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Effects of vermicompost, vermicompost tea and a bacterial bioagent against *Meloidogyne incognita* on banana in Egypt

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Abstract

The nematicidal potential of vermicompost (VC), vermicompost tea (VCT) and the bacterial bioagent Nemaless® was evaluated against *Meloidogyne incognita* infecting banana (*Musa acuminata*) cv. "Grand Nain" as compared to the nematicide fenamiphos 40% EC under laboratory and field conditions. Experiments were carried-out during two successive seasons (2016 and 2017). Results of the laboratory tests showed that the toxicity of VCT and Nemaless® to *M. incognita* second-stage juveniles (J_2) was increased as their concentrations and exposure times were increased. The LC_{50} of VCT ranged from 30.86×10^3 to 83.77×10^5 mg/l, while the LC_{50} of Nemaless® ranged from 2.69×10^8 to 3.17×10^8 cell/ml. Results from the field experiments revealed that all of the tested treatments greatly suppressed ($P \leq 0.05$) the numbers of galls, egg-masses and nematode final populations. The most potent materials in reducing the numbers of *M. incognita* in banana soil, in a descending order, were: fenamiphos, Nemaless®, vermicompost and vermicompost tea (VCT).

Keywords: *Serratia marcescens,* fenamiphos, root-knot nematode, biological control

Agricultural production plays an important role in the global economy and food security for humans and animals. Unfortunately, plant pests and diseases cause damage and shortages in both human and animal foods. Thus, the management of these pests and / or diseases is very necessary. Banana (*Musa* sp.) is such a large and important feeding crop in many tropical and sub-tropical countries as well as in Egypt, where the cultivated area reaches up to 65.791 feddan with a 20.39 tons of fruits/feddan (FAO, 2016). However, root-knot nematodes (*Meloidogyne* spp.) are among the most destructive pests which limit banana production in Egypt and were found in 76% of the rhizosphere soil samples collected from banana plantations (Mokbel *et al*., 2006). *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *M. incognita* (Kofoid and White, 1919) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949 and *M. hapla* Chitwood, 1949 are the most common species of root-knot nematodes, being responsible for at least 90% of all the plant damages caused by nematodes (Castagnone-Sereno, 2002). It was also found that the root-knot nematodes are the most predominant group that are targeted by 48% of the global nematicides used for all crops (Haydock *et al*., 2006).

There are various tools to manage plant-parasitic nematodes (PPN) such as chemical nematicides, bio-pesticides, resistant varieties, soil solarization and soil amendments. Unfortunately, most farmers, especially in the developing countries, prefer the use of chemical nematicides as an effective and rapid solution, even though they may adversely affect human life and the environment. Recently, there has been considerable pressure to eliminate the use of the chemical pesticides and to look for

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alternatives that may be safer and better for the environment. Some of these approaches include plant residues, animal wastes, compost, vermicompost, and bio-fumigants (Nath & Singh, 2011; El-Sherbiny & Awd Allah, 2014; Devi & Das, 2016).

Serratia marcescens Bizio is a gram-negative bacterium that has been considered as a natural and environmentally acceptable alternative to chemicals (Abd-Elgawad & Mohamed, 2006; Zeinat *et al*., 2010). It is one of the most effective bacteria for degradation of chitin (Monreal & Reese, 1969) due its ability to produce a variety of chitinolytic enzymes and chitin-binding proteins (Fuchs *et al*., 1986).

Vermicompost, as soil amendment, can be used to improve soil health, and to control some soil-borne pathogens and pests. It is a kind of organic products produced by certain species of earthworms, especially *Eisenia fetida* Savigny, 1826. Earthworms accelerate the biodegradation of different types of waste such as farm, kitchen, market, bio-wastes of agro-based industries, livestock wastes etc. Vermicompost increases the level of macro nutrients such as N, P, and K (Suhane, 2007), in addition to the amount of organic matter and improvement of soil fertility. Some reports have declared the effectiveness of vermicompost and vermicompost tea in reducing the populations of plant-parasitic nematodes in the soil (Arancon *et al*., 2003; Sinha & Herat, 2012; Ramrao & Pathak, 2013). The objective of this study was to evaluate the efficacy of vermicompost, vermicompost tea, and the biocontrol product Nemaless® for managing the root-knot nematode, *M. incognita* on banana.

Materials and Methods

The nematicidal potential of vermicompost (VC), vermicompost tea (VCT), the bacterial bioagent, Nemaless,® and the nematicide, fenamiphos 40% EC, was evaluated for reducing *M. incognita* infecting banana, *Musa acuminata* Colla, cv. "Grand Nain" in laboratory and field conditions.

