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Control of root-knot nematodes by biological agents (Nematophagous Fungi) in field experiments

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Experiments were conducted in three commercial fields in Northern Thailand to examine the effectiveness of isolates of nematophagous fungi to increase lettuce growth parameters and manage root galls caused by *Meloidogyne incognita*. Experimental nematophagous fungi recovered from that same region included *Arthrobotrys* spp., *Pochonia* sp. and *Paecilomyces*sp. One experiment investigated application of each of the biocontrols grown on two different media (B1 and B2) to lettuce seedlings before transplantation. *Paecilomyces*sp. isolate WJI1-003 produced the highest lettuce yields representing 201% and 162% increases in shoot fresh weight combined with medium B1 or B2, respectively. Seedling applications of all fungal isolate-media combinations significantly reduced the number of galls per root and caused generally equivalent gall reduction percentages ranging from 31-73%. Another experiment examined the performance of three of the experimental biocontrols alone and in combination when applied to lettuce at planting in a commercial field fertilized chemically (Area 1) or one that was managed organically (Area 2). The biocontrols (*A.oligospora* MTI2-001, *A. conoides* API3-001, *Paecilomyces*sp. WJI1-003) generally produced plant growth increases equivalent to or greater than the commercial biocontrol and Dazomet in both Areas 1 and 2. Experimental biocontrols and their combinations, the commercial biocontrol and Dazomet produced equivalent reductions in galls per root and very dramatic gall reduction percentages ranging from 64-99% in both areas. Nematophagous fungi isolates *A.oligospora* MTI2-001, *A. conoides* API3-001 and*Paecilomyces*sp. WJI1-003 consistently reduced root galls caused by *Meloidogyne incognita* in three commercial fields.

Keywords: Root-knot nematodes, *Meloidogyne incognita*, biological control, nematophagous fungi, vegetables

Introduction

Meloidogyne root-knot nematodes are worm-like animals. They have a wide host range, and cause problems in many annual and perennial crops. Affected plants have an unthrifty appearance and often show symptoms of

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stunting, wilting or yellowing. Control strategies for these nematodes should be based on density reduction in soil through sustainable and eco-friendly approaches. However, biological control including improvement of bio-agent establishment is a current challenge.

Scientists have evaluated using competent nematophagous fungi for controlling many plant parasitic nematodes for decades (Nordbring-Hertz*et al*., 2012). The majority of the principal research has investigated culture and sporulation techniques including their appropriate application to decrease the population of nematodes as well as to permit fungal establishment in the soil complex. In addition, field application, formulation for delivery, the most appropriate farm management practices to enhance biological control and the education of farmers on the use of the technology have also been addressed (Cook, 1994). Nematophagous fungi parasitize and kill nematodes through the balance of nature and buffering capacity of soil biodiversity (Sobita&Anamika, 2011). Furthermore, the capturing efficiency of predacious fungi may be influenced by the environmental condition and nature of the soil (Jaffee *et al*., 2001). Some nematophagous fungi such as *Arthobotrysoligospora*, *Pochoniachlamydosporia*, *P. rubescens*, *Nematoctonusrobustus* and *Drechslerelladactyloides* have the potential to multiply rapidly and colonize the rhizospere and plant roots as a probable survival strategy. These fungi probably induce plant defense reactions and cause a higher chance to parasitize nematodes and to decrease their succeeding spread and root infection (Jean&Kishan, 2011). Kumar and Singh (2011) studied the effect of *Dactylariabrochopaga* (isolate D) on the management of wheat root-knot disease. Their results showed that applying a mass culture (10 g/pot) and a spore suspension of the fungus with and without cow dung manure to soil infested with 2,000 *Meloidogynegraminicola*juveniles per pot significantly improved plant height, root length, weights of shoots, roots, panicles and grains per hill compared to the control. Furthermore, the fungus significantly reduced the number of root-knots, the number of egg masses, juveniles, and females per hill compared to those in the control. Bio-efficacy of the fungus was increased when the mass culture and a spore suspension were used in combination with cow dung manure to improve the plant growth parameters and reduce the number of root-knot and reproductive factors. The objective of this research was to evaluate the capability of competent nematophagous fungi for controlling root-knot nematodes in the field by means of various application methods and fungal combinations.

Materials and methods

Experimental design

Experiment I. The ability of nematophagous fungi-amended seedling application against root-knot of lettuce caused by M. incognita in the field

The disease level of the root-knot-infested area of lettuce plantation at Mae Sapok Royal Project Foundation, Chiang Mai province was observed and assessed. A selected area was harrowed and divided into five plots.

