




**ORIGINAL PAPER**

## Efficiency of Humic Acid and Three Commercial Biocides Against *Meloidogyne incognita* and *Tylenchulus semipenetrans* Associated with Olive Plants

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### ABSTRACT

In comparison to a chemical nematicide (Oxamyl), humic acid and three commercial bioagents namely Abamectin (*Streptomyces avermitilis*), Clean Root (*Bacillus subtilis*) and Nemastrol (chitinase, glycosynolates, Glucanase, cytokinins and flavonoids) were tested in a greenhouse and field to see how they affect the population density of root-knot and citrus nematodes and, as a result, the effects on plant growth parameters. In greenhouse test, humic acid gave the highest results in terms of increasing total plant fresh weight (403.8 %) and shoot dry weight (400%) compared to untreated plants. Regardless chemical nematicide used, Abamectin showed the best results in suppressing *Meloidogyne incognita* reproduction with a reduction percentage of (85.4%), while Nemastrol resulted the best reduction percentage of *Tylenchulus semipenetrans* population (84.4%). Six months after treatment, Nemastrol showed the highest decrease percentage of root-knot nematode (82.4%) and citrus nematode (75.6%) in a field experiment. On the other hand, Oxamyl and humic acid outperformed other treatments in terms of improving the percentage of fruit weight (166.84 and 145.87 %), respectively. Furthermore, all treatments had an impact on the fatty acid composition of olive oil. At the same time, compared to untreated trees, such treatments resulted in lower peroxidase and polyphenoloxidase activities. In terms of phenol content, it was clear that Nemastrol, followed by humic acid improved total phenol content. However, Clean Root gained the highest overall protein concentration. Furthermore, humic acid increased the total carbohydrate level.

**Keywords:** Olive, *Meloidogyne incognita*, *Tylenchulus semipenetrans*, *Streptomyces avermitilis*, *Bacillus subtilis*, Nemastrol, Humic acid.

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### INTRODUCTION

Olive trees (*Olea europaea* L.) have several purposes other than oil production, such as providing food and wood, and they are sometimes used as cattle feed (Guillaume *et al.*, 2018). About 90% of the world's olive production is produced in the Mediterranean basin (Fraga *et al.*, 2021). Olive production is extremely significant in Egypt, both economically and environmentally. Olive trees have been shown to host over a hundred different phytoparasitic nematode species (Nico *et al.*, 2002). *Pratylenchus vulnus*, *P. penetrans*, *Tylenchulus semipenetrans*, *Gracilacus peratica*, *Rotylenchulus macrodoratus*,

*Xiphinema elongatum*, *X. index*, and *Meloidogyne* spp. are among the few genera and species which can impact on olive tree growth (Korayem *et al.*, 2014 and Hamza *et al.*, 2015).

Many endoparasitic nematodes species belonging to *Meloidogyne*, *Tylenchulus*, *Pratylenchus*, and *Heterodera* have been identified as olive parasites. As a result of disrupting the natural process of plant root growth and function, some nematodes have parasitism effects on plant growth and yield (Karssen and Moens, 2006). Because they are polyphagous, root-knot nematodes are the most damaging plant parasitic nematodes, causing severe damage to olive seedlings and groves. The mechanical damage caused by *Meloidogyne* spp. second stage juvenile feeding on the roots of young olive trees favors penetration and infection of the root tissue by soil-borne pathogens, particularly *Verticillium dahliae* (Lamberti *et al.*, 2001), and root-knot nematodes frequently damage young olive seedlings in nurseries. Many people dismiss the warning symptoms, mistaking them for mineral deficiency or other problems (Sasanelli and D'Addabbo, 2002).

Although the citrus nematode is one of the specific nematodes found on citrus plants, it was

first discovered on olive trees many years ago (Baines and Thorne, 1952 & Lamberti and Baines, 1970). Because this genus is a semi-internal parasite, the gelatinous egg masses are placed outside the root, adhering to the female's back, causing the soil granules to adhere to the gelatinous masses. The afflicted root tissue loses its ability to absorb water and nutrients necessary for plant growth as a result of nematode infection, resulting in plant yellowing and poor growth (Duncan, 2005).

Because phytonematodes have such a big economic impact on agriculture, a variety of nematode control measures have been developed, including the use of chemical nematicides. However, because chemical pesticides are dangerous to human health and pollute the environment, there is a growing need to develop efficient nematode control methods that do not rely on them. Biocontrol, whether by using crucial enemies such as fungi or bacteria (Ibrahim *et al.*, 2020 and Soliman *et al.*, 2021), organic additives (Mostafa *et al.*, 2016 and El-Deriny *et al.*, 2020), and elements that boost plant resistance are all key approaches to tackle nematodes (Ibrahim *et al.*, 2021, Nagachandrabose and Baidoo, 2021).

In Egypt, applying humic acid is one of the agricultural strategies that could improve citrus production by increasing microbial activity and accessible elements (Ennab, 2016). According to Nagachandrabose & Baidoo (2021), humic acid is harmful to several phytonematode species, including *R. reniformis*, *Radopholus similis*, *Meloidogyne* spp., and *Helicotylenchus multicinctus*. Nemastrol is a commercially available native product that comprises active components like chitinase and glucanase. The product's suppressive effects are due to the inclusion of combination enzymes that break down nematode eggshell chitin (Mostafa *et al.*, 2018).

A number of scientists have looked into the interaction between nematodes and some helpful bacteria. *M. incognita* second stages died at a higher rate when *Streptomyces hydrogenis* was used, which resulted in a lower rate of egg hatching (Kaur *et al.*, 2016). Meidani *et al.*, 2020, found that culture supernatants, cell solvent extracts, and spore suspensions of *Streptomyces* strains from Greece have nematicidal action against *M. incognita* and *M. javanica*, as well as negative crop production consequences.

