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Field Application of Biological Control on Root-Knot Nematode and *Fusarium* Root Rot Fungus in Banana *Cv.* Grand Naine

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ABSTRACT

Two commercial bio-control agent products (CBAP), i.e. Fornem x5® (containing *Rhodotorula pustula*, , *Serratia entomophila*, *Serratia marcescens, Pseudomonas fluorescens* and *Pseudomonas putida*) and Micronema@ (containing *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*) , compared to chemical nematicide of Nemacur® were applied for controlling nematode root-knot (*Meloidogyne incognita*) and Fusarium root rot (*Fusarium solani*) fungus in banana *Cv*. Grand Naine under field application. Each CBAP was applied at rate of 10, 20 and 30 ml/plant, while Nemacur@ was applied at 10 kg/fed. Effects of CBAP on nematode parameters [i.e. number of juveniles (J_2) in soil (200g) and number of each J_2, third stage (J_3), females and eggs in roots (5g)] and pathological parameters [i.e. total microbial counts (spore forming bacteria ,bacteria and fungi) , Frequency (%) of common mycoflora and *Fusarium* root rot incidence] were studied. Fornem x5® and Micronema® significantly reduced the numbers of nematode parameters after 2,4 and 6 months of treatment. The highest percentages reduction of juveniles (J_2) in soil and each J_2, third stage (J_3), females and eggs in roots were achieved by Fornem x5® followed by Micronema® at 30 ml/plant after six months. The biological treatments increased the total counts of spore forming bacteria, bacteria and fungi in treated soil, than their counts at initial time. Results showed that the treatments increased the frequency of *Aspergllius* spp. and *Penicillium* spp., while they decreased the frequency of *Fusarium* spp, moderately inhibitory effect on *Fusarium* root rot was obtained by CBAP. Effect of treatments on yield parameters of banana *Cv*. Grand Naine was recorded. Yield parameters (i.e. The number of fingers/ hand, no. hands/bunch, hand and bunch weight) were increased with Fornem x5® and Micronema® at 30 ml/plant compared to Nemacur® and untreated control.

Key words: Banana, biological control, Meloidogyne incognita, Fusarium solani, yield

Introduction

Banana (*Musa* sp.) is one of the most economic tropical fruit crops in the world. Bananas grow in a wide variety of soils. In Egypt, its cultivation area reached about 59518 fed with average of 18.8 ton / fed. (FAO, 2009). Root-knot nematode and root rot diseases play an important role in limiting banana productivity. The root-knot nematode received an attention in banana orchards (El-Nagdi, 2001 and Eissa *et al*., 2005). *Meloidogyne* spp. were the most prevalent nematodes in banana samples with about 76% frequency of occurrence (Mokbel *et al.*, 2006). *Rhizoctonia solani* and *Fusarium oxysporum* were isolated from banana plants showing root rot-wilt complex disease (Abdel-Kader *et al*., 2004). A survey study in banana roots revealed that *Fusarium solani* and *Rhizoctonia* spp. were the predominant fungi in soil. *R. solani* was consistently isolated from crown lesions and brown decaying roots of Banana Passionflower (*Passiflora mollissima*) in Italy (Polizzi *et al.,* 2011). *F. oxysporum*, *R. solani* and *Macrophomina phaseolina* were isolated from blue pine during nursery surveys, where the frequencies were 47.3, 29.7 and 13.0%, respectively (Dar *et al*., 2011).

The management of the previous pests by using chemicals is not recommended because of risks to humans and the environment. Thus, alternative control strategies such as bio-control agents are needed. The use of bio-control agents were successfully used in controlling root -knot nematode and the fungal soil borne pathogens. Esnard *et al*. (1998) have found that *Penicillium* sp., *Paecilomyces* sp. and *Bacillus* sp. showed high activities as nematicidal effect against *M. incognita* on banana plants. *Pseudomonas fluorescens*, *Trichoderma viride*, *Glomus fasciculatum, Bacillus subtilis* and *Paecilomyces lilacinus* were applied against lesion nematodes in banana. Application of *P. fluorescens* at 20 g/plant gave the greatest bunch length (95 cm), bunch weight (24 kg), number of hands per bunch (10) and number of fingers per bunch (176). The increases in yield parameters ranged from 59 to 110%. *P. fluorescens* reduced the populations of *Radopholus similis, Pratylenchus coffeae* and *Helicotylenchus multicinctus* by 48.7, 46.3 and 44.3%, respectively. Colonization by *P. fluorescens, T. viride, G. fasciculatum*, and *B. subtilis* was $10^5 \times 10^8$ CFU/g, 60×10^6 CFU /g, 44% and 58×10^8 cells/g of root

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(Shanthi and Rajendran, 2006). *P. fluorescens* at 10^9 CFU/ml decreased *Meloidogyne javanica* infections, compared to the control. Also, it was able to cause destruction of nematode egg mass matrix and significantly decreased nematode egg hatching level (Norabadi *et al*., 2014).

P. fluorescens showed less severe wilting due to *F. oxysporum* f.sp. *cubense* infection in *Musa balbisiana* seedlings in greenhouse experiments (Sivamani and Gnanamanickam, 1988). *P. fluorescens* isolated from banana rhizosphere had significant inhibitory action on the growth of *F. oxysporum* f.sp. *cubense*. After three months of inoculation, *P. fluorescens* showed lesser vascular discoloration index than the control (Saravanan *et al*., 2004). *T. harzianum* and *T. viride* reduced the mycelial growth of *F. oxysporum* and *R. solani* by 33.0-73.3% and 29.5-70.8%, respectively (Dar *et al*., 2013).

The objective of this research was to study the antagonistic potential effect of two commercial products of Fornem x5® and Micronema®, compared to chemical nematicide of Nemacur® , against the root-knot nematode, *M. incognita* and fungal root-rot disease incidence under naturally field infection conditions.

