BIOLOGICAL CONTROL OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM SOLANI* IN SUGAR BEET

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Summary. *Meloidogyne incognita* and *Fusarium solani* cause the root-knot and root-rot diseases of sugar beet (*Beta vulgaris* L.) in Egypt. Therefore, the effect of several commercial products containing either the fungi *Paecilomyces lilacinus*, *P. fumosoroseus* and *Trichoderma album* and the bacteria *Bacillus subtilis* and *B. megaterium* were tested against both pathogens on sugar beet, and compared with the nematicides fenamiphos and cadusaphos, under *in vitro*, greenhouse and field conditions. *In vitro*, the mortalities of *M. incognita* were within the range of 61-94% with bio-control products, compared to 96-98% with fenamiphos and cadusaphos. All treatments significantly reduced the mycelial growth of *F. solani*, with the greatest reduction given by *T. album*. In the greenhouse, *B. megaterium* greatly reduced the numbers of galls, females and egg-masses of the nematode in the roots of sugar beet, followed by *B. subtilis*, *P. lilacinus*, *P. fumosoroseus* and *T. album*, respectively. All treatments increased shoot length and weight, root weight and percentage of total soluble solids (TSS) in sugar beet plants. In the field, all treatments greatly reduced the population densities of second-stage juveniles in soil, and the numbers of galls, females and egg masses of *M. incognita* in the roots, more so at the recommended than at half the recommended dose. *Aspergillus* spp., *A. niger*, *F. solani*, *F. oxysporum*, *Penicillium* spp., *Rhizoctonia solani*, *Rhizopus nigricans* and *Trichoderma* spp. were the most common fungi found in the rhizosphere of treated and untreated sugar beet plants. All treatments affected the frequency of the isolated fungi. Soil treatments with *P. lilacinus*, *P. fumosoroseus*, fenamiphos and cadusaphos increased plant weight, foliage weight, length, diameter and weight of roots, survival of plants and tap root yield. The treatments also affected total soluble solids, sucrose content, the sucrose purity of tap roots and sugar yield.

Keywords: *Beta vulgaris* var. *saccharifera*, biocontrol products, root-knot nematode, root-rot fungus.

After sugar cane, sugar beet (*Beta vulgaris* L. var. *saccharifera* Alefeld) is the second most important sugar crop in Egypt, from which 28% of sucrose is produced. In Egypt, sugar beet is susceptible to seedling diseases caused by several soil-borne pathogens. The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., is the most important nematode attacking sugar beet (Abd El-Massih *et al*., 1986; El-Nagdi *et al*., 2004). As for other crops, the rhizosphere of sugar beet also harbours a number of fungal pathogens that are known to severely damage seeds, sprouting seeds and seedlings. Among them is *Fusarium solani* (Mart.) Appel *et* Wollenw. emend. Snyder *et* Has (Srivastava, 1998), whose association with plant-parasitic nematodes is reported to cause greater losses than either pathogen alone (Francl and Wheeler, 1993). Infection of roots by the root-knot nematode predisposes crop plants to infection by soil-borne root-infecting fungi, resulting in the development of root-rot and wilt diseases (Armstrong *et al.,* 1976). Soil application of pesticides has been used successfully to control soil-borne diseases. However, the toxicity of these materials to animals and humans, concerns for the environment and economic costs prevent the use of the most effective chemicals (Ruzo, 2006). On some occasions, bio-control products, especially bacteria such as *Pseudomonas* spp. and *Bacillus* spp. and fungi of the genera *Paecilomyces* spp. and *Trichoderma* spp., have proved successful as alternative methods to control soil-borne diseases (Siddiqui and Mahmood, 1996; Martin and Loper, 1999; Youssef *et al*., 2008).

Paecilomyces lilacinus Samson is an egg-pathogenic fungus, attacking also sedentary stages of root-knot and cyst nematodes. It is considered one of the most promising and practicable biological control agents for the management of plant-parasitic nematodes (Siddiqui and Mahmood, 1996).

Species of *Trichoderma* are free-living fungi that are highly interactive in root, soil and foliar environments and have been used successfully in field trials to control many crop pathogens (Reino *et al*., 2008). *Trichoderma* spp. are known to attack other fungi, produce antibiotics that affect other microbes, and to act as bio-control microbes (Harman, 2006). Excellent management of damping-off disease has been obtained with integration of soil application of *Trichoderma harzianum* Rifai and seed treatment with metalaxyl (Mukhopadhyay and Chandra, 1982; Abada, 1994). *Trichoderma hamatum* Bonod showed the greatest control of sugar beet damp-

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ing-off disease *in vivo*, followed by *T. viride* Pers, whereas *Bacillus subtilis* Chon showed the least (Tohamy *et al.*, 2002). In a pot experiment, *P. lilacinus* reduced significantly the numbers of galls, second stage juveniles and egg masses of *M. incognita* attacking tomato, similarly to the nematicide fenamiphos (Oclarti and Cumagun, 2009).

Therefore, the objective of this investigation was to evaluate the activity of the antagonists contained in the commercial products Bio-Nematon®, Priority®, Stanes sting®, Bio-Arc® and Bio-Zeid® against *M. incognita* and *F. solani* in sugar beet, in comparison with the commercial granular formulation of the synthetic nematicides Nemacur® (10% of the active ingredient fenamiphos) and Rugby® (10% of the active ingredient cadusaphos), under *in vitro*, greenhouse and field conditions.

MATERIALS AND METHODS

The sugar beet cv. Dema Poly, characterized as France polygerm cultivar, was used in the study. Females and egg-masses of *M. incognita* were isolated from infected sugar beet roots collected from Nubariya region. Cultures of the nematode were then established from single egg-masses that had previously been identified by observation of the morphological characteristics of the perineal patterns of the adult females (Taylor and Sasser, 1978). The nematode was reared on eggplant cv. Pusa Purple long in a glass-house at 30 ± 5 °C. Second-stage juveniles (J_2) of *M. incognita* were obtained from these cultures by incubating infected roots in water for three days at 30 ± 5 °C and the hatched J_2 were collected and counted.

