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IMPACT OF ENTOMOPATHOGENIC NEMATODES AND TWO BIOCONTROL PRODUCTS ON ROOT-KNOT NEMATODE, *Meloidogyne javanica*

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ABSTRACT: ***This research was carried out to evaluate two entomopathogenic nematodes i.e. Steinernema carpocapse ES and Heterorhabditis bacterophora HP88; two biocontrol commercial products i.e. Trichoderma album and Bacillus megaterium individually or in combination in comparison to the organophosphate nematicide Fenamiphos for control root-knot nematode, Meloidogyne javanica under laboratory and greenhouse conditions on tomato plants. Results of in vitro assays indicated that both tested entomopathogenic nematodes at three different population densities i.e. 100, 200 and 400 infective juvenile stages (IJS) significantly reduced the percentage of egg hatching and increased the mortality percentage of Meloidogyne larvae either after 96 and/or 144 hrs incubation period compared to control. Population density of 400 IJS of both entomopathogenic nematode genera was the most effective density either in reducing egg hatching or in increasing larval mortality of M. javanica at both incubation periods compared with the other densities. Results of in vivo experiment reported that all applied materials significantly reduced number of galls, egg-masses; females/root system; eggs/egg-mass as well as number of second stage juveniles/250g soil when comparing with root-knot nematode treatment only. Results indicated that the combined application of H.bacterophora HP88 plus T. album was the most effective one in reducing the percentages of galls numbers; egg-masses/root system; eggs/egg-mass and juveniles/250 g soil with values of 95.1; 97; 95.2 and 83.7%, respectively, followed by the combination of H.bacterophora HP88 and B. megaterium giving reduction percentages of 94.7; 90; 91.1 and 82%, respectively. The lowest effective treatment was recorded with the applications of entomopathogenic nematodes individually in soil. Results also revealed that all tested treatments markedly enhanced the plant growth parameters i.e. fresh shoot and root weights, plant height, root length and dry weight. The significant increase was accomplished with the combination of H.bacterophora HP88 and T. album. Generally it can be concluded that all tested treatments showed promising results through suppression the root-knot nematode population and encouraging the plant growth parameters.***

Key words: *Bio-agents, Bio-pesticide, Entomopathogenic nematodes, Nematicide, Root-knot nematodes, Tomato.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important solanaceous crops in Egypt either for local consumption or exportation. Tomato is considered as one of the highest nutrional crops because of its high contents of vitamin C as well as

many chemical compounds and elements which are not found in the other solanaceous crops.

Root-knot disease is prevalent throughout the world caused by root-knot nematodes, *Meloidogyne* spp., which considered an economically important polyphagous group of highly adapted obligate plant parasites and are considered major problems in agricultural production throughout the world as they caused significant economic damage and yield losses of a wide range of crops as well as reduction the quality of crops (Moens, *et al.*, 2009). Root-knot nematodes, *Meloidogyne* species are serious pests of agricultural crops and cause tremendous yield loss to them, particularly under subtropical and tropical climates (Sasser, 1979). This genus is an important one of plant-parasitic nematodes that has a world-wide distribution especially in Egypt, as it has extensive host ranges and is able to interact with other pathogens i.e. plant-parasitic nematodes, fungi, bacteria and virus to form disease complex syndromes (Agrios, 1988). Netscher and Sikora (1990) reported that species of *Meloidogyne* cause severe damage to many crops especially vegetable crops as they reported that crop losses exceed 32% on tomato according to one estimation by the International Meloidogyne Project (IMP). Root-knot nematodes, *Meloidogyne* spp. are considered to be among the most damaging plant pathogens, causing estimated annual crop losses of more than 5% worldwide (Sasser and Carter, 1985).

The most frequently methods used for managing nematodes in agriculture include crop rotation with plants that are not hosts of plant parasitic nematodes, using resistant plants, and applying chemical nematicides. Additional methods include soil solarization and the use of organic amendments, trap crops, plants that are antagonistic to nematodes and microbial biocontrol agents (Wang, *et al.*, 2007). Generally, a chemical method was the most effective control of all these methods followed by the biological control to avoid the hazard effects of using the nematicides on human, animal and environment and because they are often not favorite to growers, therefore, biological control is a safe alternative control method of plant-parasitic nematodes. During the last two decades, studies have reported an antagonistic interaction between entomopathogenic and plant-parasitic nematodes (Pérez and Lewis, 2002).

This publication focused on the developing of the non-chemical alternatives for the management of root-knot nematode, *Meloidogyne javanica* on tomato plants under laboratory and greenhouse conditions.

MATERIALS AND METHODS

The *in vitro* assays were conducted under the laboratory of Agric. Botany Dept. and the *in vivo* experiment under greenhouse conditions at the Experimental farm of Fac. of Agric., Minoufiya Univ., Shebin El-Kom to evaluate the entomopathogenic nematodes i.e. *Steinernema carpocapse* ES and *Heterorhabditis bacterophora* HP_{88} at the rate of 4000 IJS/pot and two commercial microbial formulations registered in the Egyptian market as a bio-pesticides i.e. BioZeid 2.5%WP containing 1 x 10^7 spores/g (10 million spores/g) of the fungus *Trichoderma album* and BioArc 6% WP which contains 2.5 x 10^7 spores/g (25 Million spores/g) of the bacterium *Bacillus megaterium* both of them were recommended to be applied at the rate of 40 kg/feddan (1g / pot) and the organophosphate systemic nematicide Fenamiphos 40% EC recommended to be applied at the rate of 3 liter/feddan (0.6 ml/plant).

In Vitro Assays

In vitro assays were carried out in sterile 72-well microtitre plates to

evaluate three population density of entomopathogenic nematodes: *S. carpocapse* ES and *H. bacterophora* HP_{88} i.e. 100; 200 and 400 IJS, on the egg hatching and larval mortality of *M. javanica* after incubation period of 96 and 144 hrs. Each well received eggs and larvae in sterile water were served as control. After 96 and 144 hrs of incubation at room temperature, number of hatched eggs and number of dead larvae in 50 eggs and 50 larvae for egg hatching and mortality, respectively were examined and counted under stereomicroscope.

