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Influence of Some Bionematicide and Entomopathogenic Nematodes Against *Meloidogyne incognita* and *Rotylenchulus reniformis* Infecting Papaya Plant.

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ABSTRACT

The efficacy of Agrein, Bioarc, Bionema, Biozeid, Micronema and two entomopathogenic nematodes (EPNs), *Steinernema carpocapsae* (All), *Heterorhabditis bacteriophora* (B20) as biological control agents of *Meloidogyne incognita* and *Rotylenchulus reniformis* nematodes infecting papaya, *Carica papaya* L. were evaluated under greenhouse conditions. All the tested bionematicide and (EPNs) significantly reduced in the numbers of nematodes in soil, galls formation, eggmasses and eggs per eggmass than those in the untreated (check) and improved plant growthparameters. The highly significant increase in weights of both shoot and root of plant grown in soil treated with Agrein and *Steinernema carpocapsae*. This research may contribute in the novel direction of using bionematicide and entomopathogenic nematodes (EPNs) a biological agent against plant parasitic nematode.

Keywords:-Papaya, *Meloidogyne incognita*, *Rotylenchulus reniformis,* Bionematicides, Entomopathogenic nematode.

INTERDICTION

Papaya, *Carica papaya* L. is one of the major fruit crops cultivated in tropical and sub-tropical zones due to its fast growth, high yield, long fruiting period and high nutrient value as well (Teixeira da Silva *et al*., 2007).

In Egypt papaya cultivation is lately spreaded in old land of Nile Delta governorates i.e. Sharkeya, Quliobia and Giza and new reclaimed lands at Noubareia.

Two nematode species, *Meloidogyne incognita* and *Rotylenculus reniformis* infecting papaya and reduced growth and production (El-Borai and Duncan 2005 and Kesba *et al*., 2012). Bacterial isolated of *Bacillus subtilis*, *B. thuringiensis*and *Pseudomonas aeruginosa* were found to reduce the number of galls, eggmasses and population of *Meloidogyne* species infecting the economic crops. Eggs of *Meloidogyne* spp. were penetrated and parasitized by few hypha of *Trichoderma harzianum, T. viride, T. megatrium* (Kavitha *et al.,* 2007; Al-shalaby & Sedik, 2008 and Huang *et al.,* 2009).

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are found in many region throughout the world including Egypt (Shamseldean and Abd-Elgawad, 1994). Limited nematicide availability and high costs of nematicides development have created a need to discover alternative methods for controlling plant-parasitic nematodes. Other studies have shown that EPN and their associated bacteria possibly may interfere with the infection and reproduction of some PPN (Grewal *et al*., 1997). Some nematologists are interested in determining this interaction between EPN and PPN were first shown by Bird and Bird (1986), who showed that a reduction of the infection of *M. javanica* in tomato plants was caused by *Steinernema glaseri* (Steiner) application in greenhouse pot tests. The present study was to evaluate the efficacy of some bionematicide and the EPN in the infection of *M. incognita* and *R. reniformis* under papaya plants in the greenhouse condition.

MATERIALS AND METHODS

Nematode species stock cultures:

Pure culture of root-knot, *Meloidogyne incognita* and reniform, *R. reniformis* nematodes were obtained from isolates belonging to the Nematology Research Center (NRC), Faculty of Agriculture, Cairo University and propagated separately on eggplant Nematodes were extracted from soil by sieving and modified Baermann technique (Goodey, 1957).

Entomopathogenic nematode :

Two species of Heterorhabditis bacteriophora (B20) and Steinernema carpocapsae (All) were used. The entomopathogenic nematodes population used in this research originated from Plant Protection Research Inst. Agricultural Research Centre (ARC) Dokki, Giza., Egypt, where greater wax moth Galleria mellonella was used as host insect to invivo culture; to be used in this work as the biological agent.

Bionematicides:

Five bionematicides used in this study were obtained from Organic Company and apply on papaya plants.