To isolate *Fusarium* spp., samples of feeder roots of sugar beet were collected from commercial fields and then transferred to the laboratory. Roots were surface sterilized by dipping in 2% sodium hypochlorite solution for 2 minutes, washed several times in sterile distilled water, dried between two filter papers, cut into small pieces and plated on PDA medium in sterile Petri dishes. The plates were incubated at 25 °C for 7 days to isolate *Fusarium* spp. according to isolation procedures described by Dhingra and Sinclair (1985) and Raviv *et al.* (2005). Identification of *Fusarium solani* was made on the base of morphological and culture characteristics (Nelson *et al.*, 1983).

The commercial bio-control products used were Bio-Nematon®, containing *Paecilomyces lilacinus* at 10^8 units/cm^3, Priority®, containing *Paecilomyces fumosoroseus* Apopka at 10^8 units/cm^3, Stanes sting®, containing *Bacillus subtilis* at 10^9 bacterial cells/ml, Bio-Arc 6%®, containing *Bacillus megaterium* De Bary at 25 × 10^6 bacterial cells/g, and Bio-Zeid 2.5%®, containing *Trichoderma album* Freuss at 10^6 fungal cells/g. They were obtained from the Agricultural Research Center (Giza), Egypt. The two commercial nematicides Nemacur® (fenamiphos) [3-methyl-4-(methylthio) phenyl (1-methylethyl) phosphoramidate] and Rugby® (cadusafos) [*O*-ethyl S, S- bis (1- methlpropyl) phosphorodithioate] were used as control treatments.

Tests *in vitro*

Paecilomyces lilacinus, *P. fumosoroseus* and *B. subtilis* were tested at aqueous concentrations of 0.5, 1.0 and 1.5% of the commercial product against *M. incognita, F. solani* and *Fusarium oxysporum* Schlecht. emend. Snyd. *et* Hans. *Bacillus megaterium* and *T. album* were tested at concentrations of 0.3, 0.6 and 0.9 % of the commercial product. Nemacur and Rugby were tested at concentrations of 1.25, 2.5 and 5 ppm of the commercial product suspended in water.

Effect against M. incognita. Nine ml of distilled water instead of the nematicide solutions or biocontrol product suspension were added to 1 ml of a nematode suspension containing 400 J_2 of *M. incognita* in a 50 ml plastic capsule to serve as a nematode only control. Each treatment was replicated five times according to a completely randomized design. The numbers of viable and dead nematodes were counted under a light microscope after 24, 48 and 72 h exposure periods at 25 °C. Nematodes were considered alive if they moved or assumed a winding shape, and dead if they were straight and immobile. After the various exposure periods the nematodes in each treatment were transferred to distilled water and left for 24 h to see whether immobile nematodes resumed activity or not. The corrected percentages of nematode mortality were calculated according to Abbott's (1925) formula:

$$\text{Mortality (\%)} = (m - n)/(100 - n) \times 100$$

where m and n indicate the percentages of mortality in treatments and control, respectively.

Effect against F. solani *and* F. oxysporum. The antifungal activity of the treatments against *F. solani* and *F. oxysporum* was evaluated in Petri-dishes containing PDA medium, by the dual culture technique. A disc of the pathogenic fungi was inoculated on the surface of media treated with each treatment concentration, separately. As a reference control, *F. solani* was grown on PDA in Petri dishes without any treatment. Three replicated dishes for each treatment as well as for the control were prepared. Petri dishes were incubated at 25 °C for 7 days. Inhibition of fungal growth (%) was calculated using the formula:

$$\text{Inhibition of fungal growth \%} = (A - B)/A \times 100$$

where A = diameter of linear mycelial growth in the control, and B = diameter of linear mycelial growth in the treated dish.

Greenhouse experiment

The experiment was conducted to assess the effects of the treatments on *M. incognita* and *F. solani* in a glasshouse at the Sugar Crops Research Institute (SCRI), Agricultural Research Centre (ARC), Egypt. The soil for each pot (6 kg soil) was mixed separately with the corresponding amount of each bio-product at the various rates. The rates of application were 5, 10 and 15 cm^3/pot for each of *Paecilomyces lilacinus*, *P. fumosoroseus* and *B. subtilis,* and 3, 6 and 9 g/pot for both *Bacillus megaterium* and *T. album*. Nemacur and Rugby were applied at the rate of 2.5 g/pot at plant thinning. The plastic pots (30 cm diameter), containing 6 kg of a sterilized mixture of sandy and loamy soil (1: 1, sandy : loamy), were arranged according to a completely randomized design on a bench in the glasshouse maintained at 20 ± 5 °C. After seed germination, 21 days from sowing, each pot was thinned to one plant. One week later, the pots were inoculated (in four holes made around the plant) with 2,000 newly hatched J_2 of *M. incognita* per pot. At the same time, *F. solani* at a rate of 3% of fungal culture by soil weight was applied on pot surface and carefully incorporated into the soil. Six replicated pots were used per treatment as well as for the control.

The nematode variables were recorded as numbers of galls, females and egg-masses in the entire root apparatus of sugar beet plants (six plant roots per treatment), 6 months after nematode infestation. Also, feeder roots (six plant roots per treatment) were collected 6 months after fungal inoculation to examine the presence of *F. solani* infection. Root samples were then transferred to the laboratory. Isolation of *F. solani* from the roots was made by the standard method described by Dhingra and Sinclair (1985). The fungus was detected and identified according to morphological and culture characteristics by microscopy examination (Nelson *et al.*, 1983).

Plant growth components, such as shoot (length and fresh weight) and fresh root weight were also recorded. The percentages of total soluble solids (TSS%) of fresh tap root juice in treated and non-treated plants were determined using a traditional handheld refractometer.