*Bacillus* spp. efficiency has been determined in agricultural experiments. It has been discovered that it improves soil properties by

fixing atmospheric nitrogen and solubilizing phosphate (Huang *et al.*, 2010 and Keneni *et al.*, 2010). As a result, it can be considered a plant growth promoting and biocontrol agent, as well as its ability to produce phytohormones (Martinez-Viveros *et al.* 2010). Furthermore, certain *Bacillus* spp. secondary metabolites, such as ammonia and hydrogen cyanide HCN, have nematocidal activity (Abd El-Rahman *et al.*, 2019).

Numerous industries have turned to the development of biocides derived from plants, fungi, bacteria, certain minerals, or bioactive components due to the necessity of biological control in combating many disease pests. So, in greenhouse and field experiments, the possible use of humic acid and three commercial biocides against *M. incognita* and *T. semipenetrans* associated with olive was explored.

## MATERIALS AND METHODS

In a greenhouse and field experiments, the nematicidal effects of humic acid and three commercial bioagents namely Clean Root (*Bacillus subtilis*), Abamectin (*Streptomyces avermitilis*), and Nemastrol (chitinase, glycosynolates, Glucanase, cytokinins and flavonoids), were compared to Oxamyl as nematicide against root-knot and citrus nematode and subsequent influence on plant growth parameters of olive plants. The induced resistance (IR) of each treatment was assessed based on chemical composition and enzyme activity.

### 1. Preparation of nematode inoculum:

#### 1.1. Preparation the inoculum of *Meloidogyne incognita* (Kofoid and White) Chitwood:

Hussey and Barker, 1973 approach was used to obtain root-knot nematode eggs from egg-masses on the roots of *Coleus blumei* plant. According to Coyne *et al.*, 2007, the eggs were moved to flasks and incubated at 28°C for three days, after which the eggs hatched in water and active juveniles (J2) of *M. incognita* were collected. As an inoculum, a calculated suspension containing about 1000 viable juveniles per plant was used.

#### 1.2. Preparation the inoculum of *Tylenchulus semipenetrans* Cobb:

*T. semipenetrans* was collected from soil of a citrus grove, 12 years old. According to Goodey (1963), the juveniles were extracted from soil using extraction trays. The juveniles were standardized and concentrated in water for the studies. As inoculum, a calculated suspension containing about 3000 viable juveniles per plant was used.

## 2. Tested materials and Bioagents:

### a. Black power:

A native commercial formulation of humic acid (20 percent) was applied at a rate of 2L/feddan.

### b. Abamectin:

*Streptomyces avermitilis* commercial bioproduct of Abamectin (2 percent SC) and administered at a rate of 2.5L / feddan.

### c. Clean Root:

*Bacillus subtilis* commercial bioproducts comprise  $3 \times 10^7$  spores per gram of bacterium and administered at 1L / feddan.

### d. Nemastron:

A native commercial formulation of active components containing chitinase ( $12 \times 10^5$  IU), glycosinolates (12 percent), 1-3, Glucanase ( $2 \times 10^5$  IU), cytokinins (200ppm), and flavonoids (5 percent) at a rate of 5L/feddan.

### e. Vydate:

Chemical nematicide 10 percent G (Oxamyl): Methyl N N-dimethyl-N [(methyl) carbamyloxy]-1-thioxamidate was utilized at a rate of 20kg/feddan.

## 3. Experimental design in greenhouse:

In thirty-five plastic pots (25-cm-d) each containing 3.5 kg steam-sterilized soil, six-month-old plant seedlings of the olive plant (*Olea europaea* L.) cv. Manzanillo were inoculated with 3000 viable citrus nematodes, *T. semipenetrans*, and 1000 viable root-knot, *M. incognita* second stage juveniles. One week later, the plants were treated with the selected materials as a soil drench. Five pots were treated with Oxamyl as a conventional nematicide at a rate of 0.5g /pot. Five pots were left free of nematode infestation and any treatment as a control (Ck1). For both nematodes, five pots of nematode alone were used as controls (Ck2). The pots were then placed in a greenhouse at  $27 \pm 3^\circ\text{C}$  in a randomized complete block configuration with five replicates and watered as needed. As a result, the following treatments were used:

1. Humic acid @ 20 ml/pot
2. Abamectin @ 2 ml/pot
3. Clean Root @ 1ml/pot
4. Nemastron @ 0.5 ml/pot
5. Oxamyl @ 0.5g /pot
6. Uninoculated healthy plants (Ck1)
7. Nematode alone (*M. incognita*) and (*T. semipenetrans*) (Ck2).

Sixty days after nematode inoculation, roots were washed to remove any adhered soil. Weight of the fresh and dry shoots, as well as

the length of the shoots and roots, were determined. A modified Baermann method was used to extract the nematodes from the soil (Goodey, 1963). Roots were stained in lactic acid with 0.01 acid fuchsine and examined under a stereomicroscope for nematode counting (Byrd *et al.*, 1983). Females, developmental stage, galls, and egg masses were counted during root-knot nematode development. Root galling or egg masses were rated on a scale of 0-5 with 0 indicating no galls or egg masses, 1 indicating 1-2 galls or egg masses, 2 indicating 3-10 galls or egg masses, 3 indicating 11-30 galls or egg masses, 4 indicating 31-100 galls or egg masses and 5 indicating more than 100 galls or egg masses per root system (Taylor and Sasser, 1978). The number of juveniles, males, and females of citrus nematode were also counted. According to Hussey and Barker's (1973) approach, one egg mass of *T. semipenetrans* was separated to count the number of eggs per egg mass.

