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Efficacy of Five Biopesticides for the Management of Root-Knot Nematode, *Meloidogyne incognita* Infecting Pepper (*Capsicum annum* L.) Plants

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ABSTRACT

A greenhouse experiment was conducted to assess the impact of five commercial biopesticides on root knot nematode, *Meloidogyne incognita* infecting pepper plants cv. Kortoba. Biopesticides used in the present study were: Anti-Nema (*Serratia marcescens*) Bio-Arc (*Bacillus megaterium*), Bio-Zeid (*Trichoderma album*), Bio-Nematon (*Purpureocillium lilacinum*), and Nema-Stop (*Streptomyces avermitilis*), compared to chemical nematicide, Krenkel as well as untreated check. All biopesticides were applied as soil drench after nematode inoculation (1000 *M. incognita* second stage juveniles) which spread around the base of plant. Applications of such biopesticides achieved a significant ($P \leq 0.05$) improvement in plant growth parameters and significant suppression in nematode population. Bio-Arc recorded maximum values in improving total plant fresh weight (89.7%), plant length (123.0%), and shoot dry weight (143.8%), followed by Bio-Zeid. Also, Bio-Arc recorded the highest percentages of reduction in number of juveniles in soil, root galling and egg masses with values 66.06, 65.79 and 62.32% respectively comparing to nematode alone. Furthermore, it is also worth noting that the majority of treatments had the same values for both root gall and egg masses indices (2.0) compared to nematode alone (3.0).

Keywords: *Capsicum annum*, *Meloidogyne incognita*, *Bacillus megaterium*, *Trichoderma album*, *Purpureocillium lilacinum*, *Serratia marcescens*, *Streptomyces avermitilis.*

INTRODUCTION

Pepper (*Capsicum annum* L.) is one of the most important vegetable crops in many countries of the world. In Egypt, pepper being a vegetable crop having marketing and exporting values, where the total area under production reaches 91,840 feddan (Annual Bulletin 2019). Pepper plants are recognized to be attacked by *Fusarium solani*, *Rhizoctonia solani* and *Meloidogyne incognita*. Root-knot nematodes are obligate parasites that damaging plant and limiting the agricultural productivity. Most cultivated pepper species are susceptible to root-knot nematode infection (Kamali et al., 2015). In Egypt, root-knot nematodes, *Meloidogyne* spp. became a real threat to almost all vegetable crops, especially in the newly reclaimed areas and have been considered as limiting factors in crop production (Ibrahim et al., 2000).

Traditionally, the main control technique involves the use of chemical nematicides; however, the negative consequences of these products to the environment and their inefficiency after long-term use have resulted in the prohibition or restrictions on various molecules employed. Therefore, there is an increasing requirement for developing non-chemical alternative methods for the management of the root-knot nematode (Huang et al., 2016). One beneficial alternative that is gaining popularity in nematode control is the biological control, predominantly utilizing the microorganism groups like fungi and bacteria already present in the soil biota (Crawford and Clardy, 2011).

Many bacterial and fungal agents are effective in controlling *Meloidogyne* spp. (Vos et al., 2013; Mohamed et al., 2020; Migunova and Sasanelli, 2021). These microorganisms can induce resistance through activation of salicylic acid (SA), jasmonic acid (JA) and ethylene pathways that promote the production of phenolic compounds, reactive oxygen species and PR proteins Pathogenesis-related proteins (Hao et al., 2012). Some species of *Trichoderma* can induce resistance against *M. incognita* and improve plant growth (Mascarin and Junior, 2012; Al-Hazmi and Javeed, 2016; Poveda, 2020) that adopts JA and SA related defense responses according to nematode stages (De Medeirosa et al., 2015 & Martínez-Medina et al., 2017). Sharon et al. (2001) reported that *T. harzianum* was antagonistic organism to root-knot pathogen, *M. javanica* in soil. Other *Trichoderma* species and isolates have also exhibited significant biocontrol activity against *M. javanica* in growth chamber experiments.