Materials and Methods

Primary pathogens detection:

Eight samples of rhizosphere soil and roots were collected from the commercial banana fields at Gazzart El-Dahab, Giza Governorate, Egypt and then immediately transferred to Plant Pathology Department, National Research Center (NRC) for detection of infection with the root-knot nematode (*M. incognita*) and fungal root rot (*F. solani*) infection using standard fungal isolation and nematode extraction methods.

Identification of Meloidogyne spp.:

Adult females of root-knot nematode were isolated from galled roots of banana plants and identified as *M. incognita* by examination of their cuticular perineal patterns and morphological characteristics according to Taylor and Sasser (1978).

Bio-control agents:

Two commercial bio-control agents, i.e. Fornem x5® (containing $2X10^3$ CFU/ml of *Rhodotorula pustula*, $6x10^3$ CFU/ ml of *Serratia entomophila*, $9x10^8$ CFU/ml of *Serratia marcescens,* $3x10^5$CFU/ml of *Pseudomonas fluorescens* and $3x10^3$ CFU/ml of *Pseudomonas putida*) and Micronema® (containing10^9 CFU/ml of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis* were applied. The chemical nematicide of Nemacur® (fenamiphos) 10G [3-methyl-4- (methylthio) phenyl (1-methylethyl) phosphoramidate] was applied as comparison.

Field experiment:

This investigation was conducted during 2013 (third ratoon) on Grand Naine banana plants (*Musa cavendishii* L.), grown in a private orchard at Gazzart El Dahab, Giza Governorate. Plants were grown at 3.5 x 3.5 m. The soil texture was clay loamy, soil pH in water suspension (l: 2.5) was 7.5 and EC of soil paste at 25 ^{o} C was 1.3ds.m^{-1}(AOAC 1980), under drip irrigation system. All recommended horticultural practices were made. During April, one off-shoot per hole was selected beside the mother plant for the third and fourth ratoon, according to complete block design with 4 replicates each of 3 holes. Fertilizers injected into the irrigation system as recommended by The National Program for Improving Banana Productivity. Basal application of Nitrogen was 500g N/plant /year as ammonium sulphate (20.5 %) and potassium at 600 g K_2O/plant/year (as potassium sulphate 48 %). Fornem x5® and Micronema® were applied at rate of 10, 20 and 30 ml per plant, while Nemacur® was applied at 10 kg /fed as control. All treatments were applied as soil drench and added twice on 7 and 21 April of the same season.

Samples:

Four samples of roots and soil were collected from each treated banana as well as untreated control after 2, 4 and 6 months of treatment for different examinations. Then, the samples were immediately transferred to Plant Pathology Department, NRC, for the following studies:

Effect on Meloidogyne incognita:

Effect of Fornem x5® and Micronema® as well as Nemacur® on *M. incognita* parameters i.e., numbers of second stage juvenile (J_2) in soil per 200 g, J_2 in roots (5g); third stage (J_3), females and eggs in roots per 5g were counted according to Franklin and Goodey (1957). Reductions (%) of *M. incognita* population in soil and roots were determined according to the formula of Handerson and Tilton (Puntener, 1981):

$$\text{Nematode Reduction (\%)} = 1 - (PTA/PTB \times PCB/PCA) \times 100$$

Where: PTA = Population in the treated banana plant after application.
PTB = Population in the treated banana plant before application.

PCB = Population in the check banana plant before application.
PCA = Population in the check banana plant after application.

Effect on total microbial counts:

Effect of Fornem x5® and Micronema® as well as Nemacur® on total counts of fungi, aerobic bacteria and spore forming bacteria were determined by the plate count technique using suitable media (Ghini *et al.,* 2007).One gram of each soil sample, separately, was shaken in 90 ml of sterilized distilled to give a dilution of 10^{-1}. Then, serial dilutions up to 10^{-7} of fresh suspension of each sample were prepared. About of 1 ml of each dilution from 10^{-3} to 10^{-7} were transferred to each Petri dish and four petri dishes for each dilution were used as replicates. Martin medium (glucose 10g, peptone 5g, KH_2PO_4 1g, $MgSO_4$ 0.5g, rose Bengal 30µg, streptomycin 0.03g, distilled water 1L) was used for counting the common fungi after 7 days of incubation at 25±1°C. Nutrient agar medium (peptone 5 g, beef extract 3 g, agar 20 g, distilled water 1L, pH 7) was used for counting both total aerobic bacteria and spore forming bacteria after 2 days of incubation at 28 °C (Bridson, 1995).The results are presented as a number of colony-forming units (CFU) per gram of soil sample.

Effect on frequency % of common fungi:

Effect of Fornem x5® and Micronema® as well as Nemacur® on the percentages frequency occurrence (population) of common mycoflora in the rhizosphere of banana plants was determined in one gram of soil using the pour plate method and dilution technique (Ghini *et al*., 2007) using PDA medium. The plates were incubated at 25±1°C for 7 days. The resulted fungi were counted and identified to level of genus according to the morphological and culture characters (Barnett & Hunter, 1972 and Nelson *et al*., 1983). Each isolated fungus was counted and the frequency percentage of fungaus was calculated according to the following formula:

Fungus frequency percentage (%) = Fungus no. / Total fungi no. x100

Effect on fungal root rot disease incidence (%):

Effect of Fornem x5® and Micronema® as well as Nemacur® on disease incidence of fungal root rot disease was determined. Standard procedures were applied for isolation and detection of fungal root rot pathogens (*F. solani*.) from banana root parts .

Yield parameters:

Effect of Fornem x5® and Micronema® as well as Nemacur® on yield parameters such as bunch weight (kg), number of hands/bunch, average hand weight and number of fingers per hand was recorded.

Statistical Analysis:

Data were subjected to analysis of variance using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA. Means of values were compared by the Least Significant Difference (LSD) test at $P \leq 0.05$ level of significance (Snedecor and Cochran, 1980).