## 4. Experimental design in olive orchard:

Before starting the treatments, a nematode sample was taken from the chosen soil and used as a zero-day. The open-field experiment was carried out in Nubaria, Behera governorate, during 2019 on 7-year-old Manzanillo olive trees naturally infested with different species of nematodes i.e., *Ditylenchus* spp. 238 individual / 250g soil, *Helicotylenchus* spp. 330 individual / 250g soil, *Meloidogyne* spp. 2975 individual / 250g soil, *Pratylenchus* spp. 280 individual / 250g soil, *Rotylenchulus* spp. 780 individual / 250g soil, *Tylenchus* spp. 95 individual / 250 g soil, *T. semipenetrans* 1378 individual / 250 g soil, and *Tylenchorhynchus* spp. 227 individual / 250g soil. The most common nematode species on olive trees in Nubaria locality turned out to be *M. incognita* (which was identified according to Whitehead (1968) & Taylor and Sasser (1978) and *T. semipenetrans*. The trees were set 5x5m apart and a drip irrigation system was used to irrigate an experimental area. The same treatments used in the pot experiment were repeated in soil naturally infested with *M. incognita* and *T. semipenetrans* in March-April 2019. Each treatment comprised five duplicates, each containing ten trees. As a soil drench, many applications were applied to the soil. All therapies were applied three times monthly, except for Oxamyl and samples were taken 2, 4, and 6 months later.

Using sieving and modified Baermann techniques, root-knot, and citrus nematodes were extracted from soil (Goodey, 1963). Roots were stained with acid fuchsine in lactic acid

and examined under a stereomicroscope for counting root-knot nematode developmental stages and females, according to Byrd *et al.* (1983). The number of second-stage juveniles (J2), females, and eggs/egg mass of *T. semipenetrans* were also counted. In either soil or roots, the efficiency percent of nematode reduction was measured. For each treatment, the reproduction factor (Rf) was calculated by dividing the final population (Pf) by the initial population (Pi). At the end of experiment, the total weight of fruits for each treatment was measured.

## 5. Biochemical analysis:

### 5.1. Fatty acid isolation and extraction:

Gas chromatography was used to identify the fatty acids in the extracted olive oil using procedures for producing fatty acid methyl esters (FAME) from fats and oils (ISO 12966, 2015).

### 5.2. The assay of peroxidase (PO) and polyphenol oxidase (PPO) activity:

Peroxidase activity (PO) was determined in fresh olive leaves by detecting the oxidation of pyrogallol to pyrogallin in the presence of H<sub>2</sub>O<sub>2</sub> at 425 nm using a UV spectrophotometer, according to Allam and Hollis (1972).

Polyphenol oxidase (PPO) activity was determined, according to Cho and Ahn (1999). Using a UV spectrophotometer, the activity of (PPO) was calculated as the change in the absorbency of 1.0 mL of extract per minute at 420 nm.

### 5.3. Phenolic contents estimation:

Conjugated, free, and total phenols were determined by mixing 1 ml of sample extract with 0.25 ml of HCl and boiling for 10 minutes in a water bath, then cooling. The reagent folin Denis (1ml) and Na<sub>2</sub>CO<sub>3</sub> (6ml) were then added to the sample. The mixture was finished to volume using distilled water (10ml). The colour optical density of the reacting mixture was measured on an absorbance spectrophotometer Miltonroy Spectronic 601 at 520 nm (Snell and Snell, 1953). The content of phenol was calculated as mg/g fresh weight/min.

### 5.4. Crude protein and total carbohydrate estimations:

Bradford's 1976 approach was used to assess crude proteins. Furthermore, the total carbohydrate content was determined using Hodge and Hofreiter's methods (1962).

## 6. Data Analysis:

To compare means, the data were evaluated statistically using analysis of variance (ANOVA) (Gomez and Gomez, 1984) and Duncan's multiple range tests (Duncan, 1955).

## RESULTS

### Greenhouse experiment:

The effects of three bio-agents, namely Nemastrol, Abamectin, and Clean Root, as well as humic acid compared to Oxamyl, on the plant growth response of olive seedlings inoculated with root-knot nematode, *M. incognita* and citrus nematode, *T. semipenetrans* are illustrated in Table (1). All treatments improved plant growth parameters as indicated by length and fresh weight of root and shoot as well as shoot dry weight when compared to nematode treatment alone. Humic acid exceeded the other treatments, with percentage increase in length of shoots and roots as well as total plant fresh weight and dry shoot weight of 67.4, 138.1, 403.8, and 400.0 %, respectively when compared to nematode treatment alone. The Nemastrol treatment gave the lowest plant length increase, being 32.1 and 28.6% on the average for the shoots and roots, respectively. However, in terms of percentage increase of plant fresh weight (21.9%) and shoot dry weight (44.3%), the Clean Root was the least effective treatment. Oxamyl, a systemic nematicide, caused a significant increase in plant growth criteria at a concentration of 0.5 g/plant, with percentage increases in shoot length (40.2%), root length (73.3%), plant fresh weight (91.5%) and shoot dry weight (135.0%).

Data in Table (2) reveal that *M. incognita* parameters such as numbers of galls, egg masses, females/root system of olive seedling, and rate of buildup were suppressed by application of humic acid, Nemastrol, Abamectin, and Clean Root. Whereas, Oxamyl showed more suppressive effect on nematode reproduction (RF=0.27), being 93.2% 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 71.5% for humic acid treatment to 85.4 % with Abamectin treatment. Oxamyl treatment resulted in the lowest number of galls (37.2 galls/root), followed by the treatment with Abamectin (80.4 galls/root). Oxamyl, Abamectin, and Nemastrol treatments produced lower numbers of egg masses/root (27.2, 33.2, and 39.2, respectively).

Parameters of *T. semipenetrans* such as the number of second stages (J2, eggs, and females/root system of olive seedlings, and rate of buildup were all suppressed by humic acid, Nemastrol, Abamectin, and Clean Root. Moreover, Oxamyl at 0.5g/plant had more

suppressive effects on nematode reproduction (0.05) and reduction (97.0 %) when compared to nematode alone. All treatments had the best results in terms of reducing nematode parameters, with average values of 74.8, 84.4,

79.4 and 75.8 % and rates of the buildup of 0.41, 0.25, 0.33 and 0.39 for humic acid, Nemaströl, Abamectin and Clean Root, respectively, compared to nematode treatment alone, which had a rate of buildup (1.61).