Laboratory assays: The effects of different concentrations of vermicompost tea and the bacterial bioagents Nemaless® on the mortality of *M. incognita* second-stage juveniles (J_2) were evaluated *in vitro*. Vermicompost tea (VCT) was prepared by soaking the finely ground vermicompost in distilled water @ 250g VC/l water and mixed thoroughly for 48 hours. The suspension was filtered using muslin tissue and kept in a clean glass flask to be used as a standard solution (S).

Four concentrations of VCT suspension were prepared as follows: 1) S = 250 g VC/l water, 2) S/2 = 125 g/l, 3) S/3 = 83.3 g/l, and 4) S/4 = 62.5 g/l). Nemaless® (N) which contains the bacterium *S. marcescens*, as a biocontrol agent, was used as 1) N = 1×10^9 CFU/ml, 2) N/2 = $0.5 \times l0^9$ CFU/ml, 3) N/3 = 0.33×10^9 CFU/ml and 4) N/4 = 0.25×10^9 CFU/ml. Thus, eight treatments were used, plus distilled water as a control. One ml of each suspension and/or dilution prepared was transferred to a well of a 24-well plate (Corning®), and approximately 200 newly hatched (J_2) of *M. incognita* were added to each well. Each treatment was replicated 5 times, and the plates were kept in the lab at room temperature. The mortality of J_2s was determined after 24, 48, and 72 hr. of exposure.

Field experiment: The field experiments were undertaken during two successive seasons (2016 and 2017). In this respect, forty uniform banana plants (age and size) were selected in a banana plantation naturally-infested with the root-knot nematode, *M. incognita*. The orchard was located at Nubaria region, Behera Governorate, Egypt. The selected plants were spaced at 2.5 × 3.5 m apart (600 plants/feddan), and received the same horticultural practices usually adopted for this area according to the guidelines of the Ministry of Agriculture, Egypt. The plants were irrigated using a surface drip irrigation system. Soil and root samples were collected from the rhizosphere of each banana plant and transferred in insulated chests to the laboratory. Samples were kept in a refrigerator at 5 °C until they

were processed for nematode extraction within a week. Whenever needed, J_2 of the root-knot nematodes were extracted from the rhizosphere soil samples using sieving and centrifugal flotation techniques (Ayoub, 1980), identified, and counted with a compound microscope using Peter`s 1 ml eelworm counting slide. Adult females of the root-knot nematodes were isolated from the banana galled roots and identified as *M. incognita* by the examination of the perineal patterns according to Taylor & Sasser (1978). The initial population density for each banana plant and treatment were expressed as mean numbers of J_2s/kg soil, numbers of root galls, and nematode egg-masses/20 g roots. Nematode populations were determined with the same methods as described in the case of initial population after 3 and 6 months of the first application. The bacterial bioagent Nemaless® (containing the bacterium, *S. marcescens* at 1×10^9 CFU/ml) was obtained from the local market, Vermicompost produced from animal manures, paper, and plant and food wastes was obtained from a private farm in Nubaria. Vermicompost tea (VCT) was prepared as previously mentioned. Treatments on April 2016 were as follows:

1- Vermicompost @ 250 g/plant
2- Vermicompost @ 500 g/plant
3- Vermicompost tea (125 g VC/l water) @ 1000 ml /plant
4- Vermicompost tea (250 g VC/l water) @ 1000 ml /plant
5- Nemaless® @ 20 ml/plant
6- Nemaless® @ 30 ml/plant
7- Fenamiphos (40% EC) @ 3 l/feddan
8- Non-treated plants

All treatments were applied second time on July, 2016 and the experiment was repeated as in 2017. Treatments were applied as soil drenches, whereas vermicompost was mixed with the soil in the rhizosphere. Each treatment was replicated five times. The reductions (%) of *M. incognita* population in soil, galls, and egg-masses in the roots were determined according to the formula of Handerson & Tilton's (1955) as follows:

$$\text{Reduction (\%)} = \left\{ 1 - \left(\frac{a}{b} \text{ X} \frac{c}{d} \right) \right\} \text{X } 100$$

Where:
a = nematode population after the treatment
b = nematode population before the treatment
c = Nematode population in the check before treatment
d = Nematode population in the check after treatment

Statistical Analysis: Data were subjected to the analysis of variance (ANOVA) using the computer program CoStat 6.303 (2005). Means were separated using the least significant difference (LSD) method at $P \leq 0.05$.