Field experiment

This study was conducted during the 2009-2010 season in a field with clay loam soil, naturally infested with *M. incognita* and *F. solani*, irrigated by overhead sprinklers, at Nubariya district, Behera Governorate, Western Nile Delta region, Egypt. The bio-control products containing *P. lilacinus* and *P. fumosoroseus* were applied at 2.5 (half recommended dose) and 5.0 (recommended dose) cm^3/m^2. Nemacur and Rugby were applied at 2.5 (half recommended dose) and 5.0 (recommended dose) g/m^2. All tested materials were spread on the surface of the soil and then incorporated to a depth of 10-20 cm using a hoe, 7 days before sowing. The experimental field (378 m^2) was divided into four blocks with nine plots per block, and each plot was 3 m wide × 3.5 m long (= 10.5 m^2 i.e. 1/400 Fadden). Each plot consisted of six rows of plants spaced 50 cm apart. There were four replicates per treatment, arranged according to a randomized block design. Sugar beet seeds, cv. Dema Poly, were sown in the last week of October, 2009. Seeds were sown at a spacing to provide the normal density of 40,000 plants/Fadden (2.4 Fadden's = 1 ha). All treatments were managed throughout the growing season by standard agricultural practices and were irrigated as needed. The average soil nematode population density before treating (Pi) was 210 J_2/200 g soil.

Effect on M. incognita. Numbers of J_2 in soil, and galls, females and egg-masses on the roots were recorded 2, 4 and 6 months after sowing. Soil samples (1000 g) were collected from the rhizosphere of five sugar beet plants (200 g soil/plant) and the roots contained in the sample used later for other examinations. Each soil sample was thoroughly mixed and a 200 g sub-samples used to extract nematodes by the sieving and decanting method (Barker, 1985). The roots of each replicate were cut into small pieces (0.5 cm long), placed in Petri dishes and then examined under a stereoscopic microscope for counting galls, females and egg-masses on the entire root systems.

Effect on soil mycoflora. The population of the soil mycoflora from the sugar beet rhizosphere was determined, 2, 4 and 6 months after sowing, as numbers of colony-forming units (CFU) per ml of soil suspension plates on PDA medium by the pour plate method and dilution technique (Ghini *et al.*, 2007). Thus, one gram of soil was suspended in 99 ml sterile water to obtain a 1/100 dilution. Then, serial dilutions were prepared up to 10^{-5}. Three replicated plates were prepared for each dilution per soil sample. The plates were incubated at 25 °C for 7 days. Fungi that grew out were counted as CFU/plate and identified to genus and species level according to the morphological and culture characters (Gilman, 1957; Barnett and Hunter, 1972; Nelson *et al.*, 1983). Each isolated fungus was counted and its frequency percentage calculated according to the equation:

$$\text{Frequency percentage} = \text{Fungus number} / \text{Total number of fungi} \times 100$$

Detection of F. solani *infection*. Root samples of sugar beet were collected from treated and untreated plants 2, 4 and 6 months after sowing to examine the presence of *F. solani* infection according to the isolation procedures mentioned above.

Effect on plant growth, yield and technical characteristics. Plant fresh weight, foliage weight, length, diameter and fresh weight of the roots, average number of surviving plants (%) and tap root yield were recorded. The technical characteristics of sugar beet roots were as-

sessed by measuring sucrose content (S%) according to Le-Dacte (1927), total soluble solids (TSS) in fresh roots using a refractometer, while juice purity (%) was determined as a ratio between S% and TSS% according to Carruthers and Oldfield (1961).

Statistical analysis

Data were subjected to analysis of variance using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA, and means compared with the Least Significant Difference (LSD) test at $P = 0.05$ (Snedecor and Cochran, 1980). The significance of the factors treatment, concentration, exposure time and their interactions were also analyzed. Nematode data were normalized before analysis by log transformation.

RESULTS

Tests *in vitro*

In the *in vitro* tests, the factors treatment, concentration and exposure time all had significant effects on the nematode and the fungi (Tables I and II).

Effect on M. incognita. The net mortality of *M. incognita* treated with the bio-control products ranged from 59 to 94% compared to that of 96 to 98% provided by Nemacur and Rugby. However, the products containing *B. subtilis*, *P. lilacinus*, *P. fumosoroseus*, *T. album* and *B. megaterium* significantly increased the mortality of the nematode after 24, 48 and 72 h of exposure compared to the control (Table I) and nematode mortality increased with increase of concentration of the bio-product. Also, the mortality was already great after 24 hours of exposure and increased only slightly up to 72 hours

Table I. Effects of commercial bio-control products and nematicides on the mortality of second-stage juveniles of *Meloidogyne incognita* in *in vitro* tests.

Treatment		% nematode mortality after hours			Recovery (%)	Net mortality (%)
Product	Concentration[1]	24	48	72		
Bio-Nematon ® (*Paecilomyces lilacinus*)	0.5	68	71	72	1.7	70
	1.0	77	83	84	0.0	84
	1.5	91	91	92	0.0	92
	Mean	79	82	82	0.6	82
Priority® (*Paecilomyces fumosoroseus*)	0.5	66	67	68	1.3	68
	1.0	80	81	82	0.0	82
	1.5	89	90	91	0.0	91
	Mean	78	79	81	0.4	80
Stanes sting® (*Bacillus subtilis*)	0.5	74	75	76	1.0	75
	1.0	77	77	79	0.0	79
	1.5	93	94	94	0.0	94
	Mean	81	82	83	0.3	83
Bio-Arc® (*Bacillus megaterium*)	0.3	61	62	63	1.3	62
	0.6	67	68	20	0.0	70
	0.9	83	85	86	0.0	86
	Mean	70	72	73	0.4	73
Bio-Zeid® (*Trichoderma album*)	0.3	59	60	62	1.3	61
	0.6	74	75	75	0.0	75
	0.9	87	88	89	0.0	89
	Mean	73	74	76	0.4	75
Nemacur	1.25	96	96	96	0.0	96
Rugby	1.25	97	97	98	0.0	98
Distilled water (control)		0	2	2	0.0	0
L.S.D 0.05						
Exposure time (H) =1.0			H × C =		1.7	
Concentration (C) = 1.0			H × T =		2.8	
Treatment (T) = 1.6			C × T =		2.8	
			H × C × T =		4.9	

[1] Concentration: (%) for biocontrol products and ppm for chemical nematicides.