**Table (1): Impact of humic acid as well as three commercial bioagents on plant growth parameters of olive plants infected with *M. incognita* and *T. semipenetrans* under greenhouse conditions (27±3° C).**

Treatments	Plant growth response									
	Length (cm)				Plant fresh wt.(g)				Shoot dry wt. (g)	Inc.%
	Shoot	Inc.%	Root	Inc.%	Shoot	Root	whole plant	Inc. %		
Humic acid	88.7 <sup>b</sup>	67.4	25.0 <sup>a</sup>	138.1	516.0 <sup>a</sup>	20.6 <sup>b</sup>	536.6 <sup>a</sup>	403.8	205.5 <sup>a</sup>	400.0
Nemaströl	70.0 <sup>f</sup>	32.1	13.5 <sup>f</sup>	28.6	124.0 <sup>e</sup>	13.5 <sup>e</sup>	137.5 <sup>e</sup>	29.1	79.5 <sup>e</sup>	93.4
Abamectin	76.0 <sup>c</sup>	43.4	15.5 <sup>e</sup>	47.6	150.0 <sup>d</sup>	16.8 <sup>c</sup>	166.8 <sup>d</sup>	56.6	84.6 <sup>d</sup>	105.8
Clean Root	73.3 <sup>e</sup>	38.3	16.8 <sup>d</sup>	60.0	113.0 <sup>f</sup>	16.8 <sup>c</sup>	129.8 <sup>e</sup>	21.9	59.3 <sup>f</sup>	44.3
Oxamyl	74.3 <sup>d</sup>	40.2	18.2 <sup>c</sup>	73.3	189.0 <sup>c</sup>	14.9 <sup>d</sup>	203.9 <sup>c</sup>	91.5	96.6 <sup>c</sup>	135.0
Plant free of N and any treatment	90.0 <sup>a</sup>	69.8	24.0 <sup>b</sup>	128.6	450.0 <sup>b</sup>	23.0 <sup>a</sup>	473.0 <sup>b</sup>	344.1	107.0 <sup>b</sup>	160.3
Nematode alone	53.0 <sup>g</sup>	--	10.5 <sup>g</sup>	--	96.0 <sup>g</sup>	10.5 <sup>f</sup>	106.5 <sup>f</sup>	--	41.1 <sup>g</sup>	--

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

**Table (2): Influence of humic acid as well as three commercial biocides on reproduction of *M. incognita* and *T. semipenetrans* associated with olive seedlings under greenhouse conditions at 27±3° C.**

Treatment	Parameters for <i>Meloidogyne incognita</i>									
	Nematode population			Final population	RF*	Red. %	No. of galls / root system	RGI	No. of Egg masses / root system	EI
	Soil	Root	Root							
Humic acid	1002.4 <sup>b</sup>	85.2 <sup>b</sup>	74.3 <sup>b</sup>	1161.9 <sup>b</sup>	1.16	71.5	130.0 <sup>b</sup>	5.0	64.2 <sup>b</sup>	4.7
Nemaströl	838.2 <sup>c</sup>	63.9 <sup>c</sup>	59.8 <sup>c</sup>	961.9 <sup>c</sup>	0.96	76.4	95.2 <sup>c</sup>	5.0	39.2 <sup>cd</sup>	4.1
Abamectin	496.9 <sup>d</sup>	52.6 <sup>d</sup>	44.4 <sup>d</sup>	593.9 <sup>d</sup>	0.59	85.4	80.4 <sup>d</sup>	5.0	33.2 <sup>d</sup>	3.2
Clean Root	952.4 <sup>b</sup>	79.3 <sup>b</sup>	63.2 <sup>b</sup>	1094.9 <sup>b</sup>	1.1	73.15	121.4 <sup>b</sup>	5.0	50.1 <sup>c</sup>	4.0
Oxamyl	220.6 <sup>e</sup>	18.4 <sup>e</sup>	35.2 <sup>e</sup>	274.2 <sup>e</sup>	0.27	93.2	37.2 <sup>e</sup>	3.2	27.2 <sup>e</sup>	2.4
N alone	3734.3 <sup>a</sup>	248.2 <sup>a</sup>	95.0 <sup>a</sup>	4077.5 <sup>a</sup>	4.08	--	289.3 <sup>a</sup>	5.0	79.2 <sup>a</sup>	4.8

Treatment	Parameters for <i>Tylenchulus semipenetrans</i>							
	Nematodes in soil			Nematodes in root		Final population	RF*	Red. %
	J2	Males	Eggs	J2	Females			
Humic acid	765.1 <sup>b</sup>	108.7 <sup>b</sup>	151.2 <sup>bc</sup>	93.4 <sup>c</sup>	97.3 <sup>b</sup>	1215.7 <sup>b</sup>	0.41	74.8
Nemaströl	434.5 <sup>d</sup>	75.4 <sup>c</sup>	124.0 <sup>cd</sup>	85.7 <sup>d</sup>	33.7 <sup>d</sup>	753.3 <sup>c</sup>	0.25	84.4
Abamectin	592.4 <sup>d</sup>	92.7 <sup>d</sup>	134.4 <sup>c</sup>	94.5 <sup>c</sup>	79.3 <sup>c</sup>	993.3 <sup>d</sup>	0.33	79.4
Clean Root	682.3 <sup>c</sup>	102.2 <sup>b</sup>	162.4 <sup>b</sup>	120.3 <sup>a</sup>	99.3 <sup>b</sup>	1166.5 <sup>c</sup>	0.39	75.8
Oxamyl	98.2 <sup>e</sup>	11.4 <sup>d</sup>	19.5 <sup>d</sup>	12.2 <sup>e</sup>	4.5 <sup>e</sup>	145.8 <sup>f</sup>	0.05	97.0
N alone	2942.4 <sup>a</sup>	815.3 <sup>a</sup>	478.2 <sup>a</sup>	113.1 <sup>b</sup>	477.8 <sup>a</sup>	4826.8 <sup>a</sup>	1.61	--

Each value presented the mean of five replicates.

