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Comparative Efficacy of Commercial Bioagents Against *Rotylenchulus reniformis* Infecting Cowpea under Greenhouse, Micro-Plots and Field Conditions.

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Abstract: In a pot experiment, the recommended doses of the commercial microbial products, Omega (*Bacillus* spp. + *Pichia* spp.), Biofertile, Biocontrol (mixed bacterial solutions), isolates of *Pseudomonas fluorescens*, *Serratia marcescens* and vesicular arbuscular mycorrhiza (*Glomus* spp) were applied as soil treatments to test their bioefficacy against *Rotylenchulus reniformis*. All microbial agents used significantly reduced the nematode populations on roots and soil as compared with untreated check. Between microbial treatments there was no significant difference and all imposed more or less similar suppressive effects on *R. reniformis* counts. *P. fluorescens*, *S. marcescens* and Biocontrol achieved the highest percentages of reduction. Meanwhile, such bioagents failed in micro-plot experiment to impaire *R. reniformis* reproductivity except *S. marcescens* and Mycorrhiza which significantly inhibited the nematode development. Moreover, their efficacies overwhelmed those achieved in pots. Under field conditions, percent of nematode reductions were increased as compared with those of micro-plot experiment except treatments of *S. marcescens* and Mycorrhiza. Biofertile was the uppermost in reducing nematode population achieving 72.69% reduction. No obvious proportional relation was noticed between microbes capabilities in reducing nematode reproduction and plant growth improvement.

Key words: Biocontrol · Reniform nematode · Cowpea

INTRODUCTION

The reniform nematode, *Rotylenchulus reniformis* [1], is one of the most noxious pests to legume crops in Egypt. The reniform nematode is a semi-endoparasitic nematode that is widely distributed in subtropical and tropical regions of the world and documented to cause major losses to cowpea which is one of the important food legumes in the drier regions of the tropics and subtropics [2-5].

Bioagents have been successfully used to minimize the nematode injurious effect upon their hosts. A variety of nematophagus bacterial groups have been isolated from soil and/or host-plant tissues. Their mode of action may be through and/or parasitizing, producing toxins, antibiotics, enzymes. Also, interfering with plant-host recognitions, competing for nutrients, inducing systemic resistance to plants and promoting plant health [6-9]. *Pseudomonas* strains are known to co-inhibit with parasitic nematodes in the rhizosphere of a wide range of plants [10-13]. For instance, *P. fluorescens* was found to be a plant growth promoting rhizobacterium and adversely affected *R. reniformis* rate of multiplication on tomato, cotton and cowpea [14,15,3,16-19]. Also, seed treatment with *P. fluorescens* reduced population and eggs/eggmass of *R. reniformis* [20]. Other rhizospheric bacteria like *Bacillus subtilis* and *Serratia marcescens* play an important role in biocontrol of plant parasitic nematodes and promotion of plant growth [21-24]. In field trials growth of finger millet treated with *P. fluorescens* as seedling root dip were significantly increased and reduced *R. reniformis* populations and as soil application reducing females and soil population of *R. reniformis* on cotton roots and increased yield [25, 26].

The interaction between nematodes and the vesicular arbuscular mycorrhiza (*Glomus* spp.) is rated as a major biocontrol agent in modern sustainable agriculture system [27]. *G. macrocarpus* was proved antagonistic to *R. reniformis* development and reproduction on grape and on papaya [28, 29].

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The present study was conducted to investigate the effect of some commercial bioagents on *R. reniformis* reproductivity on cowpea and the plant growth response and yield under different experimental conditions.

MATERIALS AND METHODS

Nematode Stock Culture: Pure culture of the reniform nematode, *R. reniformis* [1] was obtained from isolates belonging to the Nematology Division, Faculty of Agriculture, Cairo University and propagated on pigeon pea (*Cajanus indicus*).

Materials and Doses: Seven commercial bioagents agro-products were purchased from the Egyptian market. The recommended doses as well as method of application are listed in table (1).