In Vivo Assays

This experiment was carried out under greenhouse conditions into plastic pots (15 cm in diam.) filled with sandy-clay soil (2:1, v/v). Fourteen treatments were applied in this experiment. Each was applied alone and in combination with other, except the nematicide treatment which applied alone. Four thousand infective juvenile stages (IJ_s) of entomopathogenic nematodes, *Steinernema carpocapse* ES and *Heterorhabditis bacterophora* HP_{88} were inoculated around the tomato root zone. Both commercial microbial formulations, BioZeid and BioArc were applied by mixing one gram from each in the upper layer of soil pots in the root zone. The nematicide, fenamiphos was applied by irrigating tomato roots with 0.6 ml/plant.

All treatments were applied at the same time of transplanting of three weeks-old tomato seedlings (*Lycopersicon esculentum* Mill cv. GS) into pots (one plant/pot).

Inoculums of root-knot nematode, *M. javanica* was obtained from pure culture of tomato heavily infected roots grown under greenhouse conditions at 25±2°C. Eggs of *M. javanica* were extracted from heavily galled roots by using 1.5% sodium hypochlorite solution (NaOCl) method as described by Hussey and Barker (1973). Nematode eggs were inoculated by pipetting 2000 eggs into three holes around the tomato root zone at the same time of transplanting. Each treatment was replicated five times and the non-treated pots served as control. Plants were arranged in a completely randomized block design in the greenhouse at approximately 25±2°C. Plants were watered daily and fertilized weekly with a nutrient solution.

Two months after nematode inoculation, plant growth parameters i.e. fresh weights of shoot and root (g), plant height (cm), root length (cm) and dry weight (g) as well as nematode parameters i.e. number of galls, number of egg masses/root system, number of eggs/egg mass, number of females/root system, number of developmental stages/root system, number of juveniles/250 g soil (Goodey, 1957) were determined. Egg-masses, females and developmental stages were stained prior to counting by dipping the infected roots in 0.015% phloxine-B solution for 20 minutes as described by Daykin and Hussey (1985).

RESULTS

In Vitro Assays

Results of *in vitro* assays revealed that the percentage of egg hatching and second stage larvae motality of *M. javanica* were significantly reduced by all used population densities of entomopathogenic nematodes, *S.carpocapse* ES and *H. bacterophora* HP_{88} at both incubation period either 96 or 144 hrs. (Figs. 1,2,3&4)

Data in Fig. (1&2, a&b) found that the least mean number and percentage of egg hatching of *M. javanica* as well as second stage larvae mortality of *M. javanica* were observed at the population density of 400 IJS of *S. carpocapse* ES at both incubation period compared with the control. The highest significant inhibition of egg hatching was observed with incubation period of 96 hrs. with the density of 400 IJS of *S. carpocapse* ES (Fig. 1, a). The lowest percentage of egg hatching was obtained with 400 IJS after 96 hrs. (Fig. 1, b).