Initial population of *M. incognita* = 1000 infective juveniles/plant.

Initial population of *T. semipenetrans* = 3000 infective juveniles/plant.

\*Reproduction factor (RF) = final population/initial population.

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

**Field experiment:**

During 2019, an open-field experiment was conducted on 7-year-old Manzanillo olive trees naturally infested with *M. incognita* and *T. semipenetrans*, as well as numerous species of nematodes, in Nubaria, Behera governorate. Humic acid, Nemastrol, Abamectin, and Clean Root all have nematicidal activity against root-knot and citrus nematodes, according to the findings (Table 3). When compared to nematode treatment alone, Nemastrol administration

resulted in the largest suppression of the total root-knot nematode population after six months with 82.4% reduction. Meanwhile, the use of humic acid and Abamectin showed the least common (63.8 and 68.0 % reduction, respectively), while the use of Clean Root was the most common (71.0%). Nonetheless, when compared to nematode treatment alone, the overall population was dramatically reduced with Oxamyl administration, with reproduction percentage of 83.5.

**Table (3): Effect of humic acid and three commercial biocides on population density of *M. incognita* and *T. semipenetrans* after two, four and six months of treatments under field conditions.**

Treatment	Two months			Four months			Six months		
	Final population	*RF	Red. %	Final population	*RF	Red. %	Final population	*RF	Red. %
Parameters for <i>Meloidogyne incognita</i>									
Humic acid	1389.7 <sup>b</sup>	0.47	69.3	1592.1 <sup>b</sup>	0.54	67.0	1836.7 <sup>b</sup> <sub>c</sub>	0.62	63.8
Nemastrol	623.8 <sup>c</sup>	0.21	86.2	718.3 <sup>c</sup>	0.24	85.1	905.4 <sup>d</sup>	0.30	82.4
Abamectin	1277.0 <sup>c</sup>	0.43	71.8	1445.6 <sup>c</sup>	0.49	70.0	1623.0 <sup>c</sup>	0.55	68.0
Clean Root	1095.8 <sup>d</sup>	0.37	75.8	1336.4 <sup>d</sup>	0.45	72.3	1872.5 <sup>b</sup>	0.63	71.0
Oxamyl	447.2 <sup>f</sup>	0.15	90.1	597.5 <sup>f</sup>	0.2	88.7	834.7 <sup>e</sup>	0.28	83.5
N alone	4533.7 <sup>a</sup>	1.5	--	4829.7 <sup>a</sup>	1.6	--	5068.9 <sup>a</sup>	1.70	--
Parameters for <i>Tylenchulus semipenetrans</i>									
Humic acid	2426.6 <sup>b</sup>	1.76	69.1	2732.6 <sup>b</sup>	1.98	68.4	2939.6 <sup>b</sup>	2.13	67.2
Nemastrol	1502.3 <sup>c</sup>	1.09	80.8	1891.4 <sup>c</sup>	1.37	78.1	2186.8 <sup>c</sup>	1.59	75.6
Abamectin	2261.7 <sup>c</sup>	1.64	71.2	2452.7 <sup>c</sup>	1.78	71.6	2769.4 <sup>c</sup>	2.01	69.1
Clean Root	1886.3 <sup>d</sup>	1.37	75.98	2254.3 <sup>d</sup>	1.64	73.9	2679.7 <sup>d</sup>	1.94	70.1
Oxamyl	673.6 <sup>f</sup>	0.49	91.4	1013.2 <sup>f</sup>	0.74	88.3	1308.5 <sup>f</sup>	0.95	85.4
N alone	7853.1 <sup>a</sup>	5.70	--	8636.4 <sup>a</sup>	6.27	--	8962.3 <sup>a</sup>	6.50	--

Initial population of *M. incognita* = 2975 infective juveniles/250g soil.

Initial population of *T. semipenetrans* = 1378 infective juveniles/250g soil.

\*Reproduction factor (RF) = final population/initial population

Means in each column followed by the same letter (s) didn't differ significantly at  $P \leq 0.05$  according to Duncan's multiple range test.

After two, four, and six months, the effects of humic acid, Nemastrol, Abamectin, and Clean Root, as well as the nematicide Oxamyl, on *T. semipenetrans* numbers and reproduction factor are shown also in Table (3). Oxamyl had a more inhibitory effect on citrus nematode reproduction, according to the findings. After six months of treatment, Nemastrol treatment (75.6 %) resulted in the biggest reduction in the overall nematode population, followed by Clean Root (70.1 %), Abamectin (69.1 %), and humic acid (67.2 %).

Results presented in Table (4) show that all tested treatments resulted in a significant increase in total olive fruit weights. When compared to control, Oxamyl had the highest percentage of fruit weight increase (166.84 %), while humic acid, Nemastrol, and Clean Root treatments gave a relatively lower percentages of yield increase (145.87, 125.10 and 104.13 %, respectively), when compared to untreated plants (9.68kg/tree), Abamectin caused a lowest increase in overall fruit weight (17.56 kg fruit/tree) with a percentage increase (81.40%).