Eight isolates of nematophagous fungi were applied to seedlings whichwere transplanted in the field which had a high density of *M. incognita* root-knot (1,000 J2 per 1,000 g moist soil). Before transplanting, 200 g of cow dung manure were ground and appliedper head lettuce seedling.

This experiment was divided into eighteen treatments comprised of two seedling media (B1 and B2) and eight nematophagous fungi and controls amended with each of the media alone (A1-A9) (2 × 9 factorial experiment). Ten seedlings of each treatment were planted as subsamples into five plots (replications) using a Randomized Complete Block Design (RCBD).

Ten plants of each treatment were used to measure plant height, root length, fresh weight of shoots, dry weight of shoots, number of galls per plant and percentage of gall reduction 60 days after transplantation (Hassan, 2010). Treatment means were separated by using Duncan's Multiple Range Test (DMRT) following Analysis of Variance (ANOVA).

Experiment II. Efficiency of bio-formulations of nematophagous fungi applied in the field against root-knot of lettuce caused by M. incognita

Two other root-knot-infested areas of the lettuce plantation at Mae Sapok Royal Project Foundation (Area 1 – chemically fertilized and Area 2 – organically managed) were harrowed and divided into five plots. Three competent nematophagous fungi; *Arthrobotrysoligospora* isolate MTI2-001 (formulation 1), *Arthrobotrysconoides* isolate API3-001 (formulation 2) and *Paecilomyces*sp. isolate WJI1-003 (formulation 3) were selected to study because of their high promotion of plant growth and reduction of root knot nematode gall number per plant identified in previous studies.

Sporulation of each fungus was multiplied to a concentration of $\times 10^6$cfu/g using previously developed procedures (Mensin, 2006). Two hundred grams of the ground, completed mixture were applied to each head lettuce seedling before transplantation.

This experiment was divided into ten treatments consisting of seven nematophagous formulations (isolates A1, A2 and A3; alone and in combination), fumigation with dazomet (2,450 ppm a.i.), a commercial bio-pesticide (*Paecilomyces*) and a non-treated control. The experiment used a RCBD design with five replications (plots) per treatment. Ten head lettuce seedlings of each treatment were served as subsamples in each plot.

Ten plants of each treatment were used to measure plant height, root length, fresh weight of shoots, dry weight of shoots and number of galls per plant (Hassan, 2010). At the end of harvest 60 days after transplantation severity of root galling in the lettuce plants was assessed using a 0–5 rating scale according to the percentage of galled tissue, in which 0=0–10% of galled roots; 1=11–20%; 2=21–50%;3=51–80%; 4=81–90%; and 5=91–100% (Barker, 1985). Treatment means were separated by using DMRT following ANOVA.

Results and discussions

Experiment I

Analysis of variance of two factorial treatment effects and interaction of different nematophagous fungi-amended seedling applications comparing two seedling media at 60 days after transplantation in a root-knot nematode-infested area on head lettuce growth including the number of galls per root were significantly different at P=0.01.

Isolate A1 (*A.oligospora* DLO1-001) combined with medium B1 significantly decreased plant height (Table 1) compared to the control amended with the medium alone; combining B1 with the other isolates did not appear to significantly affect plant height. The combination of each isolate with medium B2 did not significantly affect plant height. On the other hand, the combination of each isolate with medium B1 significantly decreased root length, while root length was not significantly affected by combining the each isolate with medium B2 except in the case of isolate A2 (*A.oligospora* MTI2-001) where a significant increase in root length was observed. These reductions in root length by medium B1 did not appear to affect other plant growth parameters or the root knot nematode management efficacy of the fungal isolates.

Table 1. Effect of nematophagous fungi-amended seedling application evaluated two seedling media on head lettuce growth at 60 days after transplantation in a root-knot nematode-infested area