Greenhouse experiment:

One month old seedlings of papaya *Carica papaya* L. with uniform size were grown singly in 20 cm diam pots filled with steam-sterilized sandy loam soil (1:1, V:V) separately. Two weeks later, seedlings were separately inoculated with 2000 infective stages/plant of either *M. incognita* or *R. reniformis* by bouring the nemateode suspension into 4 holes in the soil around the root system of each seedlings. After inoculation the holes were closed by pressing the soil watered. The bionematicides Agrein (*Bacillus subtilis*), Bioarc (*Bacillus thuringiensis*), Biozeid (*Trichoderma megatrium*) at concentration 5g/pot and Bionema (*Bacillus megaterium*) and Micronema (*Pseudomonas* spp + *Bacillus* spp.) at concentration 500ml./L.water/pot in simultaneously inoculation. Inoculum level was determined according to the design of each experiment during the course of this investigation which was done in the greenhouse of the department mentioned above as follows:

1-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita*.
2-Three papaya seedlings inoculated with 2000 infective stages of *R. reniformis*.
3-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + Bioarc 5g/pot.
4-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + Agrein 5g/pot.
5-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + Bionema 5ml/pot.
6-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + Biozeid 5g/pot.
7-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + Micronema 5ml/pot.
8-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + 4000 infective juveniles *Steinernema carpocapsae*.
9-Three papaya seedlings inoculated with 2000 infective juveniles of *M. incognita* or *R. reniformis* + 4000 infective juveniles of *Heterorhabditis bacteriophora* (B20).

Pots were randomly arranged on a greenhouse bench at 27±4ºC. Plants were watered regularly and treated horticulturally as recommended. After 45 days from nematode juveniles inoculation, plants were harvested infected plant roots were examined uprooted and washed with tap water and the number of galls, nematode developmental stages, egg-masses in roots were estimated after staining by lactic acid fuchsin (Byrd *et al.* 1983) recorded. Rate of nematode build-up were then calculated. Data of shoot and roots length as well well as fresh weight were recroded and regarded as plant growth criteria. Data analyzed by means of Duncan'smultiple-range test (Duncan,1955).

RESULTS AND DISCUSSION

Data in Table (1) revealed that the experiments succeeded in reducing number of the nematode juveniles in soil, numbers of galls, egg masses in root of papaya. Use of *Steinernema carpocapsae* (All) at 4000 Infective Juveniles (IJs)/pot recorded a 50% reduction in number of egg/eggmasses *M. incognita* treatment of 4000 IJs/pot *Heterorhabditis bacteriophora* (B20) caused 62% reduction egg of eggmasses. The high percentage of reduction of eggs/eggmass 70% for Agrein with dose 5g/pot followed by Micronema 5ml/pot recorded 67%. Entomopathogenic nematodes, *Steinernema* and *Heterorhabditis* were present around the roots of papaya seedlings and produced toxic agents to plant-parasitic nematodes causing a boundary area of protection around plant roots against the infection by infective juveniles of plant parasitic nematode.

Table 1. Effect of certain commercial bionematicides and entomopathogenic nematode against *Meloidogyne incognita* infecting papaya plant under greenhouse condition at (27 ± 4 °C).

Treatments	No. of nematodes in soil/pot	No. of galls/root system	Nematode counts		Rate of nematode reproduction (Pf/Pi)	No. of eggs/ Egg mass	Reduction %
			No. of egg masses/root	Final population (Pf)			
Bioarc 5g/pot	3625 bc	146 n	63 fg	3834	1.92	449 ab	43
Agrein5g/pot	2452 jk	345 bc	140 b	2937	1.45	230 jk	70
Bionema 5ml/pot	3466 cd	277 de	82 ef	3825	1.91	364 de	51
Biozeid 5g/pot	2948 fg	225 gh	127 bc	3279	1.64	468 ab	40
Micronema 5ml/pot	2612 hi	274 de	57 hi	2943	1.47	261 ij	67
Steinernema carpocapsae(All) 4000IJ/pot	3937 b	203 jk	62 gh	4202	2.10	390 ij	50
Heterorhabditis bacteriophora (B20). 4000IJ/pot	2792 hi	290 cd	70 ef	3152	1.58	301 ij	62
Control plants (*M. incognita*) only	4545 a	479 a	223 a	5247	2.62	783 ab	

Values in a column followed by the same letter(s) are not significantly different at (P≤0.05) according to Duncan's multiple-range test.
N alone= 2000J_2 *M. incognita*
***Reduction % = $\frac{\text{N. control – N. treatment}}{\text{N. control}}$ x 100**
****Reproduction Factor (RF) = $\frac{Pf}{Pi}$**

In Table 2 showed increase on length and fresh weight of both shoot and roots of papaya plants infected with root-knot nematode improved the plant growth as compared to untreated plants. The highest shoot length of Agrein 5g/pot 70% followed by micronema and *Heterorhabditis bacteriophora* (B20). The least treatment increase of length and fresh weight of shoot and roots was recorded for the papaya plant treated with Bioarc (43%, 29%, 50% and 9%).