Results

Nematode parameters:

Results given in Table (1) indicated that the commercial products of Fornem x5® and Micronema®, as well as chemical nematicide of Nemacur®, significantly reduced ($P \leq 0.05$) numbers of each second stage juvenile (J_2) in soil, J_2 in roots and third stage (J_3), females and eggs in roots comparing with untreated (control) after 2, 4 and 6 months of treatments.

Juveniles (J_2) number in soil:

After two months of treatment, results showed that the number of J_2 / 200 g soil in banana plants were in the ranges of 163 - 475 (Fornem x5®), 140-380 (Micronema®) and 93 J_2 (Nemacur®), compared to 610 J_2 in untreated control (Table,1). It was found that the highest reduction percentage of J_2 in soil (73.8%) was achieved by Micronema® at 20 ml/plant, followed by Fornem x5® (71.6%) and Micronema® (71.6%) at 30 ml/plant (Table 2). After four months of treatment, results showed that the number of J_2 in soil of banana plants were in the ranges of 303-863 (Fornem x5®), 293-393 (Micronema®) and 338 (Nemacur®), compared to 1410 in untreated. The highest reduction percentage of J_2 in soil about 79.9% was achieved by Fornem x5® and Micronema® at 30 ml/plant, compared to Nemacur® (73.3%). After six months of treatment, the highest reduction percentage of J_2 in soil was achieved by Nemacur® (74.5%), followed by Fornem x5® (86.2%) and Micronema® (67.9%) at 30 ml/plant (Table 2).

Table 1: Effect of Fornem x5®, Micronema® and Nemacur® on the number root –knot nematode parameters (*Meloidogyne incognita*) of Grand Naine banana in field application.

Treatments	Rate (ml/ plant)	Months	No. of root –knot nematode parameters*					
			J2 in soil	J2 in roots	J3	Females	Eggs	Galls
Fornem x5®	10	Initial time	623	1250	225	188	663	17
		2	475	313	225	200	283	14
		4	863	290	205	190	283	14
		6	468	428	170	170	343	12
	20	Initial time	551	1050	238	225	663	18
		2	373	238	170	118	243	12
		4	303	273	188	153	233	13
		6	338	288	138	138	273	11
	30	Initial time	671	1250	200	225	663	19
		2	163	188	113	88	170	12
		4	388	193	110	140	195	11
		6	293	233	153	153	240	11
Micronema®	10	Initial time	495	1113	175	188	700	18
		2	380	350	230	198	315	14
		4	350	248	170	178	208	14
		6	273	383	240	240	300	11
	20	Initial time	625	1175	200	188	788	18
		2	140	265	183	188	268	13
		4	393	198	110	158	185	13
		6	303	388	148	148	243	13
	30	Initial time	733	1175	188	188	650	17
		2	178	230	183	200	213	13
		4	293	188	113	90	128	12
		6	323	225	195	195	258	15
Nemacur®		Initial time	638	988	175	225	775	18
		2	93	258	86	93	148	13
		4	338	228	80	108	175	13
		6	223	168	168	168	213	13
Untreated control		Initial time	713	1225	225	238	788	19
		2	610	833	243	163	390	27
		4	1410	1193	213	200	303	34
		6	978	240	240	240	418	38
Treatments (T) =			37.5	41.7	13.7	12.2	15.2	0.8
Con. (C) =			59.3	65.9	21.6	19.3	28.7	1.3
T x C =			83.7	93.1	30.5	27.3	40.6	1.9
Months (M) =			53.0	58.9	19.3	17.3	25.7	1.2
M X T =			74.0	83.3	27.3	12.3	36.3	1.7
M x C =			118.4	131.7	43.1	38.6	57.4	2.7
T x C x M =			167.5	186.1	61.0	54.6	81.1	3.8

*Values are averages of four replicates. * (200 g soil & 5 g roots),*

Table 2: Effect of Fornem x5®, Micronema® and Nemacur® on the percentage reduction of root –knot nematode parameters (*Meloidogyne incognita*) of Grand Naine banana in field application.

Treatments	Rate (ml/ plant)	Months	Nematode parameters reduction %					
			No.J_2 in soil	No.J_2 in roots	No.J_3	No. Females	No. Eggs	No. Galls
Fornem x5®	10	2	10.9	63.2	+10.2	+79.1	13.8	42.4
		4	30.2	76.1	+15.5	+13.1	+142.8	53.9
		6	45.2	52.1	46.5	46.5	2.2	64.7
	20	2	20.9	66.7	25.0	23.4	56.4	53.3
		4	70.3	73.2	6.0	19.1	8.6	59.6
		6	55.3	61.6	56.9	39.3	22.2	69.4
	30	2	71.6	77.9	40.7	42.9	48.2	55,8
		4	70.9	84.1	31.6	25.9	23.5	67.6
		6	68.2	73.9	51.9	32.7	31.6	71.1
Micronema®	10	2	10.3	53.8	1.0	+77.3	9.1	45.6
		4	64.4	77.0	16.0	+5.9	22.7	56.4
		6	59.8	51.8	21.9	1.0	19.0	69.4
	20	2	73.8	66.8	3.9	+68.4	28.7	49.4
		4	68.3	82.6	31.6	6.0	38,9	59.6
		6	64.7	53.8	46.0	22.1	41.7	63.9
	30	2	71.6	71.2	20.9	+69.1	33.8	46.5
		4	79.9	83.5	28.5	43.0	48.8	60.5
		6	67.9	41.3	35.4	3.5	24.9	55.9
Nemacur®		2	82.9	61.6	59.2	39.7	6.1	49.4
		4	73.3	76.2	94.6	42.9	41.3	59.6
		6	74.5	29.1	42.9	26.1	48.1	63.9

Values are averages of four replicates.