**Table (4): Fruit weight of olive trees grown in soil naturally infested mainly with *M. incognita* and *T. semipenetans* after treatment with humic acid and three commercial biocides.**

Treatment	Humic acid	Nemastrol	Abamectin	Clean Root	Oxamyl	Control
Fruit weight (kg /tree)	23.80 <sup>b</sup>	21.79 <sup>c</sup>	17.56 <sup>e</sup>	19.76 <sup>d</sup>	25.83 <sup>a</sup>	9.68 <sup>f</sup>
Increasing %	145.87	125.10	81.40	104.13	166.84	-

Means in the row followed by the same letter did not differ significantly at  $P \leq 0.05$  according to Duncan's multiple range test.

Data in Table (5) show the fatty acids percentage in olive oil obtained from fruits of the olive tree cv. Manzanillo that had been treated with the aforementioned agents in comparison to untreated trees. Oxamyl performed the best and raised the amount of fatty acid palmitic (82.9%), followed by treatments of humic acid and Nemastrol (77.4%), Abamectin (75.9%) and Clean Root

(75.7%). Similarly, all of the therapies examined affected fatty acid percentages (oleic and linoleic). Treatment with Oxamyl increased percentage of saturated fatty acid (23.1%), followed by humic acid (21.4%), Nemastrol (18.4%), Abamectin (16.9%) and Clean Root (14.2%). In contrast, all tested treatments resulted in a decrease in unsaturated fatty acids when compared to untreated trees.

**Table (5): Fatty acids percentage of olive oil from fruits of treated and untreated trees with humic acid or three biocides under stress of phytonematodes (mainly *M. incognita* and *T. semipenetans*).**

Fatty acids %	Treatments					
	Humic acid	Nemastrol	Abamectin	Clean Root	Oxamyl	Untreated plant
Palmitic	77.4	77.4	75.9	75.7	82.9	74.9
Oleic	76.42	70.74	71.41	75.5	91.3	68.0
Linoleic	20.2	19.8	13.8	21.2	23.2	7.2
Saturated	21.4	18.4	16.9	14.2	23.1	12.0
Unsaturated	78.5	81.6	78.5	85.8	76.9	87.9

Tabulated data (Table, 6) show that the activity of peroxidase (PO) and polyphenol oxidase (PPO) as indicators related to the severity of the infection, in comparison with the increase activity of PO and PPO in untreated trees. Simultaneously, treatment with humic acid recorded a decrease in percentages of PO and PPO activity (94.9%) and (67.4%), respectively, followed by Abamectin (78.6, 46.5%) and Oxamyl (78.0, 45.3%).

The presence of free, conjugated, and total phenols in the tree is shown in Figure (1). All tested treatments indicated an increase in phenolic components, when compared to untreated trees. Treatment with Oxamyl yielded the highest phenolic component, followed by treatments with Nemastrol and humic acid.

In terms of protein content, it was found that applying the nematicide (Oxamyl) followed by Clean Root and Nemastrol treatments were the most effective in raising the percentage of protein content (Figure 2).

When compared to untreated trees, the treatments showed a significant increase in total carbohydrates (Figure, 3). The most potent increase in carbs was observed with the use of humic acid, whereas the lowest one was obtained with the use of Clean Root.

**Table (6) Activity of peroxidase (PO) and polyphenol oxidase (PPO) in olive tree as affected by root knot and citrus nematodes as well as application of humic acid and three biocides.**

Treatment	Enzyme activity (mM/min/g fresh w.)			
	PO	Red. %	PPO	Red. %
Humic acid	0.112 <sup>d</sup>	94.9	0.028 <sup>d</sup>	67.4
Nemastrol	1.293 <sup>b</sup>	40.6	0.084 <sup>a</sup>	2.3
Abamectin	0.465 <sup>c</sup>	78.6	0.046 <sup>c</sup>	46.5
Clean Root	1.021 <sup>b</sup>	53.1	0.074 <sup>b</sup>	14.0
Oxamyl	0.479 <sup>c</sup>	78.0	0.047 <sup>c</sup>	45.3
Untreated plant	2.175 <sup>a</sup>	--	0.086 <sup>a</sup>	--

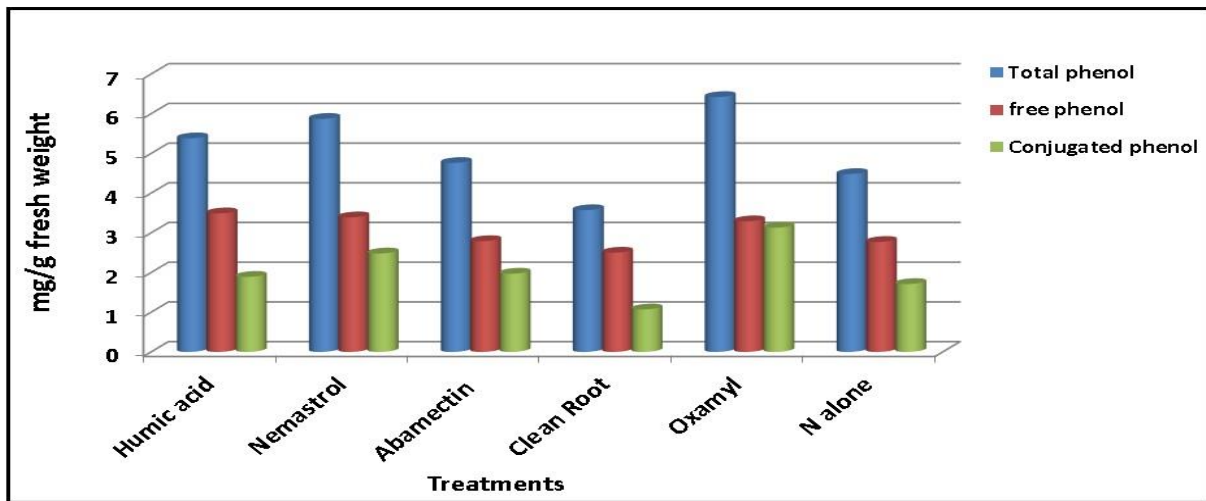


Figure (1): Influence of humic acid and three commercial biocides on free, conjugated and total phenols in olive trees as affected by phytonematodes (mainly *M. incognita* and *T. semipenetans*) under field conditions.

