure time of the MEO increased. After 24 hours of exposure, the highest proportion of juvenile mortality has occurred at 4000 and 5000 ppm concentrations (84.89 and 100 percent, respectively). After 72 hours of exposure, however, both 3000 and 4000 ppm. MEO exhibited a significant mortality rate, with percentages of 95.9 and 100.0 %, respectively. While the activity of *M. incognita* juveniles were reduced by 35.07 % after 24 hours and 69.96 % after 72 hours when exposed to MEO at 1000 ppm.

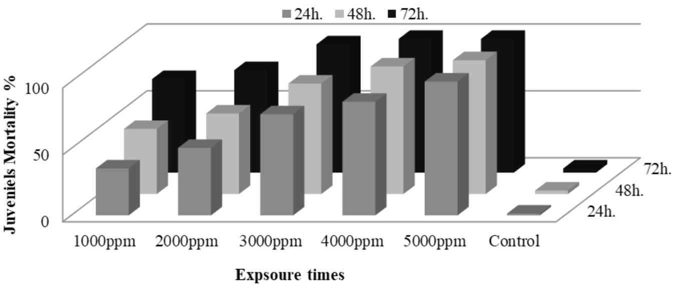

Figure 1: *Effect of five concentrations of MEO on M. incognita juvenile mortality after three intervals of exposure time (In vitro).*

Greenhouse experiments

Comparative efficacy of Marjoram emulsion oil, bio-agnate, Chitosan, Vermicompost and chemical nematicide on *M. incognita* infecting Pomegranate seedlings under greenhouse conditions: The effects of some control strategies; MEO, bio-agnate, Chitosan and Vermicompost as an alternative to chemical nematicide for control the root-knot nematode, *M. incognita* infected pomegranate seedlings were evaluated under greenhouse conditions.

The results indicated that all treatments had a significant effect on the reduction of *M. incognita* criteria. Data in Table 1 show that the criteria of *M. incognita* such as numbers of galls, egg-masses, females/ root system of Pomegranate seedling, and rate of build-up were suppressed by application of Vermicompost, *Trichoderma asperellum*, chitosan, and MEO. Whereas, Nemaphos showed a more suppressive effect on nematode reproduction (RF=0.28), being 93.0% reduction when compared to nematode treatment alone. All treatments dramatically reduced nematode final populations when compared to nematode alone, with average values of decrease ranging from 69.6% for *T.asperellum* treatment to 82.5% with MEO treatment. Nemaphos treatment resulted in the lowest number of galls (29.2 galls/root), while the treatment with Chitosan (89.3 galls/root).

On the other side, the results presented in Table 2 revealed that all the treatments improved plant growth parameters as indicated by the length and fresh weight of root and shoot as well as shoot dry weight when compared to nematode treatment alone. Nemaphos systemic nematicide exceeded the other treatments, with a percentage increase in length of shoots and roots as well as total plant fresh weight and dry shoot weight of 79.9, 201.0, 437.5, and 424.3 %, respectively when compared to nematode treatment alone.

However, in terms of percentage increase of plant fresh weight and shoot dry weight, after nematicide (437.5%, 424.3%), the MEO treatment occupied the second effective treatment (305.2%, 208.0%), followed by Chitosan (56.25%, 105.8%), and Vermicompost (29.1%, 93.4%), and in the last impact was treated by and *T. asperellum* (17.7%, 44.3%).

Table 1: *Efficacy of Marjoram emulsion oil and bio-agents on the developmental and reproduction of M. incognita infected Pomegranate plant under greenhouse conditions (27 ± 3 °C).*

Treatment	Nematode population			Final population (Pf)	RF*	Red. %	No. of galls	RGI	No. of Egg masses	EI
	J2/ 250g soil	Root								
		Develop. stages	Females							
Vermicompost	958.4[b]	72.3b	65.2b	1095.9b	1.1	72.1	124.3[b]	5.0	49.3b	4.3
T. asperellum	1032.6	87.2b	75.4b	1195.2b	1.19	69.6	132.1[b]	5.0	60.4b	4.7
Chitosan	848.2[c]	65.3c	54.8c	968.3c	0.97	75.3	89.3[c]	5.0	35.1c	4.1
Marjoram emulsion oil	565.3[d]	81.2d	41.5d	688.0d	0.70	82.5	87.5[d]	5.0	27.2d	3.8
Nemaphos	230.6[e]	16.4e	26.2e	273.2e	0.28	93.0	29.2[e]	3.2	18.3e	3.1
Nematode	3635.3[a]	203.2a	89.4a	3927.9	3.9	--	259.7[a]	5.0	76.5a	5.0
LSD 5 %	50.10	17.17	11.51	56.14	--	--	27.75	-	9.77	-