Data in Table (3) indicated that all tested bioagents reduced number of nematode juveniles in soil, galls for *R. reniformis* nematodes on root system as compared to those of the control treatment. It is interesting to observe that Agrein treatment accomplished the highest reduction percentage value of 73%. Whereas, Micronema and Bioarc gave values of 65% and 58% respectively. However, entomopathogenic nematodes (EPNS), *S. carpocapsae* (All), *H. bacteriophora* (B20) recorded (39% and 40% respectively).

Table 2. Effect of certain commercial bionematicides and entomopathogenic nematodes against *Meloidogyne incognita* infecting papaya plant on growth parameters under greenhouse condition at (27 ± 4 °C).

Treatments	Shoot				Root		Root	
	Length	Increase%	Weight	* Increase%	Length	Increase %	Weight	* Increase %
Bioarc 5g/pot	40 bc	43	11.1 de	29	30 bc	50	8.1 fg	9
Agrein5g/pot	44.8 bcd	60	13.5 ab	57	48.8 a	144	11.6 a	57
Bionema 5ml/pot	50 bc	79	14.5 ab	69	23.4 ef	17	9.4 bc	27
Biozeid 5g/pot	38.3 cd	37	11.0 de	28	30.6 bc	53	10.3 ab	39
Micronema 5ml/pot	28.8 cd	2.85	10.9 def	27	26.3 de	31.5	8.1 fg	9
Steinernema carpocapsae(All) 4000IJ/pot	61 a	117.9	11.2 cd	30	30.4 bc	52	10.7 ab	45
Heterorhabditis bacteriophora (B20). 4000IJ/pot	38.4 cd	37	12.ab	42	26.0 de	3	10.1 abc	36
Control plants (*M. incognita*) only	28 e		8.6 f	-	20.0 f		7.4 h	

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

*** Increase = treatment - control (n alone) x 100**
N alone

Table 3. Effect of certain commercial bionematicides and entomopathogenic nematode against *Rotylenchulus reniformis* infecting papaya plant under greenhouse condition at (27 ± 4 °C).

Treatments	No. of nematodes in soil/pot	No. of galls /root system	Nematode counts		Rate of nematode reproduction (Pf/Pi)	No. of eggs/ Egg mass	* Reduction %
			No. of egg masses /root	Final population (Pf)			
Bioarc 5g/pot	3400 cd	163 mn	48 ij	3611 fg	1.8	328 fg	58
Agrein5g/pot	2212 jk	148 mn	91 de	2451 k	1.2	211 k	73
Bionema 5ml/pot	3360 cd	220 hi	64 fg	3644 de	1.8	339 ef	56
Biozeid 5g/pot	3015 ef	191 im	77ef	3283 ef	1.6	465 ab	40
Micronema 5ml/pot	2672 hi	324 bc	59 hi	3055 fg	1.5	270 hi	65
Steinernema carpocapsae(All) 4000IJ/pot	3234 de	269 de	69 ef	3572 cd	1.8	473 ab	39
Heterorhabditis bacteriophora (B20). 4000IJ/pot	2716 hi	272 de	67 fg	3055 fg	1.5	465 ab	40
Control plants (*R. reniformis*) only	4554 a	476 a	220 a	5250	2.6	779 ab	

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

****Rate of reproduction = Final population (Pf)**
Initial population (Pi)

Pi= 2000 infective stages of *R. reniformis*

In Table (4) reveal influences of the treatments with studied bioagents on growth parameters of papaya plants infected with *R. reniformis* , it was found that they almost increased total length of shoot and root (60% and 58% respectively of Agrein). The second increased in fresh weight for shoot and root recorded for bionematicide Micronema at 37% and 56%.

Table 4. Effect of certain commercial bionematicides and entomopathogenic nematode against *Rotylenchulus reniformis* infecting papaya plant on growth parameters under greenhouse condition at (27 ± 4 °C).