Abamectin that belongs to macrolide metabolites is produced by the bacterium *Streptomyces avermectinius* (Khalil and Abd El-Naby, 2018). The nematocidal activity of Abamectin against different genera of plant nematodes was proved by some investigations (El-Nagdi and Youssef, 2004; El-Nagdi et al., 2015 and Radwan et al., 2019). *Bacillus megaterium* is a soil bacterium capable of dissolving unavailable phosphorus in soil rendering it available for growing crops. Also, *B. megaterium* has been reported to decrease *M. incognita* population (Radwan et al., 2012; Mostafa et al., 2018).

Therefore, the current experiment was intended to evaluate the effectiveness of five commercial biopesticides; Anti-Nema (*S. marcescens*) Bio-Arc (*B. megaterium*), Bio-Zeid (*T. album*), Bio-Nematon (*P. lilacinum*), and Nema-Stop (*S. avermitilis*) against root-knot nematode, *M. incognita* infecting pepper plants.

MATERIALS AND METHODS

Nematode culturing

Single egg masses belonging to *M. incognita* (Kofoid & White) Chitwood female identified according to Taylor et al. (1955) were used to inoculate coleus plant, *Coleus blumei* grown in 25cm diameter plastic pots filled with sterilized loamy sand soil. Pots were kept on a clean bench in the greenhouse receiving water and fertilizer as needed. Two months later, plants were uprooted, and roots were examined for nematode infection. Infected roots were used to inoculate other coleus plants. Sub-culturing and maintenance were continuously carried out to obtain sufficient inoculum for further greenhouse experiments at the Nematology Research Unit, Agricultural Zoology Department, Faculty of Agriculture, Mansoura University, Egypt where this work was carried out. Nematode eggs were extracted by macerating infected coleus roots in 0.5% (v/v) NaOCl as in Hussey and Barker (1973). The collected eggs were placed in Baermann trays (Whitehead and Hemming, 1965) to obtain nematode juveniles (J_{2s}).

Nematicide

Krenkel 75% EC (Fosthiazate), (RS)-S-sec-butyl-O-ethyl-2-oxo-1,3-thiazolidin-3-ylphosphono-thioate was used as a nematicide at the rate of 0.3 ml/plant.

Biopesticides

All commercial biopesticides were obtained from the Agricultural Research Center (ARC), Giza, Egypt as follows:

1) **Anti-Nema**, a commercial product of *Serratia marcescens* which contains 25×10^9 CFU/g of bacterium. A solution of 2.5g/100 ml distilled water was prepared.

2) **Nema-Stop** 5% CS, a commercial product of Abamectin (5% CS) *Streptomyces avermitilis,* (Presently named *avermectinius*) applied at the rate of 2.5L/feddan. A solution of 2.5 ml /100 ml distilled water was prepared.

3) **Bio-Nematon** 1.75% WP, a commercial product of *Purpureocillum lilacinum* formerly known as *Paecilomyces lilacinus* which contains 1×10^8 CFU/gm of fungus. A solution of 0.25g/100 ml distilled water was prepared.

4) **Bio-Arc** 6% Powder, a commercial product of *Bacillus megaterium* which contains 25×10^6 CFU/g of bacterium. A solution of 2.5g/100 ml distilled water was prepared.

5) **Bio-Zeid** 2.5% Powder, a commercial product of *Trichoderma album* which contains 25×10^6 CFU/g of fungus. A solution of 2.5g/100 ml distilled water was prepared.

Experimental design

Pepper seedlings cv. Kortoba (25 days old) were sown in plastic pots (15cm-d) containing one kg mixture of sterilized sandy loam soil (1:1). One week later, seedlings were inoculated with 1000 *M. incognita* J_2s spread around the base of plant. Seven days after nematode inoculation, biopesticides were applied as soil drench. Treatments were as follows: Bio-Arc (100 ml/pot); Bio-Zeid® (100 ml/pot); Bio-Nematon® (10 ml/pot); Nema-Stop® (10 ml/pot); Anti-Nema® (2ml/pot); Krenkel (0.3ml/pot). Plants receiving nematode inoculum only as well as uninoculated-untreated plants (Healthy) were served as controls.