Juveniles (J_2) number in roots:

After two months of treatment, results showed that the number of J_2 in banana roots were in the ranges of 188-313 , 230-350 and 258 J_2 with treatments of Fornem x5® , Micronema® and Nemacur ®, respectively, compared to 833 in untreated control (Table 1). It was found that the highest reduction percentage of J_2 in roots was achieved by Fornem x5® (77.9%) and Micronema® (71.2%) at 30 ml/plant, followed by Micronema® (66.8%)and Fornem x5® (66.7%) at 20 ml/plant (Table, 2). Results recorded that the numbers of J_2 in roots of banana plants were in the ranges of 193- 290 & 135-170 for Fornem x5® , 188-248 & 225-388 for Micronema® and 228, 168 for Nemacur® , compared to 1193, 240 with untreated control after four and six months of treatment, respectively .The highest reduction percentages of J_2 in roots were 84.1%, 73.9% with Fornem x5® at 30 ml/plant after four and six months of treatment, respectively (Table 2).

Third stage (J_3) number in roots:

The number of Third stage (J_3) in banana roots were in the ranges of 113 – 225 and110 – 240 , compared to 80- 168 and 213 – 243 with treatments of Fornem x5® , Micronema® ,Nemacur ® and untreated control, respectively (Table 1). The highest reduction percentage of J_3 in roots was achieved by Fornem x5® (56.9 %) , followed by Micronema® (46.0%) at 20 ml/plant after six months of treatments in Table (2).

Females number in roots:

The number of females in banana roots were in the range of 88 - 200 , 90 – 240 , 93 – 168 and 163 – 240 for Fornem x5® , Micronema® , Nemacur ® and untreated control treatments, respectively (Table,1). The highest percentages reduction of females in roots were achieved by Fornem x5® (46.5, 39.3 and 32.7%) at 10, 20 and 30 ml/plant after six months of treatments in Table (2), respectively.

Eggs number in roots:

After two months of treatments, results showed that the number of eggs in roots of banana were in the range of 170-283 and 213-315 with treatments of Fornem x5®, Micronema® , compared to 148 eggs for Nemacur® and 390 eggs in untreated control (Table,1). The numbers of eggs in roots of banana were in the range of 195- 283 & 240-343 with Fornem x5® and 128-185 &243-300 with Micronema® , compared to175 & 213 for Nemacur® and 303 & 418 eggs in untreated control after four and six months of treatment, respectively .The highest percentages reduction of eggs in roots were achieved by Fornem x5® (56.4%) at 20 ml /plant after two months , followed by Micronema® (48.8%) at 30 ml/plant after four months and Nemacur® (48.1%) after six months (Table 2).

Galls number in roots:

After 2, 4 and 6 months of treatment, results showed that the numbers of galls in roots of banana were in the range of 12-14, 11-14 and 11-15 with treatments of Fornem x5®, Micronema® and Nemacur®, compared to 27, 34 and 38 in untreated control, respectively (Table 1). The highest percentages reduction of galls in roots were achieved by Fornem x5® (55.8, 67.6 and 71.1%) at 30 ml/plant after 2, 4 and 6 months of treatment, respectively (Table 2).

Pathological parameters:

Effect of total microbial counts:

Results in Table (3) revealed that the Fornem x5® and Micronema® treatments highly increased the total counts of spore forming bacteria , total aerobic bacteria and total fungi, than their counts at initial time as well as compared to Nemacur® and untreated control. The spore forming bacteria count (SFBC) was in the range of 11.7 to 18.0 x 10^4 CFU / g soil with Fornem x5®, while it was in the range of 10.7 to 19.7 x 10^4 CFU / g soil with Micronema®, than the ranges of 10.7 to 17.0 CFU / g soil with Nemacur® and 8.7 to 15.0 CFU / g soil in untreated control within six months of application (Table 3). At initial time, the SFBC was in the ranges of 3.7 to 8.7 x10^4 CFU / g with Fornem x5® and 7.7 to 12.0 x10^4 CFU / g soil, compared to 15.7 x 10^4 and 8.0 x 10^4 CFU / g soil with Nemacur® and untreated control, respectively. After six months of application, Fornem x5® and Micronema® highly increased the SFBC, than Nemacur® as well as untreated control.

The total aerobic bacteria count (TABC) was in the range of 13.7 to 36.0 x 10^6 CFU / g soil with Fornem x5®, while TABC was in the range of 14.7 to 39.0 x 10^6 CFU / g soil with Micronema®, than the ranges of 10.0 to 12.7 CFU / g soil with Nemacur® and 10.7 to 12.7 x 10^6 CFU / g soil in untreated control within six months of application (Table 3). At initial time, the TABC was in the ranges of 11.7 to 15.o x10^6 CFU / g with Fornem x5® and 7.7 to 17.7 x10^6 CFU / g soil, compared to 21.0 x 10^6 and 10.7x 10^6 CFU / g soil with Nemacur® and untreated control, respectively. After six months of application, all bio-control agents highly increased TABC, than Nemacur® and untreated control, respectively (Table 3).

The Total fungi count (TFC) was in the range of 10.7 to 25.0 x 10^3 CFU / g soil with Fornem x5®, while the TFC was in the range of 13.0 to 24.0 x10^3 CFU / g soil with Micronema®, than the ranges of 16.0 to 21.0 x

10^3 CFU / g soil with Nemacur® and 24.7 to 31.7 x 10^3 CFU / g soil in untreated control within six months of application. At initial time, the TFC was in the ranges of 17.7 to 34.0 x10^3 CFU / g with Fornem x5® and 18.7 to 31.0 CFU / g soil, compared to 34.7 x 10^3 and 31.0 x 10^3 CFU / g soil with Nemacur® and untreated control, respectively. All bio-control agent treatments highly reduced TFC, Nemacur® as well as untreated control. Significant differences were recorded at 2,4 and 6 months after treatment with SFBC, TABC and TFC as well as among treatments in TABC, except between Fornem x5® (10 ml / plant) & Nemacur® and between (20 ml / plant) & Micronema® (30 ml / plant) [Table 3].