Table II. Effects of commercial bio-control products and nematicides on lineal mycelial growth of *Fusarium solani* and *F. oxysporum* in *in vitro* tests.

Treatment		Pathogenic fungus			
		F. solani		*F. oxysporium*	
Product	Conc.[1]	Mycelial growth (cm)	Growth Reduction (%)	Mycelial growth (cm)	Growth Reduction (%)
Bio-Nematon ® (*Paecilomyces lilacinus*)	0.5	2.20	75.6	2.80	68.9
	1.0	1.48	83.6	1.78	80.3
	1.5	0.50	94.5	0.00	100.0
	Mean	1.39	84.4	1.53	83.1
Priority® (*Paecilomyces fumosoroseus*)	0.5	4.15	53.9	6.05	32.8
	1.0	2.75	69.5	3.35	62.8
	1.5	0.00	100.0	2.15	76.1
	Mean	2.30	74.5	3.85	57.2
Stanes sting® (*Bacillus subtilis*)	5	5.50	38.9	6.13	31.9
	10	3.93	56.4	3.18	64.7
	15	2.03	77.5	0.00	100.0
	Mean	3.82	57.6	3.10	65.5
Bio-Arc® (*Bacillus megaterium*)	0.3	4.50	50.0	4.08	54.7
	0.6	2.85	68.4	2.40	73.3
	0.9	1.00	88.9	0.00	100
	Mean	2.78	69.2	2.17	76.0
Bio-Zeid® (*Trichoderma album*)	0.3	2.23	75.3	1.93	78.6
	0.6	1.00	88.9	0.00	100.0
	0.9	0.00	100.0	0.00	100.0
	Mean	1.08	88.1	0.64	92.9
Nemacur	1.25	2.90	67.8	4.20	53.3
	2.5	1.73	80.8	2.35	73.9
	5.0	1.20	86.7	1.58	82.5
	Mean	1.94	78.4	2.71	69.9
Rugby	1.25	4.58	49.2	1.53	83.0
	2.5	3.10	65.6	1.30	85.6
	5.0	2.15	76.7	1.05	88.4
	Mean	3.28	63.6	1.29	85.5
Control		9.0	-	9.0	-
L.S.D.0.05			Reduction %		
Fungus (F)	= 1.1		F × C = 1.9		
Concentration (C)	= 1.3		F × T = 2.8		
Treatment (T)	= 2.0		C × T = 3.5		
			F × C × T = 4.9		

[1] Conc. = Concentration (%) for biocontrol products and ppm for chemical nematicides.

of exposure. Small but significant differences were recorded among the bio-control products, at different exposure times and concentrations (Table I).

Effects on F. solani *and* F. oxysporum. The mycelial growth of *F. solani* was reduced by 38.9 to 100% with the bio-control products and by 49.2 to 86.7% with Nemacur and Rugby (Table II). The product containing *T. album* gave the largest reduction in linear mycelial growth of *F. solani* (88.1%), followed by that with *P. lilacinus* (84.4%), Nemacur (78.4%), *P. fumosoroseus* (74.5%), *B. megaterium* (69.2%), Rugby (63.6%) and *B. subtilis* (57.6%). For *F. oxysporum* the linear reduction of mycelial growth was in the range from 31.9 to 100% and from 53.3 to 88.4% with the bio-control products and chemical nematicides, respectively. The product with *T. album* provided the greatest reduction of linear mycelial growth of *F. oxysporum* (92.9%), followed by Rugby (85.5%), the products with *P. lilacinus* (83.1%) and *B. megaterium* (76.0%), Nemacur (69.9%), and the products with *B. subtilis* (65.5%) and *P. fumosoroseus* (57.2%). Significant differences were

recorded among treatments, concentrations and pathogenic fungi. The suppressive effect of the tested bio-control products on pathogenic fungi increased with the increase of their concentration (Table II).

Greenhouse experiment

All treatments greatly reduced the numbers of galls, females and egg masses of the nematode compared to the untreated control (Table III). However, the effects of the factors treatment and concentration and their interaction were significant on plant growth variables but not always on nematode and fungus variables.

Effect on M. incognita. The bio-product containing *B. megaterium* greatly reduced the number of galls per plant (14 galls), followed by *B. subtilis* (18 galls), *P. fumosoroseus* (19 galls), *P. lilacinus* (20 galls) and *T. album* (30 galls), compared to 12, 13 and 95 galls on the roots of chemically treated and untreated plants (Table III). The bio-control products also reduced the numbers of females in the roots, which were 10, 12, 12, 13 and 20 in the pots treated with *B. megaterium*, *B. subtilis*, *P. fumosoroseus*, *P. lilacinus*, and *T. album*, and 5, 13 and 95 in those treated with Rugby, Nemacur or untreated, respectively (Table III). A similar trend was observed for the effects of the treatments on numbers of egg masses of the nematode.

Effects on F. solani. The percentages of sugar beet roots infected by *F. solani* were within the range 2.5-43.8% in the treatments with the bio-control products, compared to 12.5 with both Nemacur and Rugby and 75.0% in untreated pots (Table IV). Infection by *F. solani* decreased with the increase of the rates of the bio-control products.

Effect on plant growth and total soluble solids. The bio-control products and the nematicides significantly

Table III. Effects of commercial bio-control products and nematicides on numbers of galls, females and egg masses of *M. incognita* per plant and infection (%) by *F. solani*, in sugar beet under greenhouse conditions.