Pots were arranged in a completely randomized design with four replicates for each treatment under greenhouse conditions 27±2°C. The experiment was terminated forty-five days after nematode inoculation. Data on plant growth parameters i.e., fresh shoot and root weights, dry shoot weight, shoot and root lengths were measured. Juveniles were extracted from an aliquot (250g) of soil using sieving and modified Baermann technique (Goodey, 1957). Roots were stained with acid fuchsin in lactic acid (Byrd et al., 1983). Thus, root galling, and egg masses were recorded. Galls (GI) and egg masses indices (EI) were assessed on 0-5 scale according to Taylor and Sasser (1978) where 0= no galls or egg masses per root system; 1=1-2; 2=3-10; 3=11-30; 4=31-100; and 5= >100 galls or egg masses per root system.

Statistical analysis:

Statistical analysis of the data was performed using version 6.303 of a computer program Costat (2005). Statistically significant differences between means were compared using analysis of variance (ANOVA) with the least significant difference (LSD) at a probability of 0.05.

RESULTS AND DISCUSSION

As shown in Table (1) and Figure (1) treatments with five commercial biopesticides i.e., Anti-Nema, Bio-Arc, Bio-Zeid, Bio-Nematon, and Nema-Stop induced a significant difference ($P \leq 0.05$) in reducing number of juveniles in soil, root galling and egg masses of *M. incognita* on pepper roots cv. Kortoba comparing to check under greenhouse conditions at 27±2°C. It is interesting to observe that among the tested products as a biotic factor, Bio-Arc accomplished the best results in reducing number of juveniles in soil, root galling and egg masses on root system of pepper plants since their reduction percentage values were amounted to 66.06, 65.79 and 62.32%, respectively comparing to nematode alone.

Table 1: Efficacy of tested biopesticides applied as soil drench, on number & reduction of juveniles, root galling and egg masses of *Meloidogyne incognita* infecting pepper plants**.**

Treatments	J_2/pot	Red. %	No. of galls	Red. %	RGI	No. of egg masses	Red. %	EI
Anti-Nema	750.0^{cd}	59.59	10.65^{a}	43.95	2.0	9.25^{b}	46.38	2.0
Nema-Stop	760.0^{cd}	59.06	7.25^{cd}	61.84	2.0	8.25^{b}	52.17	2.0
Bio-Nematon	1300.0^{b}	29.96	14.5^{b}	23.68	3.0	9.25^{b}	46.38	2.0
Bio-Arc	630.0^{d}	66.06	6.5^{d}	65.79	2.0	6.5^{b}	62.32	2.0
Bio-Zeid	660.0^{cd}	64.44	9.0^{cd}	52.63	2.0	7.0^{b}	59.42	2.0
Krenkel	795.0^{c}	57.17	10.0^{cd}	47.37	2.0	8.5^{b}	50.72	2.0
Nematode alone	1856.2^{a}	----	19.0^{a}	----	3.0	17.25^{a}	----	3.0
LSD 0.05	104.16	----	2.86	----	----	4.02	----	----

Each value is the mean of four replicates. Means in each column followed by the same letter (s) did not differ at $p<0.05$ according to Duncan's multiple-range test. Reduction % $= \frac{\text{Nematode alone} - \text{Treatment}}{\text{Nematode alone}} \times 100.$

Results of Bio-Zeid treatment were averaged 64.44, 52.63 and 59.42%, respectively for the same previous nematode criteria, while Nema-Stop treatment stated the moderately values 59.06, 61.84 and 52.17%, respectively comparing to nematode alone. Moreover, among the tested biotic agents, Bio-Nematon treatment accomplished the least reduction percentages of juveniles in soil (29.96%) galls (23.64%), and egg masses (46.38%). However, Krenkel as a nematicide recorded reduction percentages as 57.17, 47.37 and 50.72%, respectively (Fig.1). Furthermore, it is also worthy to note that the most tested treatments gave equal values of both nematode criteria i.e., root gall and egg masses indices that averaged 2.0 while it was 3.0 for nematode alone (Table 1).