Greenhouse Experiment: Cowpea, *Vigna sinences* cv. Kareem 7 (a susceptible host to *R. reniformis*) was used in the present study. Individual seedlings (one week old) were grown in 15 cm diameter clay pots filled with loamy sand soil (1:1, v:v) and inoculated with 2000 infective stage of *R. reniformis* by pipetting nematode suspension into 4 holes around the root system of each plant. One week later, the plants were treated with the tested products as mentioned in (Table 1). Each treatment was replicated 8 times. Eight inoculated plants were left without adding materials to serve as untreated check, as well as another 8 plants were left without inoculation as healthy check. All pots were arranged in a complete randomized design on a clean bench in a greenhouse at 30°C ± 5 and horticulturally treated the same.

Nematode Assay

Soil Population: Forty five days from nematode inoculation, plants were harvested and nematodes were extracted. The soil suspension was quite stirred, then poured through sieves of 60, 200 and 325 mesh screens followed by Baermann set and collected after 48hr [30]. Hawksley counting slide was used to calculate the number of juveniles in 1 ml of suspension and then referred to the whole volume.

Root Population: Root systems were gently dried, weighed and stored in 5% formaldehyde in plastic jars. Roots were stained using acid fuchsine method [31]. Developmental stages, mature females and egg-masses were counted under a stereo-microscope.

Micro-Plots Trial: Seeds of cowpea were cultivated in one cubic meter cement micro-plots filled with solarly sterilized sandy loam soil (1:1, v:v). One week after germination, each micro-plot was divided into 3 parts, each part contains 4 seedlings equally distanced from each other and each seedling was inoculated with 2000 infective stages of *R. reniformis*. One week after nematode inoculation, the infected plants were treated with the commercial bioagents agro-products as illustrated in table (2). Each treatment was replicated 12 times and 12 inoculated plants were left without adding materials as well as another 12 un-inoculated healthy plants to serve as check treatments. Three months later, the plants were taken off and nematode counts in 250g soil and on 2g roots were determined. Plant growth criteria (plant fresh weight and shoot dry weight) and yield were also recorded.

Table 1: Doses of the experimented commercial bio-products.

Treatment	Component	Dose/plant as soil drench
Omega	*Bacillus subtillis* and other *Bacillus* spp.+ *Pichia* spp.	0.3 g
	$3.1x10^7$: $3x10^7$colones /gm	
Biofertile	Mixed bacterial solution	5 ml after dil. 1:4
Biocontrol	Mixed bacterial solution	5 ml after dil. 1:4
Pseudomonas fluorescens	$1x10^8$	5 ml
Serratia marcescens	$1x10^9$	5 ml
Mycorrhiza (*Glomus* spp)	Vesicular Arbuscular Mycorrhiza	3 g
Perfect	Oxamyl 24%+ Metalaxyl-M4%+ Tetramethrin 2%	2 ml

Table 2: Doses of tested bioagents on cowpea infected with *R. reniformis* under micro-plots condition.

Treatment	Dose/plant as soil drench
Omega (*Bacillus* spp.+ *Pichia* spp.)	0.3 g
Biofertile (Mixed bacterial solution)	5 ml
Biocontrol (Mixed bacterial solution)	5 ml
Pseudomonas fluorescens	5 ml
Serratia marcescens	5 ml
Mycorrhiza (*Glomus* spp)	3 g

Field Trial: Efficiency of the most effective microbial agents was tested under field conditions. The experimental field area (6m × 20m) was solarly sterilized, plowed, harrowed, rowed and seeds of cowpea cv. Kareem 7 were planted (3/hole). Each treatment was replicated 3 times at three different rows (1.5m length). Distance between plants was 60cm along side and 30cm apart. One week after germination plants were thinned to one seedling/site and each seedling was infected with 2000 un-swollen females of *R. reniformis*. One week later, infected plants were treated with the selected commercial bioagents (Table 3). After three months, plants were taken off and counts of nematode in 250g soil and on 2 g roots were determined. Plant growth criteria and yield were recorded.

Table 3: Doses of tested bioagents on cowpea infected with *R. reniformis* under field condition.

Treatment	Dose/plant as soil drench
Biofertile	
(Mixed bacterial solution)	5 ml
Pseudomonas fluorescens	5 ml
Serratia marcescens	5 ml
Mycorrhiza (*Glomus* spp)	3 g

Statistical Analysis: Differences among treatments were determined with analysis of variance (ANOVA) using SPSS statistical package [32]. Whenever significant differences were detected, means were separated using least significant Difference test (LSD) at 5% level of significance.