*Each value presented the mean of five replicates. M. incognita (1000 larva/ plant). Means in each column followed by the same letter(s) did not differ at P≤ 0.05 according to Duncan`s multiple range test. * Reproduction factor (RF) = final population/initial population.*

Table 2: *Efficacy of emulsion oil, bio-agnates and chemical nematicide on growth parameters of Pomegranate plant infested with M. incognita infesting under greenhouse conditions (27±3°C).*

Treatments	Plant growth response								Shoot dry wt. (g)	increase %
	Length (cm)				Plant fresh wt.(g)					
	Shoot	Inc.%	Root	Inc.%	Shoot	Inc. %	Root	Inc. %		
Vermicompost	70.0^{de}	31.6	13.5^{e}	39.2	124.0^{cd}	29.1	13.5^{d}	28.6	79.5^{de}	93.4
T. asperellum	73.3^{cd}	37.8	16.8^{d}	73.2	113.0^{d}	17.7	15.8^{cd}	50.5	59.3^{f}	44.3
Chitosan	76.0^{c}	42.9	15.5^{d}	59.8	150.0^{c}	56.25	16.8^{c}	60.0	84.6^{d}	105.8
Marjoram emulsion oil	74.3^{cd}	39.7	21.2^{c}	118.6	389.0^{b}	305.2	22.9^{b}	118.1	126.6^{c}	208.0
Nemaphos 40%	95.7^{a}	79.9	29.2^{a}	201.0	516.0^{a}	437.5	26.6^{a}	153.3	215.5^{a}	424.3
Control	90.0^{b}	69.2	24.0^{b}	147.7	402.0^{b}	318.8	23.1^{b}	120.0	147.0^{b}	257.7
Nematode only	53.2^{e}	0.0	9.7^{f}	0.0	96.0^{e}	0.0	10.5^{e}	0.0	41.1^{g}	0.0
LSD	3.67	-	2.10	-	34.53	-	1.75	-	14.6	-

Each value presented the mean of five replicates. Means in each column followed by the same letter(s) did not differ at P≤ 0.05 according to Duncans multiple range test.

Table 3: *Structure of phytonematodes community inhabiting the rhizosphere of Pomegranate trees in different localities of Beheira Governorate.*

Phytonematodes Genera	South Tahrer n=150						Wadi Natrun n=150						Nubaria n=150						Mean of total prevalence	
	Umm saber n=50 cv. Altaayifaa		Omar Shaheen n=50 cv. Altaayifaa		Omar Makram n=50 cv. Altaayifaa		Banaa Salama n=50 cv. Manfaluty		Alhamra n=50 cv. Manfaluty		Alwadaa Alfarigh n=30 cv. Manfaluty		Elshaarawy n=50 cv. Wonderful		Naguib Mahfouz n=50 cv. Wonderful		Abu Bakr Alsidiyq n=50 cv. wonderful			
	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %	PD	FO %
Aphelenchus	58	64	17	40	0.0	0.0	32	50	0.0	0.0	14	18	39	48	17	26	8	14	20.56	28.9
Criconemella	87	84	36	60	17	32	0.0	0.0	95	74	21	24	123	88	85	78	32	38	55.1	53.1
Helicotylenchus	123	88	176	16	97	78	243	24	139	18	115	8	56	34	118	82	79	72	109.67	46.7
Longidorus	22	40	0.0	0.0	30	58	0.0	0.0	16	26	9	14	32	60	43	78	11	22	18.1	32.0
Meloidogyne*	2245	100	980	96	1462	100	780	100	543	94	590	92	2478	100	3653	100	3196	100	1769	98.0
Paratrichodorus	0.0	0.0	0.0	0.0	13	26	5	6	0.0	0.0	10	14	20	34	0.0	0.0	8	18	6.2	10.9
Pratylenchus	15	26	22	38	76	66	0.0	0.0	27	36	0.0	0.0	80	72	43	70	77	82	37.8	43.3
Rotylenchulus	327	92	237	100	487	82	578	84	423	78	879	94	574	82	240	72	670	78	490.6	84.7
Tylenchorhynchus	182	100	84	92	134	78	108	82	149	100	86	72	203	88	128	100	275	100	149.9	90.2
Xiphinema	27	40	0.0	0.0	56	86	32	54	17	30	9	18	28	38	56	82	0.0	0.0	25.0	38.1