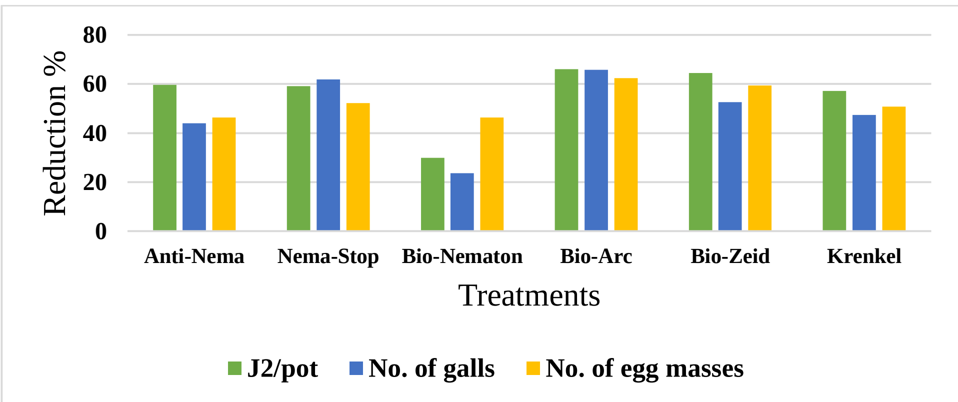

Figure 1: Reduction percentages of juveniles in soil, root galling and egg masses of *Meloidogyne incognita* infecting pepper plant cv. Kortoba as influenced by the bioagents under greenhouse conditions.

Data presented in Table (2) and Fig. (2) revealed that all tested materials obviously ameliorated significantly pepper plant growth parameters to great extent. Similarly, Bio-Arc (*B. megaterium*) performed the best and significantly ($P \leq 0.05$) improved shoot length and fresh & dry shoot weights of pepper plants infected with *M. incognita* followed by Bio-Zeid (*T. album*), then Anti-Nema. Obviously, Krenkel surpassed biopesticides treatments in increasing plant biomass as well as shoot and root lengths.
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Among the tested components, Bio-Arc showed the maximum percentages in improving total plant fresh weight (89.7%), plant length (123.0%), and shoot dry weight (143.8%), followed by Bio-Zeid as 67.9, 78.2, and 56.3% for the same plant growth parameters, respectively, whereas, Nema-Stop achieved the minimum percentages for the same plant growth criteria that averaged 15.9, 31, 6.2%, respectively comparing to nematode alone.

Table 2: Impact of five bioagents on plant growth parameters of pepper cv. Kortoba infecting with *Meloidogyne incognita* in comparison with Krenkel under greenhouse conditions (27±2°C).