RESULTS

Greenhouse Treatments: Data in table (4) infer that the tested microbial agents significantly reduced the nematode counts on roots and soil population when compared with those of the untreated check. Among treatments, the microbial treatments were not significantly different in most cases, imposing suppressive effects on rate of nematode penetration, total root population and counts of eggmasses. *P. fluorescens* and *S. marcescens* performed the highest significant reductions in nematode final populations and causing the lowest value of nematode build up. Meanwhile, biofertile achieved 43.48% reductions and 3.84 in nematode counts and build up, respectively. Although mycorrhiza impaired nematode reproduction it had lesser impact on *R. reniformis* achieving 54.7% nematode reduction. Overmatched fullback in all nematode criteria was obtained by the nematicide treatment (Perfect), however, surpassed that of all other the bioagents treatments.

In view of cowpea growth response, data in table (5) indicated variable responses due to the antagonistic effect of the tested bioproducts against the nematode. Shoot length and more or less fresh and dry weights were significantly improved when compared with the infected untreated check. *P. fluorescens* treatment showed the highest significant increase in shoot length, fresh and dry weights. *S. marcescens* improved shoot length and dry weight insignificantly but impaired fresh weight. Insignificant increase in shoot dry weight was obtained by Mycorrhiza and the rest of bioagents except Omega which resulted in 25% reduction. Roots denoted to disorder with insignificant differences among treatments.

Micro-Plot Treatments: Data presented in table (6) shows significant differences between treatments and the check in most cases. Unexpectedly, treatments of Omega, Biofertile, Biocontrol and *P. fluorescens* increased *R. reniformis* development and reproduction as indicated by the root, soil and final population comparing to nematode check. *S. marcescens* and Mycorrhiza on the contrary to that significantly inhibit all nematode criteria surpassing the others ever those achieved in pot experiment.

As for plant growth response, data in table (7) reveal that most treatments differed significantly in improving growth criteria and yield in terms of plant fresh and dry shoot weights, podding and seeds yield as compared to nematode check. Among the microbial treatments, Omega and Biocontrol recorded the best insignificant improvement in dry shoot weight, number of pods, fresh and dry pods weights and number of seeds per plant.

Field Treatments: Data presented in table (8) reveal that significant reductions were apparent in root, soil and final population of *R. reniformis* among most treatments when compared to those of the check. The microbial treatments significantly reduced root, soil, final populations and the subsequent build up. Biofertile was the uppermost in reducing nematode population among the other bioagents and treatments as well. Significant reductions were visible with the use of *S. marcescens*, *P. fluorescens* and Mycorrhiza, respectively. Percentages reduction with all bioagents increased when compared in micro-plots except *P. fluorescens* which relapsed down from 15.26% population improve in micro-plots to 48.59% reduction in field.

Table 4: Development of *R. reniformis* infecting cowpea as influenced by some commercial bioagents in a pot experiment.

Treatment	Dose/plant	*Rate of penetration	Nematode counts				Pf/Pi	% Nematode reduction
			On roots	Egg-masses/root	In soil	Final population		
Omega (*Bacillus* spp.+ *Pichia* spp.)	0.3 g	0.76 c	1526.83 c	1456.00 b	4837.56 c	6364.39 c	3.18 c	-53.13
Biofertile (Mixed bacterial solution)	5 ml	0.78 c	1564.16 c	1253.88 bc	6110.56 b	7674.72 b	3.84 b	-43.48
Biocontrol (Mixed bacterial solution)	5 ml	0.80 c	1596.20 c	1318.38 bc	3774.81 d	5371.02 de	2.69 de	-60.45
Pseudomonas fluorescens	5 ml	0.61 c	1220.72 c	957.20 c	3451.56 d	4672.28 e	2.34 e	-65.59
Serratia marcescens	5 ml	0.79 c	1574.66 c	1200.58 bc	3414.19 d	4988.85 e	2.49 e	-63.26
Mycorrhiza (vesicular arbuscular mycorrhiza)	3 g	0.99 b	1975.49 b	1522.99 b	4175.56 cd	6151.04 cd	3.08 cd	-54.70
Perfect (Oxamyl 24%+ Metalaxyl-M4%+ Tetramethrin 2%)	0.4 ml	0.10 d	190.24 d	185.34 d	74.67 e	264.91 f	0.13 f	-98.05
Check (Nematode only)		1.54 a	3089.16 a	2558.36 a	10489.63 a	13578.79 a	6.79 a	0.00

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

* Rate of penetration = Nematode counts on roots / Initial population.