**: Larvae form; PD: Population density (number of individuals/200g.soil); FO: Frequency of occurrence.*

Table 4: *Efficacy of emulsion oil and commercial bio-products on M. incognita infected pomegranate after two, forth and six month of application under field conditions.*

Treatments	Two month			Four month			Six month		
	Final population	*RF	Red. %	Final population	*RF	Red. %	Final population	*RF	Red. %
Vermicompost	1384.7^{b}	0.47	69.4	1587.1^{b}	0.54	67.1	1831.7^{b}	0.62	63.8
T.asperellum	1272.0^{c}	0.43	71.9	1440.6^{c}	0.49	70.2	1618.0^{c}	0.55	68.0
Chitosan	618.8^{e}	0.21	86.3	713.3^{e}	0.24	85.1	900.4^{e}	0.30	82.2
Marjoram emulsion oil	1090.8^{d}	0.37	75.9	1331.4^{d}	0.45	72.4	1460.5^{d}	0.49	71.2
Nemaphos	442.2^{f}	0.15	90.2	592.5^{f}	0.2	87.6	829.7^{f}	0.28	83.5
N.alone	4528.7^{a}	1.5	--	4824.7^{a}	1.6	--	5063.9^{a}	1.70	--
LSD 5%	64.34	--	--	72.24	--	--	80.0		--

*Number of initial population = 2970 / 250g. soil and root. * Reproudction factor (RF) = final population/initial population. Means in each column followed by the same letter (s) didn't differ at P≤ according Duncans multiple range test.*

Table 5: *Effect of tested bio-agents against M. incognita on the fruit yield of pomegranate trees.*

Treatment	Vermicompost	*T. asperellum*	Chitosan	Marjoram emulsion oil	Nemaphos	Control
Fruit weight (kg/tree)	18.53d	16.42e	21.45c	23.91b	28.36a	9.12f
Increase %	103.18	80.04	135.20	162.17	210.96	-

Means in each column followed by the same letter(s) did not differ at P ≤ 0.05 according to Duncan`s multiple range test.

Open field experiment

Structure of plant-parasitic nematodes community inhabiting the rhizosphere of pomegranate fields in different localities of El-Beheira Governorate: The structures of plant parasitic nematodes community inhabiting the rhizosphere of Pomegranate fields in different localities at Beheira Governorate were classified into three regions South Tahrer, Wadi Natrun, and Nubaria through three distracts for each. The data shown in Table 3 indicated that the Pomegranate parasitic nematode community consists of ten genera belonging to seven families, these genera were, *Aphelenchus* (Aphelenchoididae), *Criconemella* (Criconematidae), *Helicotylenchus* (Hoplolaimidae), *Longidorus* (Longidoridae), *Meloidogyne* (Meloidogynidae), *Pratylenchus* (Pratylenchidae), *aratrichodorus* (Trichodoridae), *Rotylenchulus* (Hoplolaimidae), *Tylenchorhynchus* (Tylenchorhynchidae) and *Xiphinema* (Longidoridae).

Generally, the study noted a marked variation in the community that was associated with tested cultivars of Pomegranate and the nature of the soil in the study locality. Where some genera were absent and associated with cv. Altaayifaa in some localities such as absence *Paratrichodorus* spp. in Umm Saber distract, and *Xiphinema* spp in Omar Shaheen distract. Also in *cv.* Manfaluty, absence of *Aphelenchus* spp. and *Paratrichodorus* spp. in Alhamra distract and *Criconemella* spp. and *Longidorus* spp. in Banaa Salama distract. Based on population density and frequency of occurrence as the index of prevalence, the genus of *Meloidogyne* ranked in first-order (1769, 98.0 %) as the dominant genus, followed by *Tylenchorhynchus* (149.89, 90.22%). In the middle-ranked were *Pratylenchus*, *Rotylenchulus*, *Criconemella*, and *Helicotylenchus* genera. While in the last ranked with a significant difference came both of *Xiphinema* spp. (25.0, 38.11%), *Longidorus* spp (18.11, 32.0%), *Aphelenchus* spp. (20.56, 28.89%) and *Paratrichodorus* spp. (6.22, 10.89%), respectively.