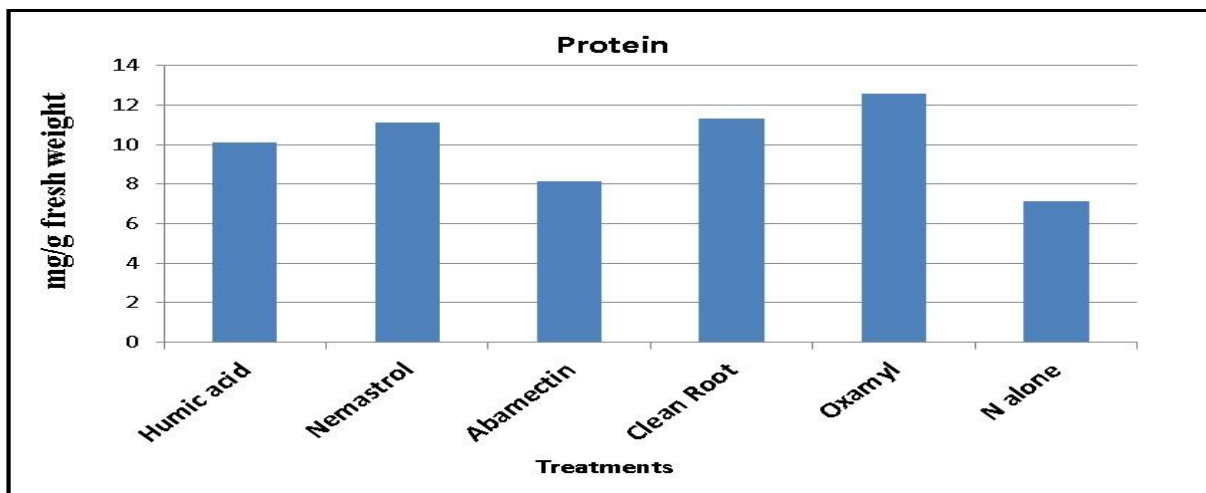


Figure (2): Impact of humic acid and three commercial biocides on protein content in olive trees as affected by phytonematodes (mainly *M. incognita* and *T. semipenetans*) under field conditions.

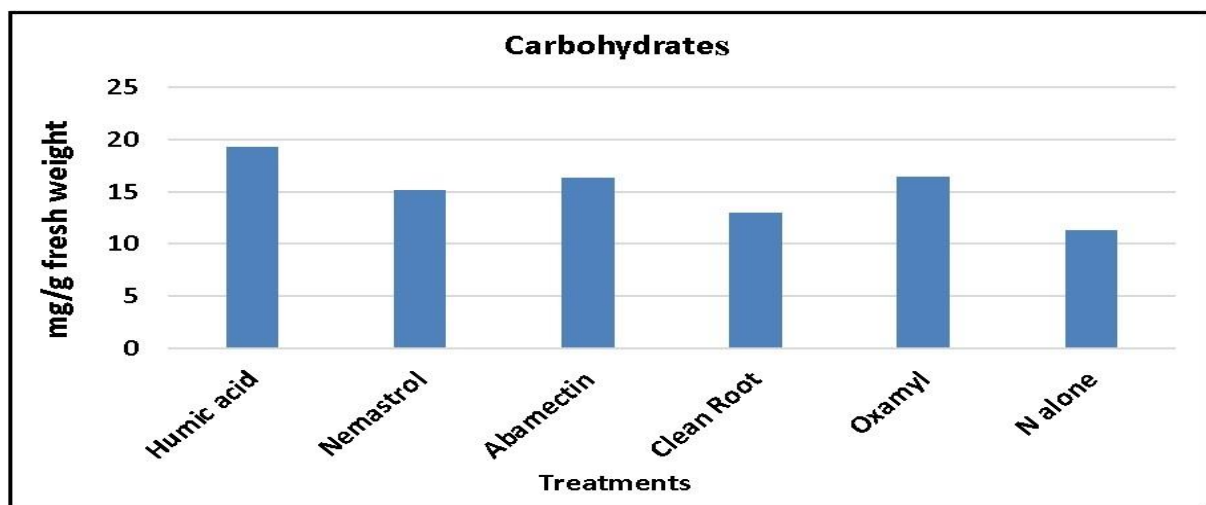


Figure (3): Effect of humic acid and three commercial biocides on carbohydrates content in olive trees as affected by phytonematodes (mainly *M. incognita* and *T. semipenetans*) under field conditions.



## DISCUSSION

Root-knot and citrus nematodes are two of the most frequent soil-borne diseases that affect a wide range of crops (Kavitha *et al.*, 2012). Crop protection is still mostly achieved through the use of pesticides. People and the environment are at risk as a result of this plan. It's a good idea to use biocontrol agents to minimize plant disease-causing activities in the soil. According to Junaid *et al.*, (2013) bioagents have a variety of processes, including myco-parasitism, cell wall disintegration, colonization of the plant rhizosphere, antibiosis, and competition induces resistance promotion and stimulation of plant development. Under greenhouse and field circumstances, the nematicidal capabilities of humic acid and three bio commercial treatments, viz. Nemastrol (chitinase, glycosynolates, Glucanase, cytokinins and flavonoids), Abamectin (*S. avermitilis*), and Clean Root (*B. subtilis*), were tested against root-knot and citrus nematodes associated with olive trees. Under greenhouse conditions, all treatments improved plant growth parameters, while under field conditions, all treatments increased yield. On the other hand, humic acid came in first in terms of enhancing plant productivity and having a minor influence on the nematode population. This result is consistent with the findings of Seenivasan & Senthilnathan (2017) who reported that a reduction in nematode population was occurred as a result of treatment with humic acid in banana. Humic acid-soaked three times in the field reduced *M. incognita* and *T. semipenetrans* by 63.8 and 67.2 percent, respectively. Our findings matched results recorded by Ismael *et al.* (2017) who found a ten-fold reduction in *M. incognita* proliferation in eggplant roots following three humic acid drenches in the soil. Humic acid has an influence on nematodes in a variety of many ways: a) hatching inhibition (Jothi *et al.*, 2009); b) direct nematode toxicity (Saravanapriya & Subramanian, 2007); c) improved uptake of salicylic acid (Molinari and Loffredo, 2006); d) increased mineral elements on roots (Al-Sayed *et al.*, 2007) and e) increased activity of defense enzymes and compounds in plants (Seenivasan & Senthilnathan, 2017).