Table 3: Effect of Fornem x5®, Micronema® and Nemacur® on total counts of spore forming bacteria , aerobic bacteria and Fungi in rhizosphere of Grand Naine banana

Treatments	Rate (ml / plant)	Months	Microbial counts as CFU / g at ml / plant		
			Spore forming bacteria at 10^4	Total aerobic bacteria at 10^6	Total fungi at 10^3
Fornem x5®	10	Initial time	8.7	11.7	22.7
		2	16.0	17.7	25.0
		4	17.0	13.7	23.7
		6	11.7	20.0	13.0
	20	Initial time	3.7	13.7	34.0
		2	12.7	28.0	10.7
		4	18.0	34.0	21.7
		6	16.0	36.0	16.0
	30	Initial time	8.0	15.0	17.7
		2	18.0	22.0	22.0
		4	11.7	33.0	18.0
		6	15.7	35.0	13.7
Micronema®	10	Initial time	7.7	7.7	18.7
		2	18.0	14.7	13.0
		4	10.7	38.0	18.7
		6	12.0	39.0	14.0
	20	Initial time	12.0	16.0	30.0
		2	11.7	17.7	15.3
		4	13.6	37.7	17.0
		6	19.7	33.3	18.7
	30	Initial time	10.7	17.7	31.0
		2	13.7	14.7	16.0
		4	17.7	34.7	21.7
		6	17.7	34.7	24.0
Nemacur®		Initial time	15.7	21.0	34.7
		2	17.0	10.0	16.0
		4	11.0	12.7	18.7
		6	10.7	11.7	21.0
Untreated control		Initial time	8.0	10.7	31.0
		2	15.0	12.7	24.7
		4	10.0	10.7	28.0
		6	8.7	12.0	31.7
L.S.D. 0.5					
Treatments (T) =			0.6	1.0	1.3
Con. (C) =			0.9	1.6	2.0
T X C =			1.2	2.2	2.8
Months (M) =			0.8	1.4	1.8
M x T =			1.1	2.0	2.5
M x C =			1.7	3.1	4.0
T x C x M =			2.5	4.4	5.6

Effect on population of common mycoflora:

Results in Table (4) revealed that *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *Trichoderma* spp., *Rhizopus* spp. and others were the common fungi in rhizosphere of treated banana as well as untreated control. The frequency of *Aspergillus* spp. was highly increased than frequencies of other fungi, followed by *Penicillium* spp.. Details of the frequencies of isolated fungi are listed in Table (4).The frequency of *Aspergillus* spp. was in the range of 11.4 – 55.3% with Fornem x5®, while it was in the range of 21.4 – 50.0% with Micronema® at 10, 20 and 30 ml/ plant after 2, 4 and 6 months of treatment, compared to the ranges of 29.0 – 35.5% and 26.1 - 56.4% for Nemacur® and untreated control, respectively. The frequency of *Penicillium* spp. was in the range of 9.1 – 37.1 with Fornem x5®, while it was in the range of 16.3 – 28.6%, compared to the ranges of 23.5 – 25.0% and 5.6 – 30.6% with Nemacur® and untreated control, respectively. The frequencies of *Fusarium* spp. were in the ranges of 4.8 – 22.2% with Fornem x5® and 9.1 – 19.4% with Micronema®, compared to 12.9 –35.5% and 8.2 – 36.1% with Nemacur® and untreated control, respectively. Results indicated that the highest frequencies of

Trichoderma spp. were about 19.2 and 17.9% at 10 ml / plant for Fornem x5® and Micronema® after 6 months of treatment , respectively (Table 4).

Table 4: Effect of Fornem x5®, Micronema® and Nemacur® on the frequency of common fungi in rhizosphere of Grand Naine banana

Treatments	Rate (ml/ plant)	Months	Frequency % of common fungi at ml/plant					
			Aspergillus spp.	*Penicillium* spp.	*Fusarium* spp.	*Trichoderma* spp.	*Rhizopus* spp.	Others
Fornem x5®	10	Initial time	26.7	13.3	17.8	31.1	0.0	11.1
		2	35.8	37.1	15.7	0.0	2.9	8.5
		4	55.3	21.3	12.8	0.0	2.1	8.5
		6	34.6	19.2	19.2	19.2	0.0	7.8
	20	Initial time	35.3	25.0	16.2	4.4	7.4	11.7
		2	28.6	28.6	4.8	19.1	9.5	9.4
		4	30.2	25.6	11.6	20.9	7.0	4.7
		6	34.4	18.8	12.5	9.4	9.4	15.5
	30	Initial time	8.6	8.6	14.3	54.3	0.0	14.2
		2	11.4	9.1	6.8	61.4	0.0	11.3
		4	43.4	34.2	9.2	3.9	3.9	5.4
		6	33.3	18.5	22.2	11.1	0.0	14.9
Micronema®	10	Initial time	21.6	18.9	21.6	24.3	0.0	13.6
		2	30.8	26.9	19.2	0.0	0.0	23.1
		4	33.3	24.2	9.1	21.2	6.1	6.1
		6	28.6	25.0	21.4	17.9	0.0	7.1
	20	Initial time	45.0	13.3	25.0	3.3	0.0	13.4
		2	50.0	23.5	14.7	0.0	5.9	5.9
		4	21.4	28.6	14.2	14.2	7.1	14.5
		6	24.3	18.9	16.2	16.2	10.8	13.6
	30	Initial time	29.0	14.5	19.4	29.0	1.6	6.5
		2	37.5	21.9	15.6	9.4	6.3	9.3
		4	41.9	16.3	16.3	14.0	4.7	6.8
		6	33.3	27.1	18.8	0.0	8.3	12.5
Nemacur®		Initial time	40.6	13.0	20.3	0.0	5.8	10.1
		2	34.4	25.0	21.9	0.0	9.4	9.3
		4	35.5	29.0	12.9	0.0	14.5	8.1
		6	29.0	23.5	35.5	0.0	0.0	12.0
Untreated control		Initial time	56.4	11.3	24.2	0.0	1.6	6.5
		2	40.8	30.6	8.2	12.3	0.0	8.1
		4	39.9	5.6	36.1	0.0	8.3	10.1
		6	26.1	21.7	34.8	8.7	0.0	8.7
L.S.D.0.5								
Treatments (T) =		0.07		Fungi (F)=	0.07	T x M x F=	0.39	
Months (M) =		0.05		T x F =	0.19			
T X M =		0.14		F x M =	0.13			