Comparative efficacy of emulsion oil and commercial bio-products on *M. incognita* infected pomegranate trees after two, forth and six months of application under open field conditions: In the natural growing season of 2021, at the newly reclaimed land in Nubariya, Naguib Mahfouz village, Beheira Governorate, an open-field experiment was done on 5-year-old Manfaluty Pomegranate trees are naturally infested with *M. incognita*. Data are shown in Table 4 indicated that the treated with Vermicompost, *T. asperellum*, Chitosan, and MEO all have a nematicidal activity against root-knot nematode. Where, Chitosan treatment achieved the best result in the suppression of root-knot nematode population after six months of use, with an 82.2 % reduction, when compared to nematode treatment alone. Meanwhile, *T. asperellum* and Vermicompost were the least ranked (68.0 and 63.8 % reduction, respectively), while MEO was the most popular (68.0 and 63.8 % reduction, respectively) (71.2 %). Nonetheless, when compared to nematode therapy alone, Nemaphos injection significantly reduced the final population, with a reproduction percentage of 83.5%.

Effect of tested strategies on fruit yield and pomegranate juice quality

Results presented in Table 5 show that all tested treatments resulted in a significant increase in total Pomegranate fruit weights, compared to control. Nemaphos had the highest percentage of fruit weight increase (210.96 %), while Majoram, Chitosan, and Vermicompost treatments gave a relatively lower percentage of yield increase (162.17, 135.20, and 103.18 %, respectively) when compared to untreated plants (9.12kg/tree), *T.asperellum* caused the lowest increase in overall fruit weight (16.84 kg fruit/tree) with a percentage increase (80.04%).

From each treatment, fifteen ripe Pomegranate fruits were divided into three replicates for this study. Each repetition contains 5 fruits. The juice was extracted using an electric juicer. Pomegranate juices are used to conduct chemical analyses on them, such as determining acidity pH, which revealed that Pomegranate juice is acidic, ranging from 3.88 Nemaphos in the case of treated fruits to 3.41 in the case of untreated fruits (control) Table 6. As for TSS percentages in juice, Nemaphos treatments had the highest proportion of TSS (18.57%) followed by MEO (16.97%), and the lowest was *T.asperellum* treatment with 14.17 % (Figure 2).

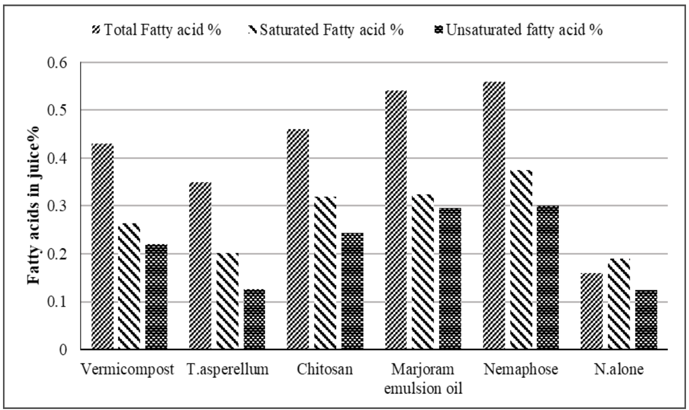

Figure 2: *Influence of Marjoram emulsion oil and three commercial bio-agents on total, saturated and unsaturated fatty acids of pomegranate juice as affected by M. incognita infection.*

The highest content of vitamin C in Pomegranate juice, measured in mg/100ml, was found in the juice produced from Nemaphos treatment, followed by Chitosan (11.66 and 11.23 mg/100ml) As for percentages of total protein, it was 3.92% in the treatment of MEO and 3.32% and 3.01% in the Chitosan and Vermicompost treatments, respectively. Regarding the percentage of total carbohydrates in Pomegranate juice, it was found that Nemaphos treatment had the highest percentage of total carbohydrates at 52.73%, while the fruits of the untreated plants (untreated) had the lowest percentage at 30.52% (Table 6). Results in Table 7 and Figure 2 show that the treatment of Nemaphos recorded the highest total fatty acid content (0.56 %), whereas *T. asperellum* occupied the lowest (0.36 %). Chitosan gave 0.320 % of saturated fatty acid in Pomegranate juices, while Vermicompost gave 0.264 percent saturated fatty acid percentages in juices and fruits. Pomegranate juice fruits of Nemaphos treatment recorded 0.301 % unsaturated fatty acid percentage, compared to 0.124 % for untreated Pomegranate juice fruits (control).