On the other hand, Abamectin (*S. avermitilis*) was found to be effective in lowering the number of nematodes in soil and olive roots. These results are similar to those obtained by Rashad *et al.* (2015). The most essential component in the fermentation of *S. avermitilis* is avermectin B1 (Abamectine) (Siddique *et al.*,

2013). Increased plant growth indices confirmed the beneficial effect of *S. avermitilis* cells and metabolites. Bioactive compounds released directly in root tissues affected tomato plant growth because nematode infection boosted *Streptomyces* penetration into plant tissues through damaged roots (Rashad *et al.*, 2015). Abamectin causes irreversible paralysis in root-knot nematodes, with larger concentrations leading to higher mortality (Faske and Starr 2006).

Clean Root (*B. subtilis*) was found to be somewhat efficient in lowering the number of nematodes infected olive plants. According to Mazzuchelli *et al.* (2020) *B. subtilis* offered effective control of root-knot and lesion nematodes on sugarcane in field circumstances and lowering the final nematode population density. *Bacillus* could enter the rhizosphere of plants in, on, or around plant tissues, boosting plant development and lowering nematode numbers by behaving antagonistically (El-Nagdi and Abd-El-Khair, 2019). Antibiotics, enzymes, and toxins produced by the investigated rhizobacteria may have numerous mechanisms of action against plant-parasitic nematodes, resulting in a drop in worm numbers (Tian *et al.*, 2007).

The Nemastrol {chitinase ( $12 \times 10^5$  IU)} treatment surpassed the majority of the treatments in terms of nematode reduction, whether in the soil or the root, and whether in the greenhouse or the field. The suppressive effect of this material could be explained by the presence of a mixture of enzymes, specifically chitinase and glucanase, which disintegrate the chitin of the nematode eggshell subsequently reduce the population of nematode (Mostafa *et al.*, 2018).

All of the above treatments were compared to the nematicide Oxamyl, which considerably reduced nematode populations for both root-knot and citrus nematode, and also enhancing development parameters in the olive plant. Oxamyl is hydrolyzed in plants to the oximino molecule, which then conjugates with glucose (Soltani *et al.*, 2013). In terms of olive production, it was discovered that all treatments increased the weight of olive output. This finding agrees with those reported by Akhtar *et al.* (2012), who stated that biological control of a nematode, plant growth stimulation and increased crop yields and quality could all be desirable outcomes.

All of these treatments boosted fatty acid-dependent olive tree yields. When olive plants were infected with *M. incognita* and *T.*

*semipenetrans*, the percentage of unsaturated fatty acids in the oil decreased, with oleic being the most important fatty acid in olive oil (Hernández *et al.*, 2021). In another study by Bernard *et al.* (2017), it was shown that the plant secretes some phenolic substances and fatty acids to self-defense against plant-pathogenic nematodes. These findings back with Al-Sayed *et al.* (2007) findings who reported that *M. incognita* infection lowered total lipid content in roots of grape compared to plants treated with pigeon dropping, smashed garlic and the nematicide.

The activation of numerous defense-related enzymes, such as peroxidase PO and polyphenol oxidase PPO, stimulates systemic resistance in response to various treatments. Recently, research has shown that stimulating the production of biochemical substances linked to host defense is beneficial (Kavino *et al.*, 2007). Interestingly, peroxidase and polyphenol oxidase activity was shown to be higher in untreated trees in the current study. Root peroxidase activity is vital in the fortification of cell walls at the infection's border in resistant plants, and it is thought to be a key component of the active defense response of nematode-infested tissue (Zacheo *et al.*, 1995).

Phenolic contents in the treated trees performed better than those in the untreated trees in this study. Role for phenolic compounds in nematode resistance has been hypothesized since the early 1960s. Larger basal and/or increased levels of phenolic compounds have been observed to correlate with nematode resistance in a variety of plant-nematode pairings (Pegard *et al.*, 2005 and Desmedt *et al.*, 2020). The use of the screening therapies increased total proteins and sugar. According to Abbasi *et al.* (2008), inhibiting root-knot nematode infection in okra and brinjal plants resulted in a similar increase in protein content. The reduced protein levels are due to root-knot nematode-induced gall formation and the development of big cells, which act as a primary sink for amino acids supplied into the roots via the vascular system (Hoth *et al.*, 2005). On the contrary, Cabello *et al.* (2013) reported that syncytial feeding sites have high quantities of sugars that nematodes may ingest, and nematode feeding sites show that most sugar pools have been enriched and starch levels in syncytia have increased

## CONCLUSION

The use of humic acid and bioagents such as Abamectine (*S. avermitilis*), Clean Root (*B.*

*subtilis*), and Nemastrol (chitinase, glycosynolates, Glucanase, cytokinins and flavonoids) to prevent root-knot and citrus nematodes in olive trees provides a feasible new control technique. By activating plant defense systems, which resulted in the synthesis of bioactive chemicals that hindered nematode development and reproduction, root-knot and citrus nematodes were reduced on olive trees. Plant growth is primarily owing to nematode suppression, as well as the effect of anti-nematode treatments on olive, which has resulted in increased weights.

## CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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