Effect on root rot disease incidence:

Results revealed that the biocontrol products as well as Nemacur® reduced the *Fusarium* root rot incidence in banana root pieces, than at initial and untreated control .The percentage of *Fusarium* root rot disease incidence was ≤ 25% with Fornem x5® after 2 months of treatment, while it was ≥ 25 - < 50% after of 4 and 6 months of application when applied at rates of 10 and 20 ml/plant. At rate of 30 ml/plant, the root rot disease incidence was ≥ 25 - < 50% after experimental time with Fornem x5® (Table 5). Micronema® reduced the root rot incidence to ≥ 25 - < 50% at rate of 10 ml/plant after 2, 4 and 6 months and at rates of 20 and 30 ml/plant after 4 and 6 months, while it was ≥ 50 - < 75% with rates of 20 and 30 ml/plant after 2 months of application. The *Fusarium* root rot with Nemacur® was ≥ 25 - < 50% after 2 and 4 months, while it was ≥ 50 - < 75% after 6 months. Results showed that the disease incidence was ≥ 75% at initial time as well as untreated control (Table 5).

Effect on yield parameters:

The number of fingers/ hand was increased from 13 to 16 and 14 to 15 with Fornem x5® and Micronema®, compared to 15 and 7 with Nemacur® and the control treatment, respectively (Table 6). Numbers of hands/bunch were increased from 7 to 8 with Fornem x5® and Micronema®, compared to 8 and 7 with Nemacur® and the control treatment, respectively. The hand weight significantly increased from 2.56 to 2.88 kg and 2.50 to 3.00 kg, than 2.94 and 2.19 kg in Nemacur® and the control treatment, respectively. Significant differences were recorded between rates of 10 ml/plant and 30 ml / plant in bio-agents treatments, while no significant differences were recorded among treatments. Bunch weight of treated banana increased from 19.00 to 23.44 kg and 17.38 to 23.13 kg with for Fornem x5® and Micronema®, compared to 21.94 and 15.88 kg with

Nemacur® and the control treatment, respectively. Significant differences were recorded only between high rates and untreated control.

Table 5: Effect of Fornem x5®, Micronema® and Nemacur® on root- rot disease incidence %, caused by *Fusarium solani* , in Grand Naine banana

Months	Root –rot disease incidence %							
	Fornem x5®			Micronema®			Nemacur®	Control
	10	20	30	10	20	30		
Initial time	++++	++++	++++	++++	++++	++++	++++	++++
2	+	+	+++	++	++++	++++	+	++++
4	++	++	+++	++	+++	+++	++	++++
6	++	++	+++	++	+++	+++	+++	++++

Root rot infection % of newly root pieces - + ≤25%, ++ ≥25-<50%, +++ ≥50 – 75%, ++++ ≥ 75%

Table 6: Effect of Fornem x5®, Micronema® and Nemacur® on yield parameters of Grand Naine banana

Treatments	Rate (ml/ plant)	Yield parameters			
		No. of fingers / hand	No. of hands / bunch	Hand weight (kg)	Bunch weight/ plant (kg)
Fornem x5®	10	13	7	2.63	19.00
	20	14	8	2.56	20.31
	30	16	8	2.88	23.44
Micronema®	10	14	7	2.50	17.38
	20	15	7	2.94	19.75
	30	15	8	3.00	23.13
Nemacur®		15	8	2.94	21.94
Untreated control		12	7	2.19	15.88
L.S.D. 0.05					
Treatments (T) =		1.04	0.48	3.26	2.01
Conc. (c) =		1.65	0.76	5.16	3.19
T x C =		2.33	1.08	7.30	4.40

Values are averages of four replicates.

Discussion

Our results of isolation study revealed that the *M. incognita* and *F. solani* were the common soil borne pathogens in the rhizosphere of in banana *Cv.* Grand Naine, where root-knot nematodes (*Meloidogyne* spp.) are important pests of many cultivated plants (El-Nagdi, 2001 and Lamovsek et *al*., 2013). *F. solani* also was the predominant fungi in nematode and non-nematode lesions on banana roots (Stover, 1966 and Abdel-Kader *et al*., 2004). Recently, the most efficient chemical control products (e.g. methyl bromide) have now been restricted due to their toxic characteristics. Therefore, research on agents that work against root-knot nematodes and do not have a detrimental impact on the environment is becoming increasingly important. Some of the well-accepted commercial products containing bacteria *Bacillus firmus* and *Pasteuria penetrans* and Fungus *Purpureocillium lilacinus* were applied (Lamovsek et *al.,* 2013). Biological control is considered as a new efficient method that becomes widely used for controlling plant parasitic nematodes, as aim to decrease the extent of environment degradation and the effect of the excessive toxic nematicides. *Pseudomonas aeroginosae* and *P. fluorescens* were potent as bio -control agents for root-knot nematodes (Rahanandeh and Moshaiedy, 2014). Especially fluorescent pseudomonads were significantly more abundant in rhizospheric soils than in bulk soils (Sutra *et al*., 2000).