% Nematode reduction = Final pop. of check – Final pop. of treatment / Final pop of check X 100.

Table 5: Growth response of cowpea plants infected with *R. reniformis* and treated, as pot soil drench, with some commercial bioagents.

Treatment	Dose/plant	Growth characters									
		Shoot						Root			
		Length (cm)	% change	Fresh weight (gm)	%change	Dry weight (gm)	% change	Length (cm)	%change	Fresh weight (gm)	% change
Omega (*Bacillus* spp.+ *Pichia* spp.)	0.3 g	36.3 b	10.0	12.2 b	-0.8	1.5 c	-25.0	20.2 bcd	-4.3	4.6 d	-31.3
Biofertile (Mixed bacterial solution)	5 ml	34.7 bc	5.1	12.9 b	4.9	2.5 ab	25.0	22.3 ab	5.7	5.0 cd	-25.4
Biocontrol (Mixed bacterial solution)	5 ml	40.3 a	22.1	12.5 b	1.6	2.3 ab	15.0	20.1 bcd	-4.7	6.2 bc	-7.5
Pseudomonas fluorescens	5 ml	42.8 a	29.7	17.0 a	38.2	2.4 ab	20.0	19.9 cd	-5.7	8.1 a	20.9
Serratia marcescens	5 ml	41.8 a	26.7	12.0 b	-2.4	2.3 ab	15.0	23.6 a	11.8	5.6 bcd	-16.4
Mycorrhiza (vesicular arbuscular mycorrhiza)	3 g	39.6 a	20.0	12.5 b	1.6	2.6 ab	30.0	22.2 abc	5.2	6.2 bc	-7.0
Perfect (Oxamyl 24%+ Metalaxyl-M4%+ Tetramethrin 2%)	0.4 ml	32.2 c	-2.4	14.1 b	14.6	2.7 a	35.0	19.0 d	-10.0	4.9 cd	-26.9
Nematode only		33.0 c	0.0	12.3 b	0.0	2.0 bc	0.0	21.1 bcd	0.0	6.7 b	0.0
Healthy plant		39.8 a	20.6	12.9 b	4.9	2.1 abc	5.0	22.0 abc	4.3	6.9 ab	2.5

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

Table 6: Development of *R. reniformis* infecting cowpea as influenced by some commercial bioagents in micro-plot experiment.

Treatment	Dose/plant	Nematode counts			% change of Pf/Pi	Final population
		Root population/2gm root	soil population/250 gm soil	Final population		
Omega (*Bacillus* spp. + *Pichia* spp.)	0.3 g	609.67 bc	3580.00 b	4189.67 b	2.09 b	38.59
Biofertile (Mixed bacterial solution)	5 ml	896.00 b	4870.00 a	5766.00 a	2.88 a	90.74
Biocontrol (Mixed bacterial solution)	5 ml	662.00 bc	3271.00 bc	3933.00 bc	1.97 bc	30.1
Pseudomonas fluorescens	5 ml	1299.50 a	2184.67 d	3484.17 cd	1.74 cd	15.26
Serratia marcescens	5 ml	396.67 cd	405.00 e	801.67 e	0.40 e	-73.48
Mycorrhiza (VAM)	3 g	680.67 bc	624.00 e	1304.67 e	0.65 e	-56.84
Nematode only		211.00 d	2812. 00 c	3023.00 d	1.51 d	0

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

Table 7: Growth response and yield of cowpea plants infected with *R. reniformis* and treated, as micro-plot soil drench, with some commercial bioagents.