Treatment[4/]		Measurement[1/]					
		Plant height (cm)	Root length (cm)	Fresh weight of shoots (g)	Dry weight ofshoots (g)	No. galls per plant[5/]	Gall reduction (%)[6/]
B 1	A1	13.68 D[2/]	9.35 E	149.89 C	9.19 BC	24.60 BC	39.11 BC
	A2	14.25 CD	9.30 E	104.82 F-H	6.38 E-G	13.60 BC	66.33 AB
	A3	15.14 B-D	9.15 E	78.68 I	5.08 H-J	20.70 BC	48.76 A-C
	A4	16.07 A-C	8.80 E	94.69 HI	4.62 IJ	24.70 BC	38.86 BC
	A5	14.64 B-D	8.15 E	79.90 I	4.15 J	21.10 BC	47.77 A-C
	A6	16.10 A-C	8.60 E	75.35 I	4.83 IJ	25.40 BC	37.12 BC
	A7	17.50 A	8.35 E	233.18 A	10.91 A	13.50 BC	66.58 AB
	A8	16.50 AB	8.10 E	130.17 C-E	8.06 CD	14.50 BC	64.10 AB
	A9	15.85 A-C	15.00 CD	115.63 D-H	5.67 F-I	40.40 A	-
B 2	A1	9.17 E	15.35 B-D	132.25 C-E	8.11 CD	20.80 BC	48.51 A-C
	A2	9.05 E	17.80 A	107.71 F-H	6.78 E-G	18.90 BC	53.21 A-C
	A3	8.30 E	15.95 B-D	112.67 E-H	6.37 EF	11.60 C	71.28 A
	A4	8.60 E	16.70 AB	100.88 GH	5.05 H-J	16.70 BC	58.66 A-C
	A5	8.00 E	15.65 B-D	124.96 D-F	6.34 E-G	17.10 BC	57.67 A-C
	A6	8.85 E	15.01 CD	120.45 D-G	6.18 E-H	27.00 B	33.16 C
	A7	9.03 E	14.65 D	182.93 B	9.70 B	24.20 BC	40.09 BC
	A8	9.50 E	16.20 BC	136.31 CD	7.21 DE	16.50 BC	59.15 A-C
	A9	15.65 A-C	15.40 B-D	112.38 E-H	5.47 G-I	40.40 A	-
	CV %[3/]	11.55	8.80	13.44	14.38	46.63	42.78

[1/]Mean of each treatment calculated from ten replications.
[2/]Means followed by the same letter are not significantly different by DMRT.
[3/]CV% = coefficient of variation 99%.
[4/]A1=*A.oligospora* isolate DLO1-001A2=*A.oligospora* isolate MTI2-001 A3=*A. conoides* isolate API3-001 A4=*A.thaumasium* isolate JDI1-001 A5=*A.thaumasium* isolate MPI1-003 A6=*A. musiformis* isolate MSO1-001 A7=*Paecilomyces*sp. isolate WJI1-003 A8=*Pochonia* sp. isolate KJO1-003 A9=Non-treated control and B1=Medium 1 B2=Medium2.
[5/]No. galls per plant counted from root system of each replication and averaged No. galls per each treatment (Hassan, 2010).
[6/]Gall reduction (%) calculated from No. galls per root of each treatment compare with averaged No. galls per root of non-treated control treatment.

Fresh weight of shoots was significantly increased by A1 (*A.oligospora* DLO1-001) and A7 (*Paecilomyces*sp. isolate WJI1-003) combined with medium B1 and by A1, A7 and A8 (*Pochonia*sp.isolate KJO1-003) combined with medium B2. Fresh weight was significantly decreased by combining isolates A3 (*A. conoides* API3-001), A5 (*A. thaumasium* MPI1-003) and A6 (*A. musiformis* MSO1-001) with medium B1. The other isolate-media combinations did not appear to affect shoot fresh weight.

Very similar results were obtained with dry weight of shoots. Shoot dry weight was significantly increased by A1 (*A.oligospora* DLO1-001), A7 (*Paecilomyces*sp. isolate WJI1-003) and A8 (*Pochonia*sp.isolate KJO1-003) combined with each medium. Dry weight was significantly decreased by the A5

(*A. thaumasium* MPI1-003) mixed with B1 and apparently unaffected by the other isolate-media combinations. Overall, *Paecilomyces*sp. isolate WJI1-003 produced the highest lettuce yields representing 201% and 162% increases in shoot fresh weight when combined with medium B1 or B2, respectively.

All fungal isolate-media combinations significantly reduced the number of galls per plant and caused generally equivalent gall reduction percentages ranging from 31-73%. Coefficients of variation, 46% and 43% for number of galls per plant and gall reduction %, respectively, were slightly higher the generally accepted upper limit (33%) possibly because of non-uniform distribution of the nematodes at this site. The performance of *Paecilomyces*sp.in this research is consistent with that reported by Bordallo *et al*., 2002, Dhawan *et al*., 2004, Thakur & Devi, 2007, Diogo*et al*., 2009, Brand *et al*., 2010. These results are encouraging and should motivate the larger-scale grower trials of the most promising of the isolates such as *A.oligospora* isolate DLO1-001, *Paecilomyces*sp. isolate WJI1-003 and*Pochonia* sp. isolate KJO1-003.

Experiment II

Combining experimental biocontrols did not appear to enhance their effectiveness in increasing plant growth or root gall reduction. All experimental treatments as well as the commercial *Paecilomyces* product and Dazomet resulted in equivalent increases in plant height compared with the non-treated control in Area 1 (chemically fertilized) and by all treatments except 1 + 2 (*A.oligospora* MTI2-001 + *A. conoides* API3-001) and the commercial biocontrol in Area 2 (organic production) (Tables 2 and 3). The experimental biocontrols generally produced height increases equivalent to or greater than the commercial biocontrol and Dazomet in both areas.