Treatments	Plant Length (cm)				Plant Fresh weight (g)				Shoot dried w.t(g)	
	Shoot	**Root**	**Total**	**Inc. %**	**Shoot**	**Root**	**Total**	**Inc. %**	**W. (g)**	**Inc. %**
Anti-Nema	21.5^{bc}	13.0^{b}	34.5^{c}	19.0	12.0^{bc}	3.4^{ab}	15.4^{ab}	77.0	2.2^{c}	37.5
Nema-Stop	21.65	12.0^{b}	33.6^{c}	15.9	8.5^{cd}	2.9^{b}	11.4^{bc}	31.0	1.7^{c}	6.2
Bio-Nematon	21.0^{bc}	13.2^{b}	34.2^{c}	17.9	8.9^{cd}	2.6^{b}	11.5^{bc}	32.2	1.9^{c}	18.8
Bio-Arc	26.0^{b}	29.0^{a}	55.0^{a}	89.7	15.4^{ab}	3.9^{ab}	19.4^{a}	123.0	3.9^{ab}	143.8
Bio-Zeid	24.0^{bc}	24.7^{a}	48.7^{b}	67.9	10.5^{c}	4.9^{a}	15.5^{ab}	78.2	2.5^{bc}	56.3
Krenkel	22.0^{bc}	13.0^{b}	35.0^{c}	20.7	12.0^{bc}	2.4^{b}	14.4^{b}	65.5	4.3^{a}	168.8
Nematode	20.5^{c}	8.5^{b}	29.0^{c}	----	6.3^{d}	2.4^{b}	8.7^{c}	----	1.6^{c}	----
Healthy Plants	30.0^{a}	28.0^{a}	58.0^{a}	100.	16.0^{a}	4.0^{ab}	20.0^{a}	129.9	3.9^{ab}	143.8
LSD 0.05	3.35	3.7	5.18	----	2.89	1.29	3.44	----	1.25	----

Each treatment is an average of four replicates, Percentage increase (Inc.%) = (Treatment – Nematode alone)/ Nematode alone ×100. Means in each column followed by the same letter(s) did not differ at $P \leq 0.05$ according to Duncan's multiple-range test. Pi (Initial population) = 1000 J_2s of *M. incognita.*

However, Anti-Nema showed considerable values of ameliorating plant parameters that averaged 19.0, 77.0, and 37.5% for plant length, total plant fresh weight, and shoot dry weight, respectively comparing to nematode alone. Moreover, Krenkel nematicide occupied the third rank after Bio-Arc and Bio-Zeid (in plant length and fresh weight only) with percentage increase values of plant length and total plant fresh weight averaged 20.7 and 65.5%, respectively as compared with nematode alone. Meanwhile, plants free of nematode and untreated with any of the tested materials showed reasonable percentage increase values that averaged 100.0, 129.9 and 143.8% for plant length, fresh weight, and shoot dry weight respectively, comparing to nematode alone (Table 2 and Fig. 2).

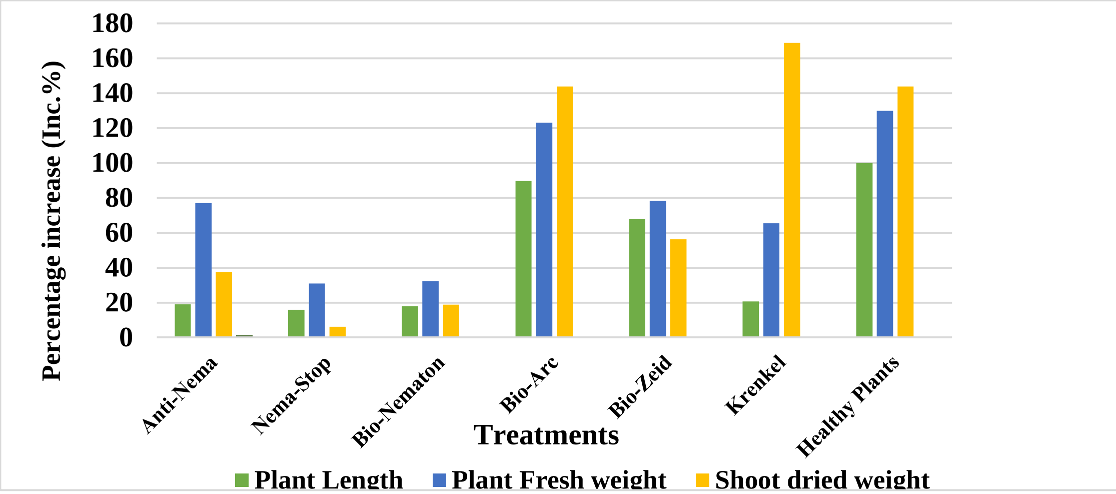

Figure 2: Increase percentages of pepper growth parameters infected with *Meloidogyne incognita* as influenced by tested bioagents applied as soil drench.