Treatments	Shoot				Root		Root	
	Length	Increase %	Weight	* Increase %	Length	Increase %	Weight	*Increase %
Bioarc 5g/pot	41 bc	43	12.4 de	44	30.4 bc	50	10.2 ab	42
Agrein5g/pot	42.8 bcd	60	11.8 ab	37	28.8 a	144	11.4 h	58
Bionema 5ml/pot	40 bc	58	15.4 a	79	26.6 ef	17	8.6 cd	19
Biozeid 5g/pot	39.4 cd	37	11.5 cd	34	33.4 bc	53	8.9 cd	24
Micronema 5ml/pot	28.5 cd	2.85	11.8 bc	37	27.5 de	31.5	11.2 ab	56
Steinernema carpocapsae(All) 4000IJ/pot	62 a	117.9	9.8 ef	14	27.2 bc	52	10.4 ab	44
Heterorhabditis bacteriophora (B20). 4000IJ/pot	38.1 cd	37	9.5 ef	10	30 de	3	7.7 gh	6.9
Control plants (*R. reniformis*) only	28.5 e		8.5 f	-	20.0 f		7.2 h	

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

N alone= 2000J_2 infective stages of *R. reniformis.*

*** Increase = treatment - control (n alone) x 100**
control (N alone)

The influence of five bionematicide in soil on plant growth of papaya infected with *M. incognita* and *R. reniformis* increased length and fresh weight of both shoots and roots. Generally, almost all tested

bionematicids succeeded to reduced the juvenile's numbers in soil, numbers of galls and eggmasses. Results are conforming to the finding of Kokalis-Burelle *et al*., (2002, 2003) and Ibrahim *et al.,* (2007). Tasted soil with *Trichoderma* spp. reduced root galling and increasing top fresh weight of plants infected with *Meloidogyne* spp. (Mayer and Roberts, 2002; Dababat and Sikora, 2007; Kumar and Jaain, 2010 and Jepathambiga *et al*., 2011).

Application of microorganisms antagonistic to plant parasitic nematode *Meloidogyne* spp. provide additional opportunity for managing the damage caused by root-knot nematode (Tian *et al.,* and Sharma and Pandey, 2009). The efficacy of Agrein, Bioarc, Bionema, Biozayed, Micronemaand Nemaless in controlling *M. javanica* infecting okra plants reduced the numbers of nematodes in soil, galls, eggmasses and egg per eggmass (Montasser *et al.,* 2012).

Entomopathogenic nematodes used successfully as biological control agents in several cropping systems to reduce populations of plant-parasitic nematodes and free-living nematodes in various way. The interaction between plant-parasitic nematodes (PPN) and entomopathogenic nematode (EPN) is especially unexpected because these nematodes do not compete for common resources nor do they interact directly in any way. The reduction of plant-parasitic nematodes is attributed at least partially to compounds produced by symbiotic bacteria associated with (EPN). These bacteria are produced in large quantities during an (EPN) infection and cadavers of insects with ongoing infections were repellents to plant-parasitic nematodes, and the cell-free extract of the bacteria in culture to be toxic to most nematodes other than their symbiotic partners.

All the above mentioned entomopathogenic nematode species reduced number plant parasitic nematode in the soil and egg-masses. This may be attributed to competition at the root surface which may affect plant parasitic nematodes behavior or entomopathogenic nematodes crowded along the roots of plants force plant parasitic away. Suppression of plant-parasitic nematode populations has been demonstrated in a number of greenhouse (Ishibashi and Choi, 1991; Ishibashi and Kondo, 1987) and field studies (Grewal *et al*., 1997; Smilety *et al*., 1992) in different cropping systems. It is also possible that experimental differences between greenhouse and field conditions significantly affected the suppressive action of *S. feltiae* on plant-parasitic nematodes.

(Kella *et al.,* 2011) tested the ability of entomopathogenic nematodes, *S. carpocapsae* and *H.bacteriophora* (B20) as biological control agents against *M. incognita* infecting tomato plants in the greenhouse. Entomopathogenic nematodes were added at three inoculum levels 1000, 2000 and 4000 IJs/pot. The results reported that the use of both entomopathogenic nematodes (EPN) effective in the biological control programs of *M. incognita* and other plant parasitic nematodes.