Results and Discussion

Toxicological effects of vermicompost tea (VCT) and the biocontrol product Nemaless® on the J_2 of *M. incognita*, after 24, 48, and 72 hrs of exposure are shown in Table (1). The calculated LC_{50} values indicated that the toxicity of VCT increased as its concentration was increased. VCT recorded LC_{50} values by 83.77×10^5, 31.49×10^3 and 30.86×10^3 mg/l after 24, 48 and 72 hrs of exposure, respectively (Table 1). The LC_{50} values of Nemaless® after 24, 48 and 72 hrs of exposure were 3.17×10^8, 2.85×10^8 and 2.69×10^8 CFU/ml, consecutively. The effect of vermicompost (VC), vermicompost tea and the bacterial bioagent in Nemaless®, on the development of *M. incognita* in banana as compared to fenamiphos (40% EC), during the first season (2016) are shown in Table (2). All treatments significantly ($P \leq 0.05$) decreased the numbers of galls and egg-masses attained by *M. incognita* on banana roots, compared with the non-treated check. The means of the reduction (%) were calculated depending on both rates of VC, VCT and Nemaless®. Fenamiphos, Nemaless®, VC and VCT greatly reduced the final population densities (Pf) of *M. incognita* in the soil.

Table 1. LC_{50}, fiducially limits, slope ± variance and regression equations for Vermicompost tea and Nemaless® against the second stage juveniles under laboratory conditions.

Treatments	Exposure times	LC_{50} (mg/l or CFU/ ml)	Fiducially Limits (Lower - Upper)	Slope ± variance	Regression equation
Vermicompost tea	24 hrs	83.77×10^5	$39.97 \times 10^3 - 1.77 \times 10^9$	0.40±0.05	-2.78+0.40 x
	48 hrs	31.49×10^3	$28.81 \times 10^3 - 34.42 \times 10^3$	3.69±0.12	-16.59+3.69 x
	72 hrs	30.86×10^3	$26.90 \times 10^3 - 35.74 \times 10^3$	2.23±0.06	-10.03+2.23 x
Nemaless®	24 hrs	3.17×10^8	$2.69 \times 10^8 - 3.73 \times 10^8$	1.49±0.05	-12.69+1.49 x
	48 hrs	2.85×10^8	$2.49 \times 10^8 - 3.27 \times 10^8$	1.97±0.05	-16.62+1.97 x
	72 hrs	2.69×10^8	$2.46 \times 10^8 - 2.95 \times 10^8$	3.17±0.08	-26.71+3.17 x

These mean reductions reached up to 74.58, 71.47, 71.27 and 63.26%, respectively by the first dose application. However, these reductions were maximized by the second application and reached up to 83.60, 79.58, 76.36, and 75.55% by fenamiphos, VC, Nemaless® and VCT, respectively. Our results showed that vermicompost and vermicompost tea were effective in controlling the root-knot nematode, *M. incognita*.

These findings are in harmony with those obtained by Dominguez *et al*., (1997); Edwards & Arancon (2004) found that solid vermicompost and vermicompost tea have an effective role in the protection of different crops from the attack by certain plant pests and increasing their productivity. Vermicompost from varied sources can be used as organic fertilizers and biocontrol agents as well. Several studies have also found that solid vermicompost and vermicompost tea were very effective in controlling some arthropod pests, plant-parasitic nematodes, and other plant pathogens (Arancon *et al*., 2007; Simsek-Ersahin*et al*., 2009; Renčo & Kováčik, 2015).

Pandey & Kalra (2010) observed that the vermicompost tea (aqueous extracts) from different plant sources inhibited the egg hatching of root-knot nematode, *M. incognita*. Also, the root galls of cluster bean plants were reduced by 21.43 to 78.57%, when vermicompost was incorporated with the soil at1, 2, 3 and 4% (Kumar *et al.*, 2011). Besides, vermicompost significantly enhanced the growth parameters (length, fresh, and dry weights of shoot and root systems) of infected plants. In another study (Xiao *et al*., 2016), vermicompost significantly decreased the root gall formation of some *M. incognita* infected tomato cultivars by 42 to 77%.

Moreover, vermicompost improves soil properties and increases the phenolic contents of plant roots. The potent of vermicompost in controlling the plant-parasitic nematodes (PPN) could be attributed to its great ability to activate the microbial communities in the soil which have an antagonistic effect towards PPN (Krause *et al.*, 2001; Scheuerell *et al*., 2005). Also, adding vermicompost to the soil augments the numbers and diversity of competitors, inhibitors, and predators of the plant-pathogenic organisms, and enriches the food sources on which these beneficial organisms depend (Sinha *et al*., 2013).