Treatment	Dose/ plant	Plant parameters				Yield criteria							
		Plant fresh weight (gm)	% change	Shoot dry weight (gm)	% change	No. pods	% change	Pods fresh weight (gm)	% change	Pods dry weight (gm)	% change	No. seeds/ pods	%change
Omega (*Bacillus* spp.+ *Pichia* spp.)	0.3 g	117.00 b	626.71	13.50 b	405.62	8.00 a	627.27	21.27 a	718.08	5.67 ab	800.00	35.33 ab	467.09
Biofertile (Mixed bacterial solution)	5 ml	91.93 c	470.99	12.33 bc	361.80	4.77 b	333.64	16.53 b	535.77	4.67 bc	641.27	31.63 abc	407.70
Biocontrol (Mixed bacterial solution)	5 ml	93.70 c	481.99	12.70 b	375.66	7.23 a	557.27	18.40 ab	607.69	6.47 a	926.98	38.57 a	519.10
Pseudomonas fluorescens	5 ml	44.37 e	175.59	8.73 c	226.97	2.00 cd	81.82	5.40 cd	107.69	2.37 d	276.19	15.00 d	140.77
Serratia marcescens	5 ml	95.50 c	493.17	13.03 b	388.01	4.20 b	281.82	15.57 b	498.85	5.00 abc	693.65	28.67 bc	360.19
Mycorrhiza (vesicular arbuscular mycorrhiza)	3 g	64.77 d	302.30	10.23 bc	283.15	3.10 bc	181.82	8.53 c	228.08	3.67 cd	482.54	15.67 d	151.52
Nematode only		16.10 f	0.00	2.67 d	0.00	1.10 d	0.00	2.60 d	0.00	0.63 e	0.00	6.23 e	0.00
Healthy plant		166.97 a	937.08	35.60 a	1233.33	6.70 a	509.09	21.70 a	734.62	3.50 cd	455.56	25.10 c	302.89

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

Table 8: Development of *R. reniformis* infecting cowpea as influenced by some commercial bioagents in field experiment.

Treatment	Dose/plant	Nematode counts				
		Root population/2gm root	Soil population/ 250 gm soil	Final population	Pf/Pi	% change of Final population
Biofertile (Mixed bacterial solution)	5 ml	400.67 b	1656.00 d	2056.67 d	1.03 d	-72.69
Pseudomonas fluorescens	5 ml	224.67 b	3646.00 bc	3870.67 bc	1.94 bc	-48.59
Serratia marcescens	5 ml	406.00 b	2940.00 c	3346.00 c	1.67 c	-55.56
Mycorrhiza (vesicular arbuscular mycorrhiza)	3 g	179.00 b	4071.00 b	4250.00 b	2.12 b	-43.6
Nematode only		1281.67 a	6248.00 a	7529.67 a	3.76 a	0

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

Variability in plant growth and yield response due to bio-commercial agro-products treatment is clearly noticed in table (9). Generally, significant differences were recorded in yield more than that of growth parameters. *S. marcesens* significantly ameliorated shoot fresh weight, number of pods, fresh and dry weights of pods and number of seeds/plant; however, insignificant increase was found in dry shoot weight.

Insignificant enhancement, but disorder is some cases with Mycorrhiza and *Pseudomonas* treatments when compared with nematode check. In case of Biofertile, insignificant improve in plant fresh and dry weights. Yield criteria showed significant increase in number of pods, pods dry weight and number of seeds when compared with nematode check.

DISCUSSIONS

The inter specific antagonistic potentials of microbial agents against *R. reniformis* infecting cowpea under greenhouse conditions were variable and depend on microbial species. Numbers of total root population, eggmasses and final population were significantly different as compared to nematode check with insignificant differences among the microbial treatments. *P. fluorescens* treatment recorded the lowest numbers parasitizing the root and consequently the lowest value of build up. *S. marcescens* and Biocontrol were in the second category. Omega (*Bacillus* spp.+ *Pichia* sp.) and Mycorrhiza were the third in reducing nematode population while Biofertile had the least potentials. The nematicide, Perfect was superior over all in reducing nematode population. Our results are in harmony with those of Siddiqui and Mahmood [6], Niknam and Dhawan [14,15], Kesba [33], Jayakumar *et al.* [16, 17], Siddiqui *et al.* [34] and Montasser *et al*. [35].