Therefore, the present study is aimed at using of two commercial bio-control products for controlling *M. incognita* and *F. solani* in banana. Results revealed that Fornem x5® and Micronema® reduced the number of J_2 in soil. The effect of bio-control products was increased by increased applied rates, but decreased by time of application. The bio-control products highly reduced the J_2 in soil than Nemacur®. Fornem x5® significantly reduced the J_3, females and galls numbers than Micronema® as well as Nemacur®, while Micronema® highly reduced the eggs number, than Nemacur® and Fornem x5®, respectively, especially after 6 months of applications .It is clear that the Fornem x5® was more effective in reducing nematode root –knot than Micronema® .This effect may be due to different bacterial species in commercial products. In this study, Fornem x5® highly increased the total count of spore forming bacteria and viable bacteria, while decreased the total count of fungi than Nemacur®, especially after 6 months of application. The frequency of *Aspergillus* spp. and

Trichoderma spp. were highly increased in bio-control products- treated soil than other treatments. The bio-control products reduced the *Fusarium* root rot by ≥ 25 – < 50 %. Treated root pieces by Fornem x5® and Micronema® significantly increased the bunch weight / plant than Nemacur®.

These results are agreement with those recorded by Medoza and Sikora (2009).They reported that the combination of *Fusarium oxyspoium* and *Paecilomyces lilacinus* reduced the nematode density of *Radophouls similis*. Combined application of *F. oxysporum* and *B. firmus* was the most effective treatment in controlling *R. similis* on banana, followed by *B. firmus* alone. Bio-control as an integral part of management is an attractive option for plant parasitic nematodes that should be pursued besides the cultural practices of crop rotation and organic amendment to include the use of microorganisms isolated, cultured and packaged in the tropics for tropical farmers (Agbenin, 2011).The highest inhibition of *F. solani* growth was obtained with *A. niger*, followed by *T. viride*, *T. harzianum* and *Penicllium* spp. (Ambikapathy *et al.*, 2002) .*B. subtilis* in presence of *F. solani* increased the percentage of healthy seedlings as well as their length, fresh and dry weight than in presence of *F. solani* alone but still less than the control (Haikal and El-Daly, 2007). *P. fluorescens* clearly inhibited *F. oxysporum* f. sp. *cubense in vitro* (Mohammed *et al*., 2011). Ajilogba *et al*., (2013) revealed that the result from *in vitro* analysis showed that *B. amyloliquefaciens*, *B pumilus* and *B. cereus* inhibited the growth of *F. solani* the most by 55.7 - 95.2% than the control .*In vivo*, *B. cereus* reduced disease incidence, followed by *B. amyloliquefaciens*, *B. pumilus* and *B. subtilis*. It is oblivious that these four *Bacillus* spp are very effective bio-control agents. Ho *et al*. (2015) indicated that *B. cenocepacia* 869T2 also decreased the disease incidence of *Fusarium* wilt on treated banana plants by 3.4 %, comparing to 24.5 % of non-inoculated plants infected in the field test. The antagonistic effect of commercial products may be due to bacteria which are the most abundant among microorganisms. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low (Sivasakthi *et al.,*2014). Application of *P. fluorescens* at 20 g/plant gave the greatest bunch length, bunch weight, number of hands per bunch, and number of fingers per bunch. *P. fluorescens* was also the most effective to control nematodes until harvest as the populations of *Radopholus similis* (Shanthi and Rajendran, 2006), Specific activities of resistance-related enzymes, namely peroxidase (POX) and phenylalanine ammonia lyase (PAL), increased significantly in *P. fluorescens*-inoculated plants. Results suggested that the destruction of eggs and plant defense mechanisms leading to systemic resistance are two main suppression mechanisms used by *P. fluorescens* against nematode (Norabadi *et al*., 2014).

References

Abdel-Kader, M.M., M.K. El-Bahr and El-Mougy, Nehal, S., 2004. Pathogenic fungi and soil conditions causing root rot and wilt disease complex during acclimatization of tissue culture-derived banana plantlets. Egypt. J. Phytopathol., 32 (1-2): 37-48.

Agbenin, N.O., 2011. Biological control of plant parasitic nematodes: prospects and challenges for the poor Africa farmer. Plant Prot. Sci., 47: 62–67.

Ajilogba, C. F., O. O. Badalona and F. Ahmed, 2013.Antagonistic effects of *Bacillus* species in biocontrol of tomato *Fusarium* wilt. Ethno. Med., 7(3): 205-216.

Ambikapathy,V., A. Panneerselvam and R. Saravanamuth, 2002. Antagonistic effect of soil fungi to *Fusarium solani*, Appel and Willenweher. Agric. Sci. Dig., 22: 14-17.

A.O.A.C., 1980. Official Methods of Analysis of the Association of Official Analytical Chemist. 12*th* ed. Washington, D.C., USA.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minneapolis, Minnesota, USA, 241 pp.

Bridson, E. Y., 1995. The Oxide Manual 7th Ed., Published by Unipath Limited ,Wade Koad, Basingstoke Hampshire , RG 248 PW, England.

Dar, Gh. H., M. A. Beig, F. A. Ahanger, N. A. Ganai, and M. A. Ahangar, 2011. Management of root rot caused by *Rhizoctonia solani* and *Fusarium oxysporum* in blue pine (*Pinus wallichiana*) through use of fungal antagonists. Asian J. Plant Pathol., 5 (2):62-74.

Dar, W. A., M. A. Beig, S. A. Ganie, J. A. Bhat, U. R. Shabir and S. M. Razvi, 2013. *In vitro* study of fungicides and biocontrol agents against *Fusarium oxysporum* f.sp. *pini* causing root rot of Western Himalayan fir (*Abies pindrow*) . Acad. J., 8 (30): 1407 – 1412.