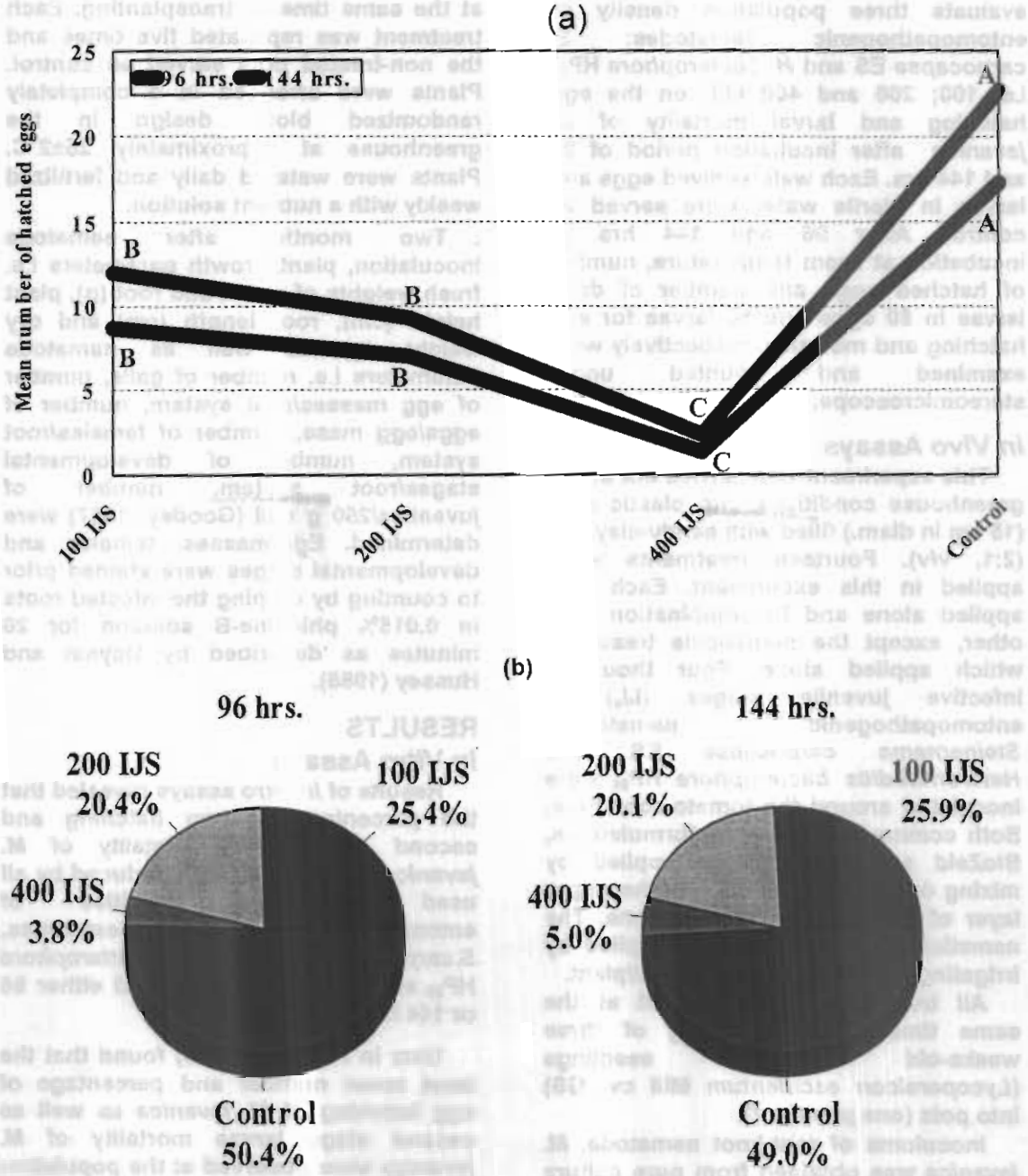
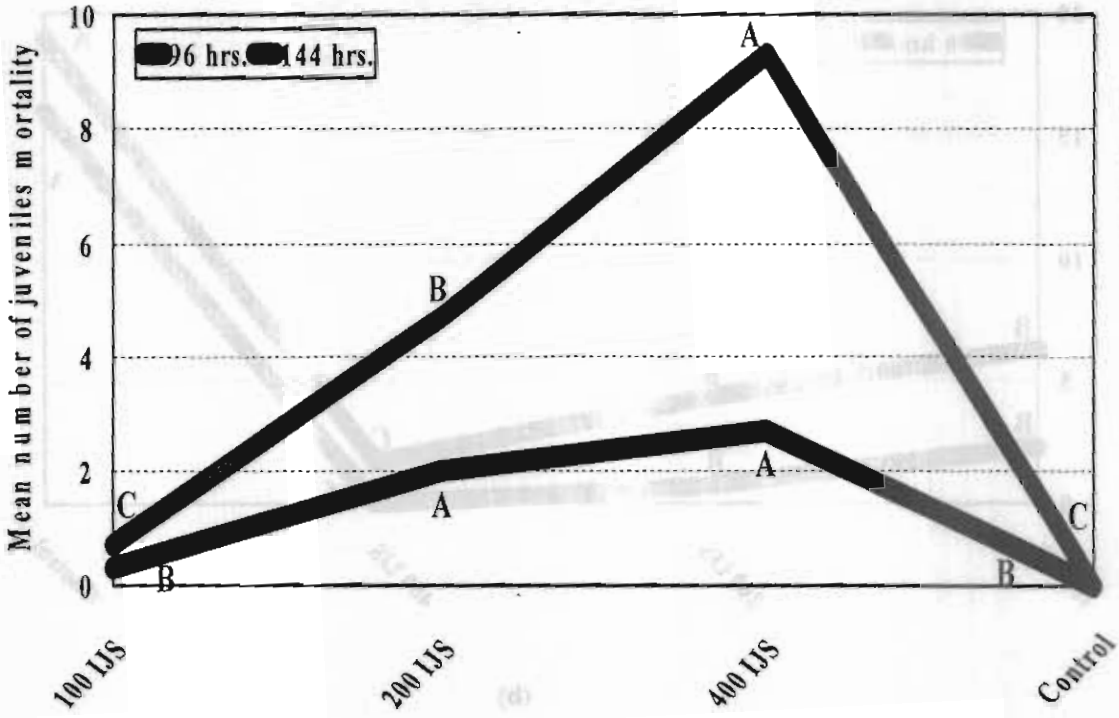


Fig. (1, a&b): Effect of entomompathogenic nematode, *S. carpocapse* ES on the mean number (a) and percentage (b) of *M. javanica* egg hatching. Lines followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

(a)



(b)