Vermicompost and vermicompost tea are rich in N, K, and P, micronutrients, beneficial soil microbes like nitrogen-fixing and phosphate solubilizing bacteria, mycorrhizal fungi, humus, and growth hormones (auxins, gibberlins, and cytokinins) which minimize the pests attack of plants (Adhikary, 2012; Sinha *et al*., 2013). From another point of view, Yasmin (2011) reported that treating plants with vermicompost can enhance the systemic resistance against different pathogens.

Table 2. The impact of vermicompost (VC), vermicompost tea (VCT), Nemaless® and fenamiphos on *Meloidogyne incognita* infecting banana during both seasons (2016 and 2017).

Treatment	The intervals of *M. incognita* on banana during the first season (2016)														
	Galls / 20g roots					Egg-masses / 20g roots					J_2 / kg soil				
		after 1st application		after 2nd application			after 1st application		after 2nd application			after 1st application		after 2nd application	
	Gi	Gf	R%	Gf	R%	Mi	Mf	R%	Mf	R%	Pi	Pf	R%	Pf	R%
VC (250g)	94.4a	75bc	40.41	62.00bc	55.34	83.40a	68.8b	38.95	53.60b	59.51	3016b	1314c	69.50	1250c	76.38
VC (500g)	89.2a	63.40de	46.69	41.20c	68.59	85.20a	56.6cd	50.84	32.80e	75.75	3224ab	1242c	73.03	974de	82.78
VCT (S/2)	93.2a	81.20b	34.66	57.20b	58.27	84.40a	70.4b	38.27	46.40bc	65.36	3060b	2026b	53.65	1550b	71.13
VCT (S)	94a	69.60cd	44.47	50.80bc	63.25	82.80a	58.8c	47.45	43.20cd	67.13	3384ab	1312c	72.86	1190cd	79.96
Nemaless®1	94.8a	72.00c	43.04	54.00bc	61.27	840a	57.2cd	49.61	44.80bcd	66.40	3218ab	1510c	67.15	1656b	70.67
Nemaless®2	95.2a	61.60de	49.34	47.20bc	64.81	82.20a	52cd	53.19	38.80de	70.26	3550a	1228c	75.79	1118cde	82.05
Fenamiphos	87.8a	57.60e	50.52	46.80bc	63.75	82.00a	50.4d	54.71	36.00de	72.42	3168ab	1146c	74.58	916e	83.60
check	89.6a	118.80a	--	131.60a	--	78.40a	106.4a	--	124.80a	--	3408ab	4850a	--	6010a	--
L.S.D. $_{0.05}$	16.93	8.24	---	14.62	---	9.45	7.61	---	9.50	---	396.91	369.40	---	254.94	---
	The intervals of *M. incognita* on banana during the second season (2017)														
Treatment	Galls / 20g roots					Egg -masses / 20g roots					J_2 / kg soil				
		after 1st application		after 2nd application			after 1st application		after 2nd application			after 1st application		after 2nd application	
	Gi	Gf	R%	Gf	R%	Mi	Mf	R%	Mf	R%	Pi	Pf	R%	Pf	R%
VC (250g)	78.8a	64bc	36.28	52.4b	59.39		54.4bc	44.59	47.2b	61.86	2526a	1257bc	70.14	1062de	79.99
VC (500g)	78a	56.4c	43.60	36.8cd	71.22	74.4a	46.8c	51.56	29.6d	75.73	2714a	1208cd	73.98	895.6fg	84.57
VCT (S/2)	79.6a	68b	33.37	46bc	64.75	76a	59.6b	39.62	38.4c	69.18	2796a	1402b	69.01	1494b	73.58
VCT (S)	77.2a	58.4bc	40.99	40.8cd	67.76	70.8a	49.6c	46.06	31.6cd	73.77	2824a	1243bc	73.59	1113d	81.08
Nemaless®1	74.8a	60.4c	37.02	41.6bc	66.07	70.4a	51.2bc	44.00	34.8cd	69.85	2648a	1353bc	69.34	1344c	75.64
Nemaless®2	79.2a	53.2c	47.61	37.2cd	71.35	74.4a	46c	52.39	30cd	75.40	2700a	1143.6d	74.59	974.4ef	82.68
Fenamiphos	80.8a	54c	47.57	38.8cd	70.67	73.2a	44.4c	53.44	31.2cd	73.97	2586a	1049.6e	75.73	781.6g	85.61
check	77.2a	98.4a	---	126.4a	---	70a	91.2a	---	114.6a	---	2910a	4866a	---	6113a	---
L.S.D. $_{0.05}$	14.37	11.04	---	11.16	---	12.90	10.12	---	8.71	---	526.1	173.81	---	138.40	---

Within a column, numbers followed by different letter(s) are significantly different using LSD at p = 0.05, * mean percent reduction.
Gi = initial galls, GF = final galls, R% = Reduction percentage, Mi= initial egg masses, Mf = final egg-masses, Pi= initial populations, Pf= final populations.VC =Vermicompost, VCT = Vermicompost tea, S= standard solution.