Eissa, M.F.M., A.Y. El-Gindi, M.M. Abd-Elgawad, A.E. Ismail, and El-Nagdi, Wafaa, M.A., 2005. Application of some bioagents and oxamyl in controlling *Meloidogyne incognita*, *Helicotylenchus exallus* and *Criconemoides spp*. infesting banana cv. Williams. Pak. J. Biotechnol., 2(1-2): 70-79.

El-Nagdi, M.A. Wafaa, 2001. Studies on Banana Nematodes in Egypt. Ph.D. Thesis, Fac. of Agric., Cairo Univ., 179 pp.

Esnard, J., M. N. Marban and B.M. Zuckerman, 1998. Effects of three microbial broth cultures and an organic amendment on growth and populations of free living and plant-parasitic nematodes on banana. Eur. J. Plant Pathol. ,104: 457-463.

FAO.,2009. World production data for crops. [Online]. Food and Agricultural Organization of the United Nations. Available: http: //faostat.fao.or/site/339/default.aspx.

Franklin, M.T. and J.B. Goodey,1957. A cotton-blue lactophenol technique for mounting plant parasitic nemato des. J. Helminthol. Abs., 23: 175-

Ghini, R.F., R.A. Patrico, W. Bettiol, M.G. de Almeida and A.H.N. Maia, 2007. Effect of sewage sludge on suppressiveness to soil-borne plant pathogens. Soil Biol. & Biochem., 39: 2797-2805.

Haikal, N. and F. A. El-Daly, 2007.Comparison between chemical and biological control of *Fusarium*-root rot disease on the response of some physiological aspects of *Cucumis sativus* seedlings. Egyptian J. Biotechnol., 25 (1): 94-101.

Ho, Y. N., H. M. Chiang, C. P. Chao, C. C. Su, H. F. Hsu, C. T. Guo, J. L. Hsieh and C. C. Huang, 2015. *In planta* biocontrol of soilborne *Fusarium* wilt of banana through a plant endophytic bacterium, *Burkholderia cenocepacia* 869T2. Plant and Soil, 387 (1-2): 295 – 306.

Lamovsek, J., G. Urek, and S. Trdan, 2013. Biological Control of Root-Knot Nematodes (*Meloidogyne* spp.): Microbes against the Pests. Acta agric. Slov., 101: 263 – 275.

Medoza, A.R. and R. A. Sikora, 2009. Biological control of *Radopholus similis* in banana by combined application of the mutuatistic endophyte *Fusarium oxysporum* strain 162, the egg pathogen *Paecilomyces lilacinus* strain 251 and the antagonistic *Bacillus firmus*. Bio control, 54:263-272.

Mohammed, A. M., L. K. T. AL-Ani, L. Bekbayeva and B. Salleh, 2011.Biological control of *Fusarium oxysporum* f. sp. *cubense* by *Pseudomonas fluorescens* and BABA *in vitro*. World Appl. Sci. J., 15 (2): 189-191.

Mokbel, A.A., I.K.A. Ibrahim, M.A.M. El-Saedy and S.E. Hammad, 2006. Plant parasitic nematodes associated with some fruit trees and vegetable crops in northern Egypt. Egyptian J. Phytopathol., 34(2): 43-51.

Norabadi, M. T., N. Sahebani and H. R. Etebarian, 2014. Biological control of root-knot nematode (*Meloidogyne javanica*) disease by *Pseudomonas fluorescens* (Chao). Archiv. Phytopathol. &Plant Prot., 47 (5): 615-621.

Nelson P.E., T.A.Toussoum and W.F.O. Marasas, 1983. *Fusarium* Species, An illustrated manual for identification .The Pennsylvania State University Press, University Park, PA., USA, 193 pp.

Polizzi, G., D. Aiello, V. Guarnaccia, A. Panebianco and P. T. Formica, 2011. First report of crown and root rot caused by *Rhizoctonia solani* AG-4 on Banana Passionflower (*Passiflora mollissima*) in Italy. Plant Dis. ,95(9): 1194.

Puntener, W., 1981. Manual for Field Trials in Plant Protection. Agricultural Division, Ciba-Geigy Limited, Basle, Switzerland, pp: 205.

Rahanandeh, H. and M. Moshaiedy, 2014. Potency evaluation of *Pseudomonas aeroginosae* and *Pseudomonas fluorescens* as biocontrol agents for root - knot nematodes in Iran. Inter. J. Biosci., 4 (12): 222 – 228.

Saravanan, T., M. Muthusamy and T.Marimuthu, 2004. Effect of *Pseudomonas fluorescens* on *Fusarium* wilt pathogen in banana rhizosphere. J. Biol. Sci., 4: 192-198.

Shanthi, A. and G. Rajendran, 2006. Biological control of lesion nematodes in banana. Nematol. medit., 34: 69-75.

Sivamani, E. and S. S. Gnanamanickam, 1988. Biological control of *Fusarium oxysporum* f. sp. *cubense* in banana by inoculation with *Pseudomonas fluorescens*. Plant and Soil , 107(1): 3-9.

Sivasakthi, G., G. Usharani and P. Saranraj, 2014. Biocontrol potentiality of plant growth promoting bacteria (PGPR) - *Pseudomonas fluorescens* and *Bacillus subtilis*: A review. Afri. J. Agric. Res., 9(16):1265-1277.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 5th Ed., Iowa State Univ. Press, Ames, Iowa, USA, 593 pp.

Stover, R. H., 1966. Fungi associated with nematode and non-nematode lesions on banana roots. Canad. J. Bot., 44 :(12) 1703-1710.

Sutra, L., J.M. Risède, and L. Gardan, 2000. Isolation of fluorescent pseudomonads from the rhizosphere of banana plants antagonistic towards root necrosing fungi. Lett. Appl. Microbiol., 31(4):289-93.

Taylor, A.L. and J.N. Sasser, 1978. Biology, identification and control of root -knot nematodes (*Meloidogyne* species). IMP. North Carolina State University Graphics, 111 pp.