In this study the potential of five commercial products namely: Anti-Nema (*S. marcescens*) Bio-Arc (*B. megaterium*), Bio-Zeid (*T. album*), BioNematon (*P. lilacinum*), and Nema-Stop (*S. avermitilis*) were applied as soil drench against *M. incognita* attacking pepper plants. Hao et al. (2012) confirmed that microorganisms can induce resistance through activation of jasmonic acid, salicylic acid and ethylene pathways which promote the production of PR proteins, reactive oxygen species and phenolic compounds However, several investigations demonstrated two actions of *Trichoderma* species against plant parasitic nematodes by direct parasitism of eggs, juveniles and females through the increase in enzymatic activities as well as inducing plant defense mechanisms (Sahebani and Hadavi, 2008 & Yang et al., 2010). *P. lilacinus* at 12 g/kg seed were found most effective followed by *Pochonia chlamydosporia* and *Glomus fasiculatum* at the same dose to enhance plant growth of bitter gourd and to reduce the infection of *M. incognita* (Bhati et al., 2019). *P. lilacinus* was more potent in reducing galling and enhancing the growth of nematode-inoculated plants than *T. harzianum* (Hano and Khan, 2016). Induced systemic resistance by plant growth promoting rhizobacteria (PGPR) has been successfully achieved in large number of agricultural crops including cucumber, potato, Arabidopsis, tomato, chili, sugarcane, rice, mango, and carnation against broad-spectrum of pathogens including bacteria, viruses, fungi and nematodes etc. (Reddy, 2012 & Alizadeh et al., 2013). Protease and chitinase enzymes produced by *P. lilacinus* were stated good effect in hyphal penetration through the cuticle of juveniles and females of *M. javanica* (Khan et al., 2006).

The study by Timmusk et al. (2005) confirmed the possibility of *Paenibacillus polymyxa* to form biofilms in plant roots after predominant colonization which was not examined in this study. Biofilms can suggest inhibition of J_2 penetration as they were found to protect pathogen infection sites. Investigation on biofilm formation is recommended for future studies to prove its possibility on protecting infection sites that may assure failure of nematode establishment and reproduction. Root colonization by rhizosphere bacteria was also reported by Siddiqui and Shaukat (2004) to reduce *M. incognita* penetration of roots, reproduction, and root-knot disease were decreased more by dual inoculations with arbuscular mycorrhizal (AM) fungi and plant growth promoting rhizobacteria (PGPR) (Li et al., 2011). Production of metabolites by these rhizosphere bacteria can also reduce attraction and penetration of nematodes as they were found to degrade specific root exudates (Oostendorp and Sikora, 1990). Efficient rhizosphere colonization and biofilm formation was found to be one of the important aspects for effective control (Haggag and Timmusk, 2008).

Joshi et al. (2012) revealed that fungal bio-agents of *P. lilacinus,* at the rate of 2 g/kg soil was the best treatment in enhancing plant growth criteria and reducing nematode reproduction over other fungal bio-agent' treatments. *P.lilacinus* was the best bio-agent in reduction root galls (57.53%). The obtained data indicated significant ($P \leq 0.05$) increase in plant growth parameters that agreed with the findings of El-Deriny (2009), Radwan et al. (2012), and El-Zawahry et al. (2015). The increase of plant growth parameters was also reported by Jung et al. (2002) and Khan et al. (2008). *Bacillus* and *Trichoderma* are well known as plant growth promoters (Resende et al*.*, 2004, Fortes et al*.*, 2007). The increased plant growth induced by *B. megaterium* that has been reported in many investigations (El-Hadad et al*.*, 2011, Radwan et al*.*, 2012 and Mostafa et al., 2018) was indicated in the present study. Several mechanisms of action of *Trichoderma* are recognized as BCA: mycoparasitism, antibiosis, competition with the pathogen, promotion of plant growth, enhanced plant-tolerance against abiotic stresses and stimulation of its defenses against pathogens (Poveda, 2020). Application of *P. fluorescens*, *P. lilacinum* and *P. guilliermondii* as biocontrol agents had also strengthen the growth of plants via production of natural growth hormones and supplying many nutritional elements, induction of systemic resistance in plants and lethal effect on nematodes (Hashem and Abo-Elyousr, 2011). Notably, *B. megaterium* gave better improvement in growth characters of cowpea infected with *M. incognita* than *T. album*. Previous studies revealed that *B. megaterium* is helpful in solubilizing of phosphorus and makes it available for plant growth and roots can gain carbon compounds i.e., sugars and organic acids, essential for bacterial growth (Khan et al., 2010 & Radwan et al., 2012).