Rhizobacteria affected nematodes by parasitizing, producing toxins metabolic products that suppress nematode reproduction or direct killing, enzymes or secondary metabolites, competing for nutrients and/or inducing systemic plant resistance[36, 6,10, 37, 38, 13].

Results of pot experiment showed improvement in plant growth with the increase in microbes' capability in reducing *R. reniformis* population. Yet, noticeable and variable improvement in shoot parameters was achieved by *P. fluorescens* treatment. Root parameters were disordered in most treatments. Obviously, *P. fluorescens* achieved the best results in reducing the nematode reproductivity and improving cowpea growth. Barua and Bora [39] reported significant increase in plant growth and reduction in nematode population at the higher levels of *P. fluorescens*. Similar results were stated by Patil and Sharma [19]. *Bacillus* and *Pseudomonas* species are known to be involved in inducing resistance to nematodes by reducing root galls caused by *M. incognita* on pepper and muskmelon[40, 41].

Mycorrhiza soil treatment exhibited considerable population reductions which were almost equivalent to those of the commercial product, Omega and significantly differenced from those of nematode check. Insignificant differences were noticed in growth parameters as compared to other treatments. It has been documented that *Glomus macrocarpus* inhibited *M. incognita*, *R. reniformis* and *T. semipenetrans* penetration, development and reproduction [28]. Schouteden *et al*. [42] proposed mechanisms of mycorrhizal actions include enhanced plant tolerance, direct competition for nutrients and spaces, induced systemic resistance and altered rhizosphere interactions. Our results are in accordance with those of Sharma and Mishra [43], Singh *et al*. [3], Kesba and Alsayed [28] and Herrera-Parra *et al*. [29].

In micro-plot and field experiments, the microbial agents, varied in their effectiveness against *R. reniformis*. Some were highly effective in pots but their ability was relapsed in microplots (*P. fluorescence*). Others were, however, more effective in micro-plots than pots (*S. marcescens* and Mycorrhiza). All microbial agents capabilities decreased with varied degrees in the field as compared to those of pots and micro-plots with congenital efficacies. Biofertile was rated as the best in the field as it accomplished the highest suppressive

Table 9: Growth response and yield of cowpea plants infected with *R. reniformis* and treated, as field soil drench, with some commercial bioagents.

		Plant parameters				Yield criteria							
Treatment	Dose/ plant	Plant fresh weight (gm)	% change	Shoot dry weight (gm)	% change	No. pods	% change	Pods fresh weight (gm)	% change	Pods dry weight (gm)	% change	No. seeds/ pods	% change
Biofertile (Mixed bacterial solution)	5 ml	135.67 c	-7.81	25.67 b	-16.66	4.40 c	88.84	6.87 c	11.35	4.90 c	206.25	43.77 b	188.53
Pseudomonas fluorescens	5 ml	167.30 bc	13.68	25.87 b	-16.01	2.00 d	-14.16	4.20 d	-31.93	1.10 d	-31.25	11.00 cd	-27.49
Serratia marcescens	5 ml	242.37 ab	64.69	40.87 ab	32.69	6.50 a	178.97	15.40 a	149.59	8.50 a	431.25	60.50 a	298.81
Mycorrhiza (vesicular arbuscular mycorrhiza)	3 g	186.60 bc	26.79	40.30 ab	30.84	1.00 e	-57.08	3.00 d	-51.38	1.00 d	-37.50	8.00 d	-47.26
Nematode only		147.17 c	0.00	30.80 ab	0.00	2.33 d	0.00	6.17 c	0.00	1.60 d	0.00	15.17 c	0.00
Healthy plant		315.80 a	114.58	47.10 a	52.92	5.50 b	136.05	9.67 b	56.73	7.30 b	356.25	40.00 b	163.68

In each column, values followed by the same letter(s) are not significantly different (P=0.05).

effects while, controversial results were obtained in micro-plots. The fluctuating malignant actions of microbial products in micro-plot and field impose the importance of different environmental factors that affect their activities in plant rhizosphere [44, 45]. Biofertile and *S. marcescens* could be recommended as candidates for *R. reniformis* integrated management programs in field.

Commercial compounds composed of microbes are recently available in the Egyptian market and acceptable as an alternative for nematicides but they can not stand alone as a control procedure. However, they could be introduced as supplementary elements in programs for nematode management.

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