Treatment		Nematode variable			*F. solani* infection (%)
Product	Dose[1]	Galls	Females	Egg Masses	
Bio-Nematon ® (*Paecilomyces lilacinus*)	5	22	13	12	31.3
	10	19	13	11	18.8
	15	19	12	11	12.5
	Mean	20	13	11	20.8
Priority® (*Paecilomyces fumosoroseus*)	5	23	15	14	37.5
	10	20	13	12	18.8
	15	13	8	8	12.5
	Mean	19	12	11	22.9
Stanes sting® (*Bacillus subtilis*)	5	24	14	14	43.8
	10	17	12	10	31.8
	15	15	11	9	18.8
	Mean	18	12	11	31.3
Bio-Arc® (*Bacillus megaterium*)	3	24	20	13	43.8
	6	11	5	4	37.5
	9	7	4	2	25.0
	Mean	14	10	6	35.4
Bio-Zeid® (*Trichoderma album*)	3	33	23	21	37.5
	6	33	20	21	31.3
	9	24	16	13	25.0
	Mean	30	20	18	31.3
Nemacur	2.5 g/pot	13	8	9	12.5
Rugby	2.5 g/pot	12	5	9	12.5
Untreated (control)		95	75	83	75.0
L.S.D 0.05					
Concentration (C) =		2.5	NS	2.0	8.2
Treatment (T) =		4.1	NS	3.3	13.4
C × T =		NS	NS	5.6	NS

[1]Dose: cm^3/pot for Bio-Nematon, Priority and Stanes sting and g/pot for Bio-Arc, Bio-Zeid and chemical nematicides.

Table IV. Effects of bio-control products and nematicides on some plant growth components and total soluble solids (TSS%) in sugar beet under greenhouse conditions.

Treatment		Growth component			
		Shoot			TSS (%)
Product	Dose[1]	Length (cm)	Weight (g)	Root weight (g)	
Bio-Nematon ® (*Paecilomyces lilacinus*)	5	29.3	87.8	80.6	14
	10	34.8	97.2	83.7	14
	15	36.3	99.0	87.6	16
	Mean	33.4	94.7	84.0	15
Priority® (*Paecilomyces fumosoroseus*)	5	29.3	63.9	84.7	14
	10	33.5	83.3	92.1	15
	15	37.0	98.6	97.3	16
	Mean	33.3	81.9	91.4	15
Stanes sting® (*Bacillus subtilis*)	5	29.5	80.5	116.9	14
	10	32.8	108.5	135.1	15
	15	36.8	142.7	157.5	17
	Mean	33.0	110.6	136.5	15
Bio-Arc® (*Bacillus megaterium*)	3	30.8	89.4	101.5	14
	6	33.5	118.8	132.3	16
	9	35.5	144.0	149.8	16
	Mean	33.3	117.4	127.9	15
Bio-Zeid® (*Trichoderma album*)	3	32.3	72.9	102.9	14
	6	32.8	107.6	117.6	15
	9	37.3	165.2	188.4	17
	Mean	34.1	115.2	136.2	16
Nemacur	2.5	38.3	149.0	109.2	16
Rugby	2.5	37.0	143.1	120.4	16
Untreated (control)		28.0	63.9	66.6	12
L.S.D. 0.05					
Concentration (C)	=	2.0	9.7	9.2	0.6
Treatment (T)	=	3.2	15.8	15.1	1.0
T × C	=	5.5	27.3	26.1	1.7

[1]Dose: cm^3/pot for Bio-Nematon, Priority and Stanes sting and g/pot for Bio-Arc, Bio-Zeid and chemical nematicides.

increased shoot length and weight and root weight of sugar beet plants (Table IV). The shoot length of the plants treated with the bio-control products was within the range 29.3-37.3 cm, compared to 38.3, 37.0 and 28.0 cm for the plants treated with Nemacur, Rugby or untreated, respectively (Table IV). *Trichoderma album* gave the greatest increase in shoot length, followed by *P. lilacinus, P. fumosoroseus*, *B. megaterium* and *B. subtilis*, respectively. Shoot length increased with increase of concentration of the biocontrol products. However, no significant differences were recorded among the bio-control products, while significant differences were recorded between their rates of application, except between the 0.3 and 0.6% *T. album* treatments.

The shoot weights were within the range 63.9-165.2 g in treatments with bio-control products and 149.0, 143.1 and 63.9 g for Nemacur, Rugby or untreated plants, respectively. *Bacillus megaterium* greatly increased shoot weight, followed by *T. album*, *B. subtilis*, *P. lilacinus* and *P. fumosoroseus*. Significant differences were recorded among different treatments and untreated plants, while the differences between *P. lilacinus* and *P. fumosoroseus* and among *B. subtilis*, *B. megaterium* and *T. album* (Table IV) were not significant.

The root weights of treated plants ranged from 80.6 to 188.4 g when treated with the biocontrol products and were 109.2, 120.4 and 66.6 g in the pots treated with Nemacur, Rugby or untreated, respectively (Table IV). The greatest increase in root weight was given by *B. subtilis*, followed by *T. album*, *B. megaterium*, *P. fumosoroseus* and *P. lilacinus*. No significant differences in root weight were recorded between plants treated with *P. lilacinus* or *P. fumosoroseus* and among those treated with *B. subtilis*, *B. megaterium* and *T. album*. Root

weight increased significantly with increase of the application rates of the bio-products compared to untreated plants, except for that containing *P. lilacinus*, which did not, and between the rates of 0.5 and 1.0% of *P. fumosoroseus*.

The bio-control products and nematicides significantly increased TSS% in the juice of treated plants (Table IV), which was in the range 14-17% in treatments with the bio-control products, whereas it was 16% with nematicides and 12% in untreated plants.

Field experiment

Effects on the nematode. All treatments reduced significantly the number of juveniles/200 g soil, which ranged from 17 to 313 in treatments with bio-control products and from 33 to 240 in plots treated with

Table V. Effects of bio-control products and nematicides on numbers of juveniles, galls, females and egg masses of *M. incognita* (per plant) and infection (%) by *F. solani* in sugar beet 2, 4 and 6 months after field application.