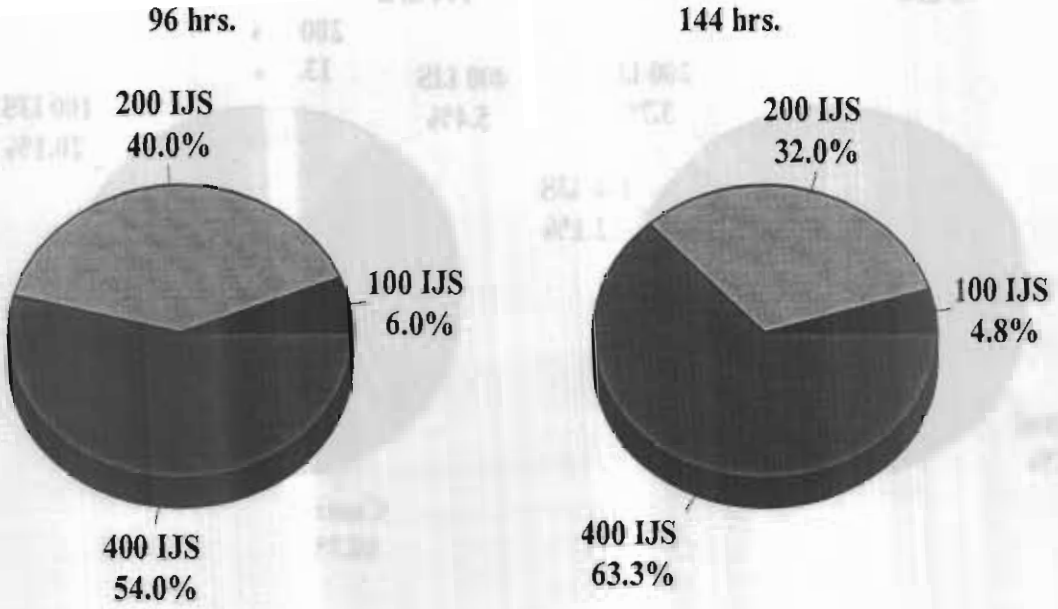
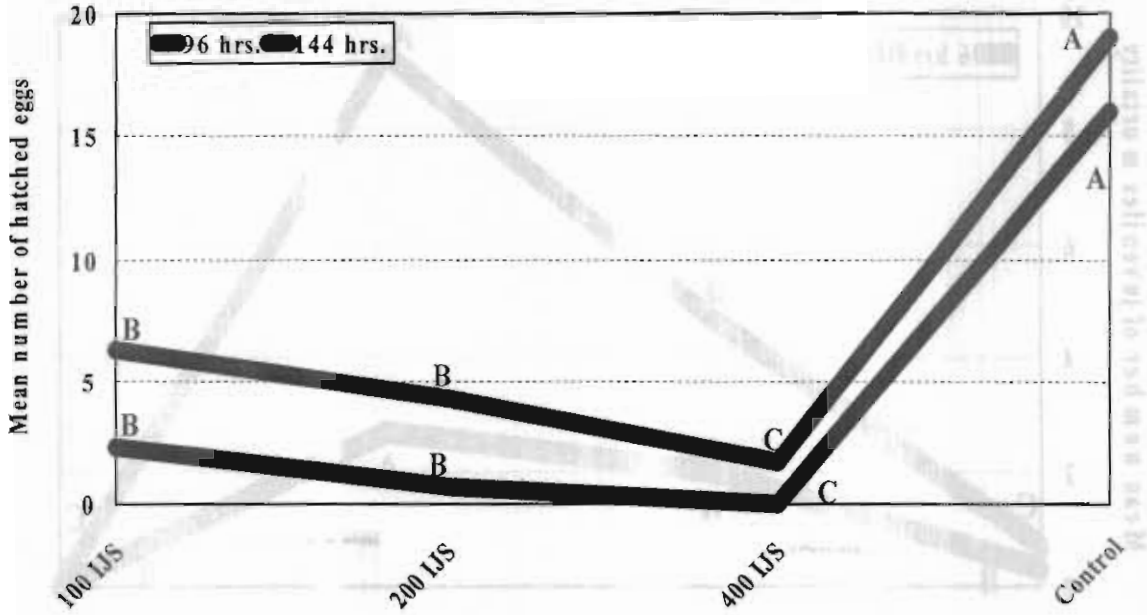


Fig. (2, a&b): Effect of entomopathogenic nematode, *S. carpocapse* ES on the mean number (a) and percentage (b) of *M. javanica* larvae mortality. Lines followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

(a)



(b)

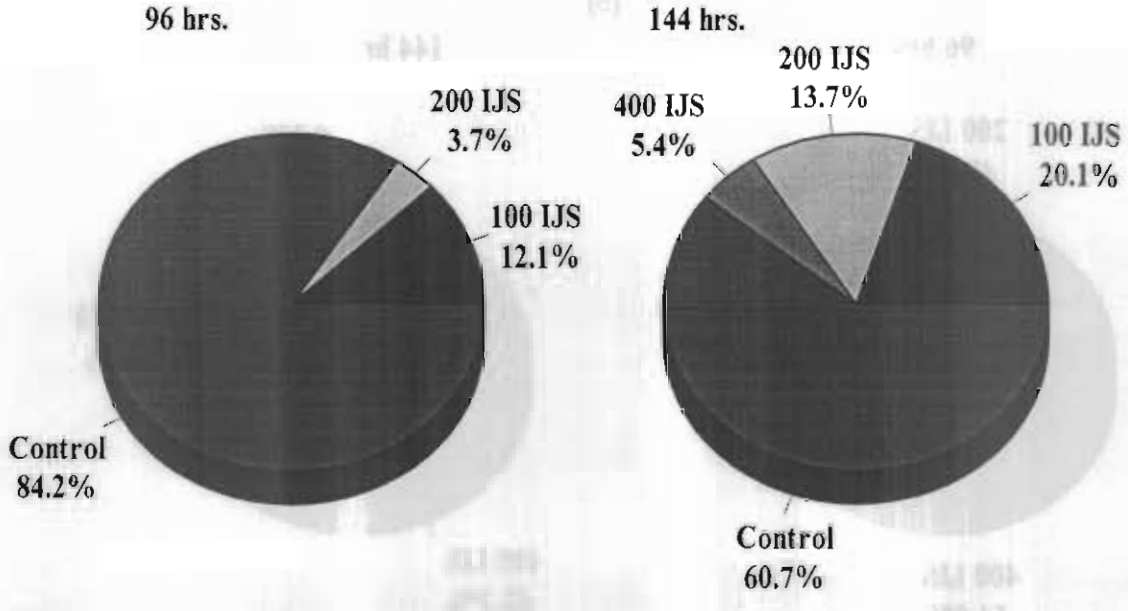
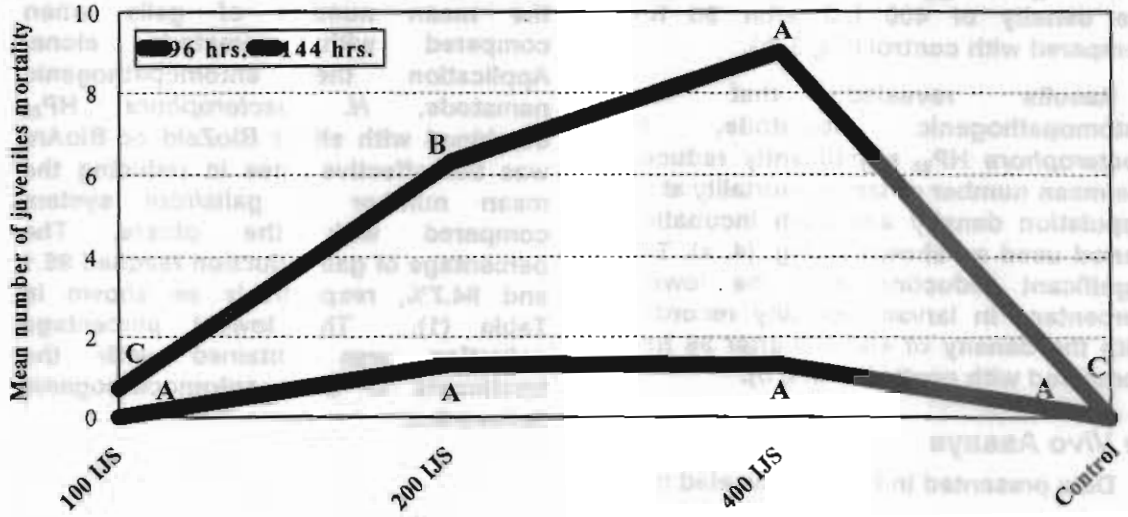