Data of the second season, 2017, showed that fenamiphos was the most effective in reducing the root galling of banana plants after the first application (47.57 %), followed by Nemaless®, VC and VCT with mean percent reductions of 42.32, 39.94 and 37.18%, respectively. After the second application, fenamiphos gave 70.67% reduction, followed by Nemaless® (68.71%), VCT (66.26%) and VC (65.31%). There were no significant differences between fenamiphos and the high and low rates of Nemaless® and VCT, as well as the high rate of VC (Table 2).

The soil treated with fenamiphos, Nemaless®, VC and VCT, sequentially were recorded for highest mean percent suppression of egg-masses of *M. incognita* by 53.44, 48.20, 48.08 and 42.84%, respectively. Application of fenamiphos, Nemaless®, VC and VCT as a second application reduced egg-masses on banana roots by 73.97, 72.63, 71.48 and 68.80%, respectively.

Two applications significantly suppressed the numbers of galls and egg-masses of infected banana plants as compared to the non-treated check (Table 2). Soil population densities of *M. incognita* were minimized significantly with fenamiphos (75.73%), VC (72.06%), Nemaless® (71.97%) and VCT (71.30%) after the first application. The high and low rates of VC and VCT had no significant differences. After the second application, fenamiphos showed the highest mean reduction in J_2 by 85.61%, followed by VC, Nemaless® and VCT, with mean reduction of 82.28, 79.16 and 77.33%, consecutively (Table 2).

According to the present results, fenamiphos was superior with suppression of the developmental parameters of root-knot nematode, *M. incognita* on banana plants. Mostafa *et al.* (2015) and Saad *et al*., (2017) found that fenamiphos (40% EC) minimized the incidence of root-knot nematode disease and significantly inhibited the nematode developmental stages in plant and soil as compared to the untreated control.

Certain investigations exhibited that the lytic bacterium *Serratia marcescens* was very effective for managing the plant-parasitic nematodes especially root-knot nematodes; *Meloidogyne* spp. (Kurz *et al*., 2003; El-Nagdi & Youssef, 2004; Darby, 2005; Zaied *et al*., 2009; Zeinat *et al*., 2009). Our results were consistent with the findings of El-Nagdi *et al.*, (2015) who mentioned that the application of Fornem X5® (containing *Rhodotorula pustula* (Buhagiar) Rodr. Mir & Weijman, *Serratia entomophila* Grimont *et al*., *S. marcescens*, *Pseudomonas fluorescens* (*Flügge*) Migula, 1895 and *P. putida* Trevisan), Micronema® and Nemacur® (fenamiphos) against *M. incognita* reduced J_2 in soil, J_2s in roots, galls and eggs ranged from 10.9 to 71.6, 52.1 to 84.1, 42.4 to 71.1 and 2.2 to 56.4%, respectively in banana cv. Grand Naine under field conditions.

Abd-Elgawad & Kabeil (2010) reported that *S. marcescens* moderately suppressed galls of *M. incognita* on roots of two tomato hybrids (Alisa and Super Strain B). While Abdel Razik *et al*., (2016) found that *S. marcescens* diminished the final population, galls and egg-masses of *M. incognita* infecting cucumber under greenhouse conditions by 84.4, 60. 30 and 59.43%, consecutively. The impacts of *S. marcescens* against the plant-parasitic nematodes may be attributed to the secretion of a variety of extracellular enzymes, including chitinase that degrade chitin (Hines *et al*., 1988; Kassab *et al*., 2017). Moreover, plants treated with *S. marcescens* induced protein content, chitinase, and peroxidase activities in treated plants as compared to untreated (Abd-Elgawad & Kabeil, 2010).

Generally, it could be concluded that vermicompost, vermicompost tea and Nemaless® in the present study showed nematicidal potential against the root-knot nematode, *M. incognita*, on banana. In addition, these applications enhanced plant growth. The safer ecological effects of these materials and their low costs are also an advantage. Nevertheless, further studies are still needed to

obviate the role of these materials in controlling the root-knot nematodes on banana plants.

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