Treatment		Months after planting	Nematode variable				Root rot infection (%)
Product	Dose[1]		J_2/200g soil	Galls	Females	Egg masses	
Bio-Nematon ® (*Paecilomyces lilacinus*)	5	2	22	11	8	ND[2]	37.5
		4	143	13	8	5	25.0
		6	83	20	14	6	6.3
		Mean	83	15	10	4	22.9
	2.5	2	52	20	15	ND	62.5
		4	200	21	11	7	31.3
		6	187	32	18	11	18.8
		Mean	146	24	15	6	37.5
Priority® (*Paecilomyces fumosoroseus*)	5	2	17	10	5	ND	37.5
		4	313	17	12	6	438
		6	100	17	10	8	37.5
		Mean	143	15	9	5	39.6
	2.5	2	35	12	7	ND	62.5
		4	143	30	18	12	37.5
		6	213	34	20	19	37.5
		Mean	130	25	15	11	45.8
Nemacur	5	2	40	13	9	ND	75.0
		4	180	17	12	7	12.5
		6	108	18	9	7	12.5
		Mean	108	16	10	5	33.3
	2.5	2	33	11	7	ND	62.5
		4	233	16	12	9	43.8
		6	240	20	10	8	37.5
		Mean	169	16	10	6	47.9
Rugby	5	2	53	9	6	ND	56.3
		4	167	12	7	2	37.5
		6	83	20	14	6	37.5
		Mean	102	14	9	3	43.8
	2.5	2	72	18	16	ND	50.0
		4	210	20	12	6	56.3
		6	187	32	18	11	31.3
		Mean	156	23	15	5	45.8
Untreated (control)		2	147	58	36	ND	87.5
		4	463	61	42	21	75.0
		6	443	105	74	65	56.3
		Mean	351	78	51	29	72.9
L.S.D. 0.05							
Treatment (T)	=		22	4	2	2	13.4
Month (M)	=		38	7	3	3	7.7
T × M	=		66	13	5	6	23.2

[1]Dose: cm^3/m^2 for Bio-Nematon and Priority and g/m^2 for Nemacur and Rugby.
[2]ND = not detected.

Table VI. Effects of treatment of the soil with bio-control products and nematicides on the frequency (%) of mycoflora in the rhizosphere of sugar beet two, four and six months after field application.

Fungus	Months after sowing	Frequency (%) of fungi at dose[2] of								
		Cont.[1]	Bio-Nematon (*Paecilomyces lilacinus*)		Priority (*Paecilomycesfumosoroseus*)		Nemacur		Rugby	
			5	2.5	5	2.5	5	2.5	5	2.5
Aspergillus niger	2	3.4	3.0	6.5	3.8	5.9	3.4	8.2	6.2	4.5
	4	3.3	7.6	6.4	10.8	8.9	6.1	4.2	3.5	3.2
	6	3.6	6.7	8.5	9.6	7.5	5.2	4.0	2.0	4.5
Aspergillus spp.	2	5.9	4.0	2.9	5.7	6.0	4.6	3.8	4.3	7.3
	4	5.0	6.2	5.2	7.9	9.3	3.9	5.8	7.1	8.7
	6	5.2	9.4	10.0	7.4	9.6	7.7	4.0	8.3	4.5
Rhizopus nigricans	2	9.2	2.0	2.9	12.8	3.9	ND[3]	ND	5.3	15.2
	4	9.0	4.6	3.9	2.4	6.9	1.9	3.2	2.0	8.8
	6	10.1	4.9	8.6	5.1	5.8	5.1	6.4	ND	4.5
Fusarium solani	2	33.7	38.3	26.3	35.8	27.6	28.5	37.5	35.3	29.1
	4	31.4	28.8	20.4	21.0	27.3	28.0	26.3	30.8	35.4
	6	35.8	22.8	18.4	14.7	23.7	20.6	25.7	27.2	35.8
Fusarium oysporum	2	11.7	21.4	22.5	15.1	11.8	18.1	17.9	21.3	12.4
	4	11.5	22.8	14.2	10.3	16.1	14.1	17.3	17.2	22.3
	6	16.7	19.7	16.3	9.6	17.0	12.8	19.3	20.8	20.1
Trichoderma spp.	2	14.3	20.1	22.7	10.0	21.4	7.3	17.3	5.7	5.3
	4	14.2	5.9	16.5	21.0	8.9	9.9	20.8	12.0	10.2
	6	6.0	6.3	11.2	26.8	11.3	10.1	16.6	15.0	16.2
Rhizoctonia solani	2	15.1	3.1	2.9	5.3	5.5	21.4	3.3	8.9	12.4
	4	14.8	15.4	18.0	7.9	6.8	16.2	7.1	13.7	10.2
	6	12.2	12.5	10.0	7.4	9.6	23.2	6.7	10.2	10.3
Penicillum spp.	2	3.3	3.0	5.2	4.1	7.9	12.0	6.0	7.3	5.5
	4	5.8	4.6	7.7	10.8	6.9	11.8	7.4	3.3	3.2
	6	5.6	8.0	5.5	9.5	5.9	5.2	8.5	6.3	4.5
Others	2	3.4	5.1	8.1	7.4	10.0	4.7	6.0	5.7	7.3
	4	5.0	4.6	7.7	7.9	8.9	8.1	7.9	10.4	8.0
	6	4.8	9.7	5.5	9.9	9.6	10.1	8.8	10.2	9.7

L.S.D. 0.05

Fungus (F)	= 1.2	F × M	= 2.0
Month	= 0.7	F × T	= 3.5
Treatment	= 1.2	M × T	= 2.0
		F × M × T	= 5.9

[1]Cont. = Control.
[2]Dose: cm^3/m^2 for Bio-Nematon and Priority and g/m^2 for Nemacur and Rugby.
[3)] ND = not detected.

Nemacur and Rugby, compared to 147 to 463 in untreated plots (Table V). Among the treatments, the greatest control was given by *P. lilacinus*, followed by Rugby, Nemacur and *P. fumosoroseus* at the recommended dose, whereas *P. fumosoroseus* was the best at half the recommended dose, followed by *P. lilacinus*, Rugby and Nemacur, respectively. *Paecilomyces lilacinus*, Nemacur and Rugby greatly reduced the numbers of juveniles in the soil at the recommended doses, but *P. fumosoroseus* did so at half the recommended dose (Table V). Six months after sowing, *P. lilacinus* gave the greatest reduction in the number of juveniles, followed by Rugby, *P. fumosoroseus* and Nemacur.