Fig. (3, a&b): Effect of entomopathogenic nematode, *H. bacterophora* HP_{88} on the mean number (a) and percentage (b) of *M. javanica* egg hatching. Lines followed by different letter (s) are significantly different compared with plants treated with nematode alone at $p \leq 0.05$.

(a)



(b)

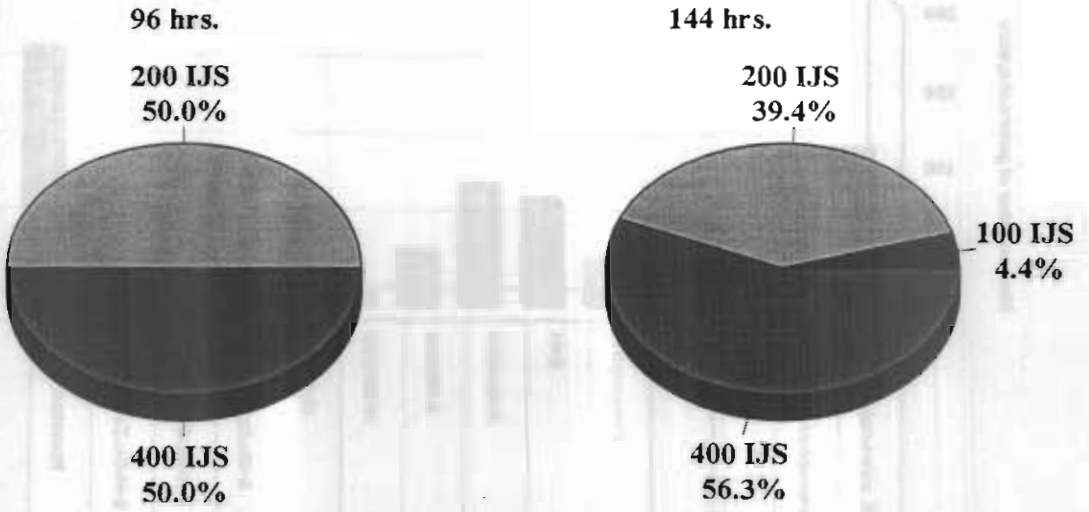


Figure (4, a&b): Effect of entomopathogenic nematode, *H. bacterophora* HP_{88} on the mean number (a) and percentage (b) of *M. javanica* larvae mortality. Lines followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

The same trend of results was obtained for the larval mortality of *M. javanica* as the highest significant and percentage mortality of larvae recorded with the population density 400 IJS of *S. carpocapse* ES after 144 hrs. compared with control (Figs. 2, a&b).

Results indicated that the entomopathogenic nematode, *H. bacterophora* HP_{88} significantly reduced the mean number of hatched eggs at all population density and both incubation period used as shown in Fig. (3, a). The significant reduction and the lowest

percentage in egg hatching showed with the density of 400 IJS after 96 hrs compared with control (Fig.3, b).

Results revealed that the entomopathogenic nematode, *H. bacterophora* HP_{88} significantly reduced the mean number of larvae mortality at all population density and both incubation period used as shown in Fig. (4, a). The significant reduction and the lowest percentage in larvae mortality recorded with the density of 400 IJS after 96 hrs. compared with control (Fig.4, b).

In Vivo Assays

Data presented in Fig. (5) revealed that all tested materials significantly reduced the mean number of galls when compared with nematode alone. Application the entomopathogenic nematode, *H. bacterophora* HP_{88} combined with either BioZeid or BioArc was the effective ones in reducing the mean number of galls/root system compared with the others. The percentage of gall reduction reached 95.1 and 94.7%, respectively as shown in Table (1). The lowest percentage reduction was obtained with the treatments of both entomopathogenic nematodes.