The symbiotic bacteria associated with Steinernematids, *Xenorhabatdus* spp., produce metabolites that are toxic to nematodes. These metabolites include indole, which produced by *Photorhabdus* in culture. Indole was associated with *M. incognita* paralysis, but was not produced in *G. mellonella* cadavers (Hu *et al*., 1999). A similar mechanism may explain the results (LaMondia and Cowles, 2002) with *P. penetrans* and *S. feltiae.* Results obtained by Kella and Hammad, 2007 have also indicated the toxic effect of Stalpene and indole on plant-parasitic nematodes. The allelochemicals produced by *Xenorhabatdus* spp. As the cause of antagonism to *M. incognita* and *T. semipenetrans* suppression using *Heterorhabditis* was more effective than using *Steinernema*. We found that pre-infestation applications of EPN suppress *T. semipenetrans* on greenhouse sour-orange. Other results by Hu *et al*., (1999) prove that alellelopathic substances produced by live or dead IJs may be toxic and/or repellent to PPN, thus reducing their population density. EPN-associated bacteria, *Xenorhabdus* spp. or *Photorhabdus* spp., produce endotoxins composed of lipopolysacarides that are toxic and could kill or affect in another way the evaluated stages (Dunphy and Webster, 1988). This results agree with reported previously to use entomopathogenic nematodes in control *Tylenchulus semipenetrans* gave reduction of number of plant parasitic nematode (Al-Ghnam *et al.,* 2015)

This data suggesting that some of entomopathogenic nematodes can suppress plant-parasitic nematode species. Generally, to achieve the second part out of a two-fold goal (Abd-Elgawad and Aboul-Eid, 2002; Abd-Elgawad *et al.,* 2008 and Kella *et al.,* 2011). In this respect, Lewis and Grewal (2005) wondered what a possible nematode control product based on EPN would look like.

CONCLUSION

Our results agree with those reported previously to use bionematicide and entomopathogenic nematodes EPN in the control of plant parasitic nematode PPN. We conclude that treatments show promise for control *Meloidogyne incognita* and *Rotylenchulus reniformis* infecting papaya plants. All tested bionematicide significantly succeeded to improve the plant growth as compared to the untreated. On the other, showed the effect of *Steinernema carpocapsae* and *Heterorhbditis bacteriophora* gave reduction of number PPN and egg masses agree with (EL-Deeb *et al*., 2004) Egyptian isolates are becoming established as biological control agents of some plant parasitic nematodes.

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تأثير بعض المركبات الحيوية والنيماتودا الممرضة للحشرات فى مكافحة نيماتودا تعقد الجذور والنيماتودا الكلوية التى تصيب نباتات الباباظ.

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تهدف هذه الدراسة لمعرفة كفاءة استخدام خمس أنواع من المركبات الحيوية المنتجة تجاريا كمبيدات نيماتودية وهى البيوارك والأجرين والبيوزيد والبيونيما والميكرونيما والنيماتودا الممرضة للحشرات *Steinernema carpocapsae* (All); *Heterorhabditis bacteriophora* (B20) فى مكافحة نيماتودا تعقد الجذور والنيماتودا الكلوية التى تصيب الباباظ تحت ظروف الصوبة الزراعية. ولقد أشارت النتائج الى أن معاملة التربة بتلك المعاملات أدى الى خفض أعداد النيماتودا بالتربة وأعداد العقد الجذرية وعدد البيض داخل كيس البيض على جذور النباتات المصابة مقارنة بالنباتات المصابة وغير المعاملة. كانت أفضل النتائج هى استخدام الأجرين حيث حققت نسبة خفض ٧٠% يليه الميكرونيما بنسبة ٦٧% اما بالنسبة للنيماتودا الممرضة للحشرات *Heterorhabditis bacteriophora* (B20) و*Steinernema carpocapsa* سجلت بنتائج جيدة فى خفض تعداد النيماتودا المتطفلة نباتيا ٥٠ و٦٢% على التوالى. وقد انعكس ذلك على معدل نمو النباتات من حيث زيادة الأوزان واللأطوال للمجموع الخضرى والجذرى حيث سجلت معدلات نمو النباتات المعاملة بالأجرين والميكرونيما أعلى النتائج يليها المعاملة بالنيماتودا الممرضة للحشرات.