The treatments also reduced the numbers of galls, females and egg masses of the nematode compared to untreated plots. The number of galls was within the range 10-34 with bio-control products, compared to 9-32 with Nemacur and Rugby and 58-74 in untreated plots. Rugby significantly reduced the number of galls at the recommended dose, while Nemacur also did so at half the recommended dose (Table V).

The numbers of females in the roots were within the range 5-20 in the plots treated with the bio-control agents and 6-18 in those treated with Nemacur or Rug-

by, compared to 36-74 in the untreated plots. Rugby reduced the numbers of female at the recommended dose by the greatest amount, followed by *P. lilacinus*, *P. fumosoroseus* and Nemacur, while Nemacur greatly reduced the numbers of females at half the recommended dose, followed by *P. lilacinus*, *P. fumosoroseus* and Rugby. No significant differences were recorded among different treatments at the recommended dose.

Egg masses were not observed until two months after sowing in all treated plots, but their numbers increased by 4 and 6 months after sowing to 5-19 with bio-control agents, 2-11 with Nemacur or Rugby, and 21-65 in the untreated plots (Table V).

Effects on F. solani *infection*. The percentages of sugar beet roots infected by *F. solani* were significantly reduced by the treatments (Table V). They were 6.3 to 62.5% (*P. lilacinus*), 37.5 to 62.5% (*P. fumosoroseus*), 12.5 to 75.0% (Nemacur) and 31.3 to 56.3% (Rugby). The observed percentages of infected roots decreased with increase of rate of application of the bio-control products but no clear trend was observed at the different observation times.

Effects on soil mycoflora. The fungi *Aspergillus* spp., *A. niger* Van Tieh, *Fusarium solani*, *F. oxysporum*, *Penicillium* spp., *Rhizoctonia solani* Kühn, *Rhizopus nigricans* Stolonifer, *Trichoderma* spp. and others were common in the rhizosphere soil of sugar beet in treated and untreated plots (Table VI). The frequencies (%) of *F. solani* were significantly reduced and were in the range 14.7-38.3% in plots treated with the bio-control products, 20.6-37.5% in those treated with Nemacur and Rugby, and 31.4-35.8% in the untreated plots.

The frequency of *F. oxysporum* was in the range 9.6-22.8% or 12.4-22.3% in the rhizosphere of plants treated with the bio-control agents or the nematicides, respectively, compared to 11.5-16.7% in the untreated plots (Table VI). At half the recommended rate, *P. lilacinus* and Rugby significantly reduced the frequency of *F. oxysporum*, while *P. fumosoroseus* and Nemacur did so only at the recommended rate. Six months after application at half the recommended rate, only *P. fumosoroseus* reduced significantly the frequency of *F. oxysporum*. The frequency of *R. solani* was in the range 2.9-18.0% and 3.3-23.2% in the rhizosphere of plants treated with the bio-control agents and the nematicides, respectively, compared to 12.2-15.1% in the rhizosphere of untreated plots. All treatments significantly reduced the frequency of *R. solani* two months after sowing and continued to do so until the end of the experiment, 6 months after sowing. Nemacur at the recommended dose had no effect on *R. solani*.

In general, both the bio-control products and the nematicides significantly increased the frequency of occurrence of *Aspergillus* spp., *A. niger*, *Penicillium* spp. and *Trichoderma* spp. *Aspergillus* spp. was from undetectable levels to 10% in treated rhizosphere, compared to 5.0-5.9% in the untreated rhizosphere. Treatment of the rhizosphere increased the frequency of *A. niger* (2.0-8.5% compared to 3.3-3.6% in untreated control), *Penicillium* spp. (3.0-12.0% compared to 3.3-5.8 % in untreated

Table VII. Effects of bio-control products and nematicides on growth components of sugar beet plants six months after their application in the field.

Treatment		Plant weight (kg)	Foliage Weight (kg)	Root			Survival plants/ Fadden	Roots yield (Ton/ Fadden)
Product	Dose[1]			Length (cm)	Diameter (cm)	Weight (kg)		
Bio-Nematon ® (*Paecilomyces lilacinus*)	5	2.88	0.54	31.3	12.8	2.23	13644	30.57
	2.5	1.97	0.42	29.7	9.8	1.55	13167	20.40
	Mean	2.42	0.48	30.5	11.3	1.89	13406	25.48
Priority® (*Paecilomyces fumosoroseus*)	5	3.08	098	30.7	12.2	2.10	13467	28.35
	2.5	3.27	0.72	31.0	12.8	2.55	12833	32.05
	Mean	3.18	0.85	30.8	12.5	2.33	13150	30.20
Nemacur	5	2.30	0.50	25.3	12.0	1.80	13200	24.01
	2.5	2.12	0.47	35.3	10.5	1.65	13870	22.42
	Mean	2.21	0.49	30.3	11.3	1.73	13,535	23.21
Rugby	5	2.60	0.52	37.3	8.8	2.08	13682	32.01
	2.5	2.12	0.50	25.3	9.5	1.62	13433	21.80
	Mean	2.36	0.51	31.3	9.2	1.85	13558	25.25
Control		1.16	0.27	19.0	8.0	0.88	11533	10.12
L.S.D 0.05								
Treatments (T) =		0.72	0.10	5.8	2.3	0.63	1052	7.91
Concentrations (C) =		NS	0.09	NS	NS	NS	NS	NS
T × C =		NS	0.18	8.2	NS	NS	NS	NS

[1] Dose, cm^3/m^2 for Bio-Nematon and Priority and g/m^2 for Nemacur and Rugby.

Table VIII. Effects of bio-control products and nematicides on technical characteristics of sugar beet tap roots under field conditions.