According to the structure of plant-parasitic nematodes community inhabiting the rhizosphere of Pomegranate fields, the *Meloidogyne* genus was the most common and harmful, although the variation of genera prevalence. Similar results were reported by some previous studies (Day and Wilkins, 2011; Shreeshail *et al*., 2015; Tariq-Khan et a l., 2017; El-Qurashi *et al*., 2019). Where Khan *et al*. (2005) revealed that infection of pomegranate roots by many populations of plant parasitic nematodes, especially root-knot nematodes, adversely affect the growth and Pomegranate productivity. The variation of genera prevalence may be due to the type of soil, agricultural processes, cultivated variety, and climatic conditions (Korayem *et al*., 2014; Singh *et al*., 2019).

Table 6: *Evaluation of Marjoram emulsion oil and three commercial bio-products as control agents against root-knot nematode on the juice quality of Pomegranate, under field conditions.*

Treatments	pH	T.S.S. mg / 100ml	Vitamin C mg/ 100ml	Total protein mg /100ml	Total carbohydrate mg /100ml
Vermicompost	3.70	15.7	10.50	3.01	42.87
T. asperellum	3.60	14.17	10.00	2.52	36.82
Chitosan	3.81	15.77	11.23	3.32	40.25
Marjoram emulsion oil	3.74	16.97	10.33	3.92	45.35
Nemaphos	3.88	18.57	11.66	4.71	52.73
Nematod alone	3.41	13.63	6.95	1.98	30.52

Table 7: *Effect of Marjoram emulsion oil and three commercial bio-products as control agents against root-knot nematode on the fatty acids of Pomegranate juice of treated and untreated trees under field conditions.*

Treatments	Saturated fatty acid %	Unsaturated fatty acid %	Total fatty acid %
Vermicompost	0.264	0.220	0.43
T.asperellum	0.202	0.126	0.35
Chitosan	0.320	0.243	0.46
Marjoram emulsion oil	0.325	0.295	0.54
Nemaphos	0.375	0.301	0.56
Nematod alone	0.190	0.124	0.16

The efficacy of emulsion oil, bio-agnates as alternative eco-friendly control strategies for root-knot nematode *M. incognita* infested pomegranate were evaluated under conditions of greenhouse and commercial open fields. The current study indicated the ability of all strategies to reduce *M. incognita* criteria, with different reduction percentages from chemical nematicide, and compared to untreated control. Where in protected conditions the use of Marjoram emulsion oil (MEO) and *Trichoderma asperellum* as separate treatments achieved the highest rate of nematode suppression after chemical nematicide. However, their ranking differs in terms of their effect on the plant vegetative growth, where Chitosan resulted in increasing percentages better than *T. asperellum* after MEO treatment. The obtained results were similar to the findings of Radwan *et al*. (2012) and Luong *et al*.

(2020) where reported that the nematicidal activity of Chitosan improved the plants' health. While, contrary findings were reported by Mota and Santos (2016), where reported that chitosan application alone had not improved the shoot growth of plants. While Kumari *et al*. (2020) reported that the soil application of *T. asperellum* improved the plant growth and suppressed root-knot nematode significantly. Moreover, the findings of Arancon *et al*. (2002), Giannakou *et al*. (2020), and Temitope *et al*. (2020) reported the ability of each of the tested strategies to suppress plant-parasitic nematodes as parasites on different varieties of plants. In agreement with the present findings, Arancon *et al*. (2002), Xiao *et al*. (2016), Giannakou *et al*. (2020), and Temitope *et al*. (2020) reported the ability of each of the tested strategies to suppress plant-parasitic nematodes as parasites on different varieties of plants. Generally, the variations of tested strategies for suppression of root-knot nematode due to the difference in the mechanism of action and the effect of the active ingredient in each strategy (Roberts, 1993; Hildalgo and Kerry, 2008; Collange *et al*., 2011; Tariq at al., 2020; Singh and Sinha, 2022).

Conclusions and Recommendations

The current study indicates the possibility of using eco-friendly control strategies such as Marjoram emulsion oil, Vermicompost and products of bio-agents, as an alternative strategy for chemical control to suppression of the root-knot nematodes.

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Novelty Statement

The current study proves the possibility of applying eco-friendly strategies to control root-knot nematodes in open agricultural fields, especially Marjoram emulsion oil and Vermicompost.

Author's Contribution

EAH conceived and designed the experiment, performed the experiments, wrote and reviewed the manuscript. AME collection and/or assembled and analyze the data, wrote, reviewed the manuscript and designed the experiment.

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The authors have declared no conflict of interest.

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