Finally, it could be concluded that the application of commercial microbial pesticides containing *Bacillus megaterium* consider a successful candidate to achieve biocontrol potential of *M. incognita* on pepper significant increase in chemical constitutions and plant defenses. The biological pesticide, Bio-Zeid ranked the next in reducing galling, nematode multiplication and promoting growth attributes as well. Further studies are needed using organic matter to improve the potentials of bio-agents and achieve safe and ecofriendly management of root-knot nematodes under greenhouse and field conditions.

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الملخص العربي

كفاءة خمس مبيدات حيوية في مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* التي تصيب نباتات الفلفل

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تناولت الدراســة الحالية تأثير خمس مبيدات حيوية هي: أنتنيم (*Serratia marcescens*) البيوأرك (*Bacillus megaterium*) البيوزيد (*Trichoderma album*) والبيونيماتون (*Paecilomyces lilacinus*) ونيماستوب (*Streptomyces avermitilis*) ضد نيماتودا تعقد الجذور *M. incognita* على نباتات الفلفل صنف قرطبة تحت ظروف الصوبة الزراعية بطريقة الإضافة للتربة مرة واحدة بعد العدوي بالنيماتودا (١٠٠٠ طور يرقي ثاني/نبات)، وقد أسفرت النتائج عن مايلي: -

١- كل المعاملات المختبرة مقارنة بمبيد الكرينكل كان لها تأثير كبير على خفض تعداد النيماتودا وزيادة نمو نباتات الفلفل المصابة مقارنة بالنيماتودا وحدها بنسب متفاوتة.
٢- حققت المعاملة بالمركب الحيوي البيوأرك أعلى القيم بين المعاملات في خفض المقاييس النيماتودية المختبرة بنسب ٦٦,٠٦، ٦٥,٧٩، ٦٢,٣٢٪ لكل من تعداد الطور اليرقي الثاني في التربة وعدد العقد النيماتودية وعدد كتل البيض على الجذور على الترتيب مقارنة بالنيماتودا وحدها.
٣- تفوقت أيضاً المعاملة بالمركب الحيوي البيوأرك على كل المعاملات المختبرة في طول النبات (٨٩,٧٪) والوزن الرطب للنبات (١٢٣,٠٪) والوزن الجاف للمجموع الخضري (١٤٣,٨٪) يليها المعاملة بالمركب الحيوي البيوزيد لنفس المقاييس النباتية.
٤- حققت المعاملة بالمبيد الكيماوي (كرينكل) خفض المقاييس النيماتودية المختبرة بقيم ٥٧,١٧، ٤٧,٣٧، ٥٠,٧٢٪ لكل من تعداد الطور اليرقي الثاني في التربة وعدد العقد النيماتودية وعدد كتل البيض على الجذور على الترتيب، مع زيادة في طول النبات (٢٠,٧٪) والوزن الرطب للنبات (٦٥,٥٪) والوزن الجاف للمجموع الخضري (١٦٨,٨٪) مقارنة بالنيماتودا وحدها.