Treatment		TSS (%)	Sucrose (%)	Purity (%)	Sugar yield	
Product	Dose[1]				Per plant (kg)	Ton/Fadden
Bio-Nematon ® (*Paecilomyces lilacinus*)	5	20	15.95	74.82	0.27	2.86
	2.5	19	16.50	85.48	0.22	2.28
	Mean	20	16.23	80.15	0.24	2.57
Priority® (*Paecilomyces fumosoroseus*)	5	25	16.65	67.54	0.24	2.20
	2.5	20	16.50	81.38	0.36	3.50
	Mean	23	16.68	74.46	0.30	2.85
Nemacur	5	22	19.11	87.11	0.30	3.13
	2.5	24	18.83	79.43	0.26	2.82
	Mean	23	18.98	83.27	0.28	2.98
Rugby	5	23	19.80	85.64	0.35	3.93
	2.5	23	17.20	73.62	0.21	2.14
	Mean	23	18.50	79.63	0.28	3.03
Control		19	14.50	70.77	0.09	0.78
L.S.D. 0.05						
Treatment (T) =		2	1.06	NS	0.14	1.63
Concentration(C) =		NS	NS	NS	NS	NS
T × C =		NS	1.51	11.82	NS	NS

[1]Dose: cm^3/m^2 for Bio-Nematon and Priority and g/m^2 for Nemacur and Rugby.

control), and *Trichoderma* spp. (5.7-26.8%, compared to 6.0-14.3 % in untreated control). Thus, the bio-agents increased the frequencies of *Aspergillus* spp., *A. niger*, *Penicillium* spp. and *Trichoderma* spp., while the nematicides increased only the frequency of *Penicillium* spp.

Effect on plant growth, yield and technical characteristics. Plant growth components, viz. plant weight, foliage weight, root length, diameter, and weight, surviving plants per Fadden and tap root yield (Ton/Fadden), significantly increased in treated plots compared to untreated plots (Table VII). In treated plots, the weights of the plants were in the range 1.97-3.27 kg and that of foliage in the range 0.42-0.98 kg, compared to 1.16 kg and 0.27 kg in untreated plots, respectively. All treatments increased root size, viz. length (25.3-35.3 cm), diameter (8.8-12.8 cm) and weight (1.55-2.23 kg), compared to 19 cm, 8.0 cm and 0.88 kg in untreated plots, respectively. On average, the numbers of surviving plants in all treatments were in the range 12833-13870/Fadden, compared to 11533 plants/Fadden in the untreated plots. Tap root yields were greatly increased (2-3 fold) by all treatments with bio-products, with *P. fumosoroseus* giving the best performance, which was comparable with that in plots treated with the recommended rate of Rugby (Table VII).

The technical characteristics, except for sucrose purity, were also affected by the treatments (Table VIII). *Paecilomyces fumosoroseus* increased the TSS% most at the recommended dose, followed by Rugby and *P. lilacinus*. Rugby significantly increased the percentage of sucrose, followed by *P. lilacinus*. The highest sugar yield per plant and/or Fadden was obtained with *P. fumosoroseus* (0.36 kg/plant and 3.50 Ton/Fadden) at half the recommended dose (Table VIII).

DISCUSSION

The results revealed that *M. incognita* (root-knot) and *F. solani* (root-rot) cause severe damage to sugar beet in new reclaimed land in Egypt. Our findings agree with those by Abo-Elnaga (2006) and Korayem (2006), who reported that *M. incognita* and fungal soil-borne pathogens were widespread in the rhizosphere of sugar beet in sandy-clay soil and damaged the roots of sugar beet plants.

The commercial bio-control products Bio-Nematon® (*P. lilacinus*), Priority® (*P. fumosoroseus*), Stanes sting® (*B. subtilis*), Bio-Arc ® (*B. megaterium*) and Bio-Zeid ® (*T. album*), in addition to Nemacur and Rugby, showed suppressive effects against *M. incognita* and *F. solani* in *in vitro* tests and reduced the incidence of root-knot and root-rot diseases in pots. These findings agree with those by Khan and Saxena (1997) and Morsy *et al.* (2009), who reported that *A. niger*, *B. subtilis*, *P. lilaci-*

nus and *T. viride* could suppress the damage caused by *M. javanica* (Treub) Chitw. and *F. solani* in tomato plants *in vitro*, in pots and in the field.

The products Bio-Nematon® and Priority® and the nematicides Nemacur and Rugby, when applied in the field, were promising in suppressing the incidence of root-knot and root-rot diseases on sugar beet, increasing soil microflora and improving growth, yield and technical characteristics of sugar beet. It is suggested that the antagonistic effects of the bio-control products may have been favored by other soil factors. The bio-product Priority® greatly increased all plant growth components, while Rugby increased significantly only the average plant survival. Our findings agree with those who state that the efficacy of nematicides in controlling soil-borne pathogens can be reduced because the pathogen strains may become resistant to pesticides and at rather high concentrations pesticides are pollutant (Alabouvette *et al*., 1993). Siddiqui and Mahmood (1996) reported that *P. lilacinus* attacks mainly the second stages of root knot and cyst nematodes and considered the fungus as the most promising and practicable biological control agent for the management of plant parasitic nematodes. Haque *et al.* (1996) reported that *P. lilacinus* controlled root-knot and root-rot in okra.

Our results suggest that the increased antagonistic efficacy of Bio-Nematon® and Priority® as bio-agents may be due to the increased frequency in the treated rhizosphere of saprophytic fungi such as *A. niger*, *Aspergillus* spp. and *Trichoderma* spp., while the nematicides increased the frequency of *Penicillium* spp. only. These mycoflora could play an important role in increasing the antagonistic effects of bio-products against soil-borne pathogens (Panneerselvan and Saravanamuthu, 1996; Harman, 2006). Also, Ambikapathy *et al.* (2002) mentioned that the greatest inhibition of *F. solani* growth was obtained with *A. niger*, followed by *T. viride*, *T. harzianum* and *Penicllium* spp. Therefore, our results suggest that nematicides can be replaced by Bio-Nematon® and Priority® for the control of root-knot and root-rot diseases of sugar beet in the conditions prevailing in the region where this study was conducted.

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