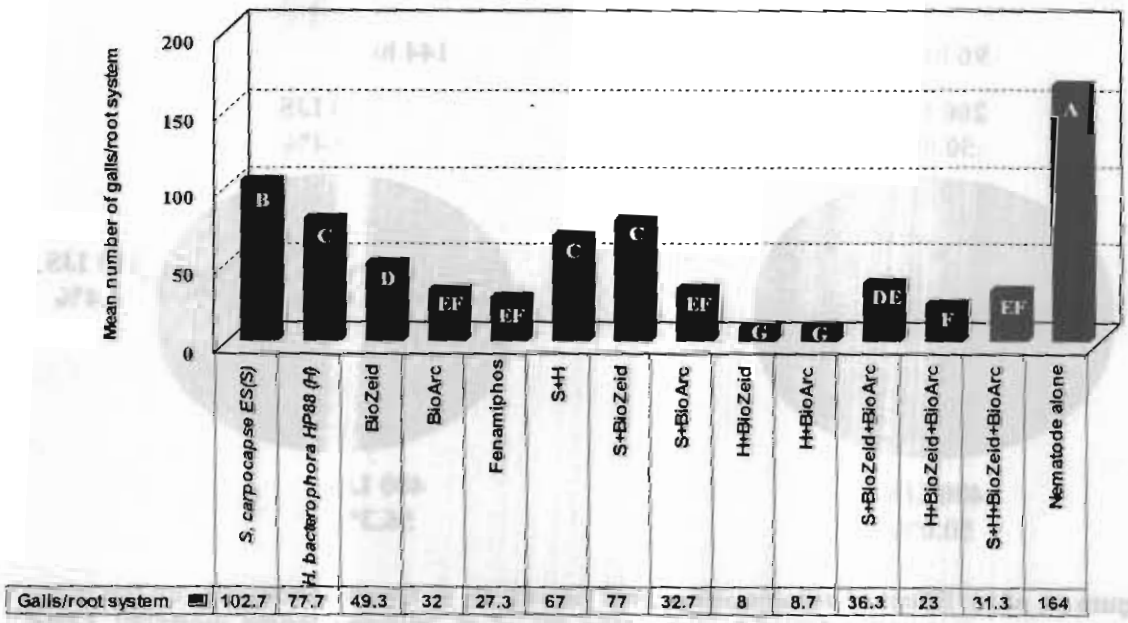


Figure (5): Effect of entomopathogenic nematodes and bio-products on the mean number of galls/root system of *M. javanica*. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at $p \leq 0.05$.

Table (1): Reduction percentages of nematode parameters as affected by entomopathogenic nematodes and bio-product applications. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

Treatments	% Reduction percentages			
	Galls/root system	Egg-masses/root system	Eggs/egg-mass	Juveniles/ 250 g soil
S. carpocapse ES (S)	37.4	23	38.1	28.1
H. bacterophora HP_{88} (H)	52.6	40	39.2	36.2
BioZeid (BioZ)	69.9	70	70.0	50.0
BioArc (BioA)	80.5	90	72.6	48.3
Fenamiphos	83.4	83	83.3	72.3
S + H	59.1	80	59.7	56.9
S + BioZ	53.0	70	75.6	68.7
S + BioA	80.1	83	79.7	57.3
H +BioZ	95.1	97	95.2	83.7
H +BioA	94.7	90	91.1	82.0
S + BioZ + BioA	77.9	90	80.3	49.5
H +BioZ + BioA	85.9	93	86.0	75.8
S + H +BioZ + BioA	80.9	87	70.3	46.6

Results presented in Fig. (6, a&b) reportrd that the mean number of egg-masses/root system and eggs/egg-mass were significantly reduced by all applied treatments compared with nematode alone. The highest significant reduction in egg-masses numbers were obtained with treatments of entomopathogenic nematode, *H. bacterophora* HP_{88} combined with either BioZeid alone or with BioZeid and BioArc together (Fig.6, a). The highest mean number of egg-masses/root system obtained with the application of both entomopathogenic nematodes alone.

Results illustrated in Fig. (7) found that the mean number of developing stage and females/root system were significantly reduced by all applied treatments compared with nematode alone. Results also indicated that application of *H. bacterophora* HP_{88} combined with either BioZeid and/or BioArc were the highest effective treatments in reducing both developing stages and females mean number (Fig.7).

Regarding to the mean number of juveniles in soil, results in Fig. (8) showed significant reduction of all applied treatments when compared with nematode alone. Results illustrated in Fig. (8) reported that the highest significant reduction obtained when the entomopathogenic nematode, *H. bacterophora* HP_{88} applied combined with both BioZeid and BioArc separately or together followed by the nematicide. The percentage of reduction reached 83.7; 82; 75.8 and 72.3%, respectively (Table 1). Application of both entomopathogenic nematode alone showed low effect on the juvenile numbers.

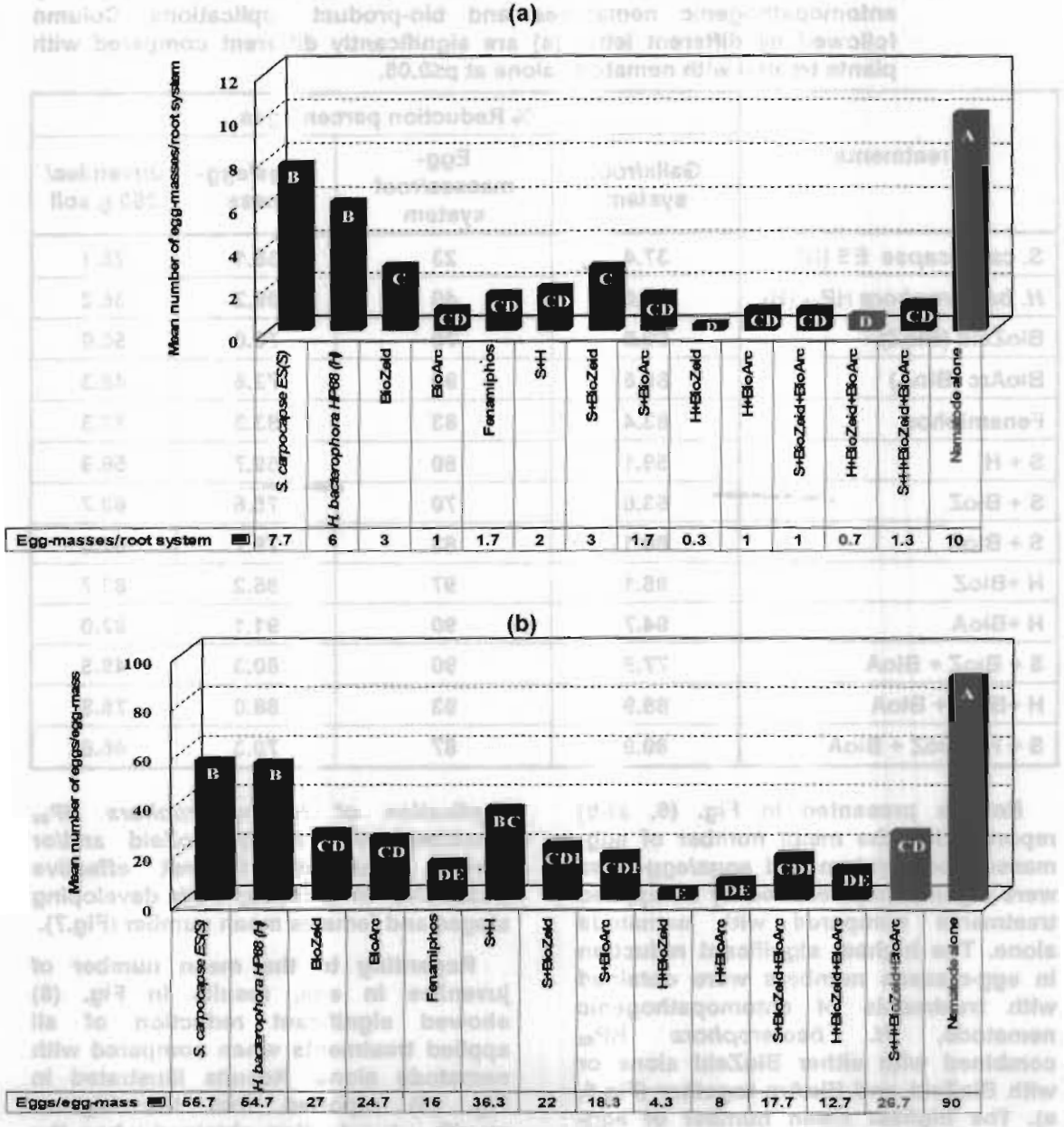


Fig. (6, a&b): Effect of entomopathogenic nematodes and bio-products on the mean number of egg-masses/root system (a) and eggs/egg-mass (b) of *M. javanica*. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

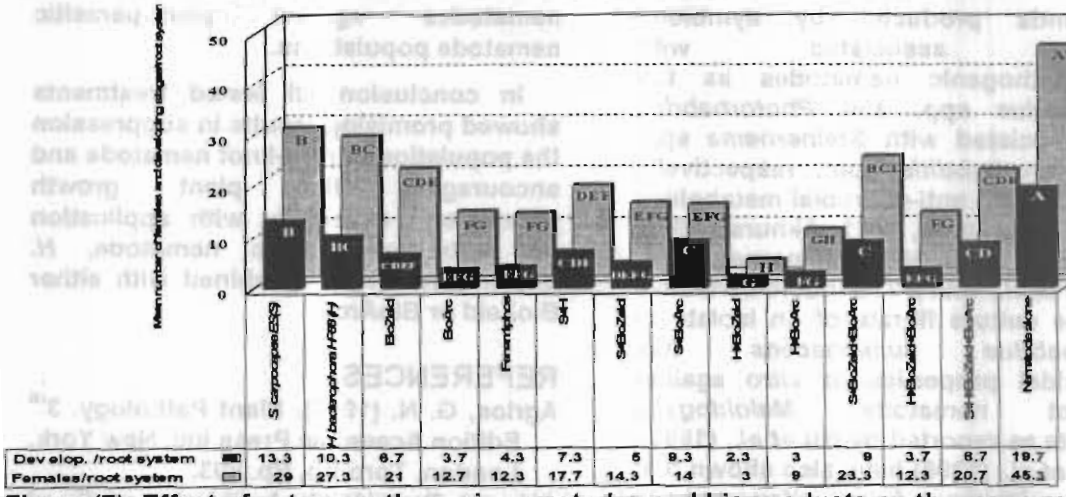


Figure (7): Effect of entomopathogenic nematodes and bio-products on the mean number of developing stages and females/root system of *M. javanica*. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

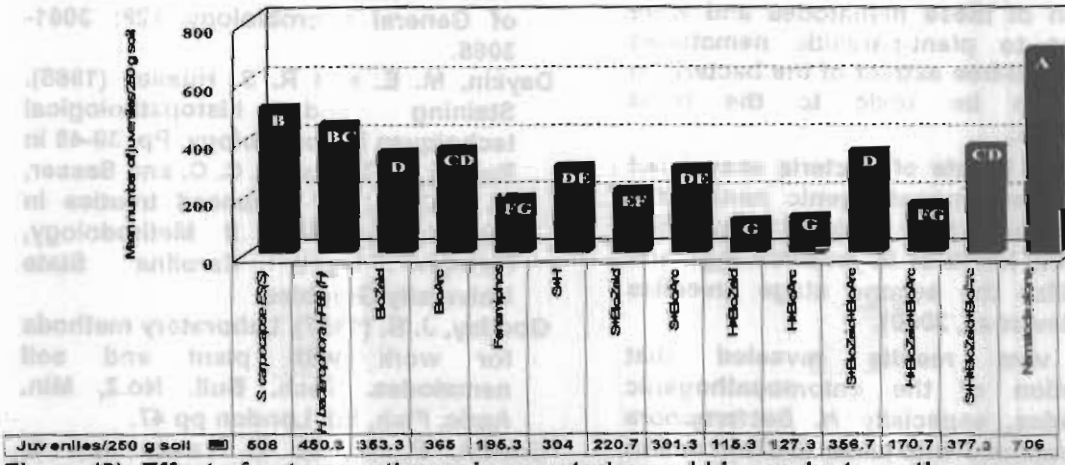


Figure (8): Effect of entomopathogenic nematodes and bio-products on the mean number of juveniles/250 g soil of *M. javanica*. Column followed by different letter (s) are significantly different compared with plants treated with nematode alone at p≤0.05.

Generally, it could be concluded that, all tested treatments showed promising future in control strategy of plant-parasitic nematodes as the reduction percentage of nematode parameters ranged between 23 and 97% (Table 1). Application the entomopathogenic nematode, *H. bacterophora* HP_{88} combined with either BioZeid and/or BioArc alone or combined together registred the promising one compared with the others.

DISCUSSION

In vitro results revealed that both tested entomopathogenic nematodes, *S. carpocapse* ES and *H. bacterophora* HP_{88} reduced markedly the percentage of egg hatching and increased the percentage of juveniles mortality of *M. javanica* at all tested densities and both incubation periods especially at 400 IJS after 144 hrs compared with control. These results could be explained that both entomopathogenic nematodes release

compounds produced by symbiotic bacteria associated with entomopathogenic nematodes as the *Xenorhabdus* spp. and *Photorhabdus* spp. associated with *Steinernema* spp. and *Heterorhabditis* spp., respectively, which produce anti-microbial metabolites *in vitro* (Paul *et al.*, 1981; Akhurst, 1982; Richardson *et al.*, 1988; McInerney *et al.*, 1991; Li *et al.*, 1997). The indole derivative from the culture filtrate of an isolate of *Photorhabdus luminescens* has nematicidal properties *in vitro* against root-knot nematode, *Meloidogyne incognita* as reported by Hu *et al.*, (1999). Grewal *et al.*, (1999) have also shown that culture filtrate from *Xenorhabdus nematophilus* are nematicidal to *M. incognita* juveniles. Lewis and Stevens (2007) found that these bacteria are produced in large quantities during the infection of these nematodes and were repellent to plant-parasitic nematodes and the cell-free extract of the bacteria in culture to be toxic to the most nematodes.

Culture filtrate of bacteria associated with the entomopathogenic nematodes that containing their bacterial metabolites inhibit the hatch of *M. javanica* eggs and immobilize the second stage juveniles (Samaliev *et al.*, 2000).

In vivo results revealed that inoculation of the entomopathogenic nematodes, especially *H. bacterophora* HP_{88} combined with either BioZeid and/or BioArc separately or combined together was the promising treatments compared with plants treated with nematode alone.

Pérez and Lewis (2004) found that application of the entomopathogenic nematode *Steinernema feltiae* to tomatoes two weeks before the release of *Meloidogyne hapla* eggs and juveniles suppressed the penetration of nematodes into tomato roots. Similarly, application of *S. glaseri* and *Heterorhabditis bacteriophora* in high concentrations diminished the penetration of *M. incognita* juveniles. Lewis *et al.*, (2001) and Kella *et al.*, (2008) reported significant effect of entomopathogenic nematodes against plant-parasitic nematode populations.

In conclusion all tested treatments showed promising results in suppression the population of root-knot nematode and encouraging the plant growth parameters, especially with application the entomopathogenic nematode, *H. bacterophora* HP_{88} combined with either BioZeid or BioArc.

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تأثير النيماتودا الممرضة للحشرات وبعض المركبات الحيوية على نيماتودا تعقد الجذور *Meloidogyne javanica*

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الملخص العربى

تم تنفيذ هذا البحث لتقييم نوعين من النيماتودا الممرضة للحشرات وهى ***Steinernema carpocapse*** ES and ***Heterorhabditis bacterophora*** HP_{88} ونوعين من المركبات الحوية وهى***Trichoderma album, Bacillus megaterium*** سواء منفردة أو متحدة معاً بالإضافة إلى المبيد النيماتودى فيناموفس كمبيد نيماتودى وذلك مكافحة نيماتودا تعقد الجذور ***Meloidogyne javanica*** تحت الظروف المعملية وظروف الصوبة على نباتات الطماطم .

أظهرت النتائج المعملية أن نوعى النيماتودا الممرضة للحشرات تحت الدراسة والتى أستخدمت بثلاثة تركيزات مختلفة وهى ١٠٠ ، ٢٠٠ ، ٤٠٠ طور معدى قد قللت جميعها معنويا من نسبة فقس البيض وزادت من نسبة موت يرقات نيماتودا تعقد الجذور سواء بعد فترة تحضين ٩٦ ، ١٤٤ ساعة مقارنة بالكنترول .

كان تركيز النيماتودا ٤٠٠ يرقة معدية لكل من نوعى النيماتودا الممرضة للحشرات هى أكثر المعاملات فاعلية في تقليل فقس البيض أو زيادة موت يرقات نيماتودا تعقد الجذور في كل من فترتى التحضين مقارنة بالتركيزات الأخرى.

أظهرت النتائج تحت ظروف الصوبة أن كل المواد المستخدمة قد قللت معنوياً من أعداد العقد النيماتودية وأكياس البيض والإناث/ المجموع الجذرى وعدد البيض/كيس بيض بالإضافة إلى أعداد الطور اليرقى المعدى لنيماتودا تعقد الجذور / ٢٥٠ جم تربة مقارنة بالنباتات المعاملة بنيماتودا تعقد الجذور فقط .

وجد أيضا أن إضافة النيماتودا الممرضة للحشرات ***H. bacterophora*** HP_{88} متحدة مع بيوزد ***T. album*** أعطت أكثر المعاملات فاعلية في تقليل نسبة العقد الجذرية وأكياس البيض/ المجموع الجذرى والبيض/ كيس بيض واليرقات/ ٢٥٠ جم تربة بمعدل ٩٥,١ ، ٩٧ ، ٩٥,٢ و ٨٣,٧% على التوالى ، تلاها في التأثير استخدام نفس نوع النيماتودا الممرضة متحدة مع ***B. megaterium*** حيث وصلت نسبة الانخفاض في تعداد نيماتودا تعقد الجذور ٩٤,٧ ، ٩٠ ، ٩١,١ و ٨٢% على التوالى . وسجلت أقل المعاملات تأثيراً عند تطبيق كل من نوعى النيماتودا الممرضة منفردة في التربة .

كذلك لوحظ أن كل المعاملات المختبرة قد حسنت من الصفات الخضرية لنباتات الطماطم مثل الوزن الطازج للمجموع الخضرى والجذرى وطول النباتات وطول الجذر وكذلك الوزن الجاف . كما لوحظت الزيادة المعنوية عند تطبيق كل من نوعى النيماتودا الممرضة معاً مع ***T. album***.

وفى العموم يمكن القول أن كل المعاملات المختبرة أظهرت نتائج جيدة من خلال تقليل جميع أطوار نيماتودا تعقد الجذور في كل من التربة والجذور وزيادة الصفات المرتبطة بالنمو لنباتات الطماطم .