Table 2. Effect of various fungal biomass formulations on head lettuce growth at 60 days after transplantation in a root-knot nematode-infested area (Area 1 – chemical fertilization)

Treatment[4/]	Measurement[1/]						
	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Gall per plant (gall)[5/]	Scale of total root system galled[6/]	% gall reduction[7/]
No. 1	21.00 AB-[2/]	10.05 A-C	229.00 CD	7.74 BC	3.50 C	0	92.69 A
No. 2	20.80 A-C	11.80 A	378.00 A	11.49 A	8.70 BC	0	81.83 AB
No. 3	18.60 E	11.70 A	323.00 AB	11.79 A	3.00 C	0	93.73 A
No. 1 + 2	19.50 DE	9.20 BC	174.00 DE	9.95 AB	1.90 C	0	96.03 A
No. 1 + 3	21.10 AB	11.45 AB	379.00 A	11.00 A	17.30 B	0	63.88 B
No. 2 + 3	20.00 B-D	10.20 A-C	214.00 CD	9.04 AB	0.40 C	0	99.16 A
No. 1 + 2 + 3	19.80 CD	10.30 A-C	218.00 CD	7.53 BC	0.30 C	0	99.37 A
Commercial *Paecilomyces*.	21.00 AB	11.80 A	228.00 CD	9.42 AB	7.10 BC	0	85.17 A
Dazomet	21.90 A	9.95 A-C	319.00 AB	8.95 AB	3.10 C	0	93.52 A
Non-treated control	16.80 F	8.30 C	133.00 E	5.75 C	47.90 A	2	-
CV %[3/]	4.71	17.38	22.73	23.97	90.96		18.44

[1/]Mean of each treatment calculated from ten replications.
[2/]Means followed by the same letter are not significantly different by DMRT.
[3/]CV% = coefficient of variation 99%.
[4/]*A.oligospora*isolate MTI2-001 (formulation 1), *A. conoides* isolate API3-001 (formulation 2) and *Paecilomyces*sp. isolate WJI1-003 (formulation 3).
[5/]No. galls per plant counted from root system of each replication (plant) and averaged No. galls per each treatment (Hassan, 2010).
[6/]Barker's 0-5 root knot nematode gall rating scale was used (Barker, 1985).
[7/]Gall reduction (%) calculated from No. galls per root of each treatment compare with averaged No. galls per root of non-treated control treatment.

Table 3. Effect of various fungal biomass formulations on head lettuce growth at 60 days after transplantation in a root-knot nematode-infested area (Area 2 – organic production)

Treatment[4/]	Measurement[1/]						
	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Gall per plant (gall)[5/]	Scale of total root system galled[6/]	% gall reduction[7/]
No. 1	21.70 AB[2/]	11.15 AB	94.60 D	4.51 DF	5.90 B	0	85.94 A
No. 2	21.80 AB	10.30 A-D	177.10 AB	5.81 CD	4.60 B	0	89.05 A
No. 3	22.20 AB	9.45 B-D	152.00 BC	9.07 AB	6.70 B	0	84.04 A
No. 1 + 2	19.60 D	11.10 AB	126.80 B-D	7.70 A-C	5.40 B	0	87.14 A
No. 1 + 3	22.40 A	9.00 D	209.90 A	7.09 B-D	4.40 B	0	89.52 A
No. 2 + 3	21.30 A-C	9.60 B-D	102.20 CD	5.20 C-E	3.00 B	0	92.85 A
No. 1 + 2 + 3	21.00 BC	11.70 A	132.40 B-D	10.36 A	9.70 B	0	76.90 A
Commercial *Paecilomyces*	20.20 CD	10.25 A-D	166.40 AB	5.89 CD	5.89 B	0	86.19 A
Dazomet	22.20 AB	9.10 D	135.50 B-D	7.37 B-D	3.60 B	0	91.43 A
Non-treated control	19.60 D	9.25 CD	31.10 E	2.84 E	42.00 A	2	-
CV %[3/]	4.95	14.18	29.58	35.18	77.00		16.92

[1/]Mean of each treatment calculated from ten replications
[2/]Means followed by the same letter are not significantly different by DMRT
[3/]CV% = coefficient of variation 99%.
[4/]*A.oligospora*isolate MTI2-001 (formulation 1), *A. conoides* isolate API3-001 (formulation 2) and *Paecilomyces*sp. isolate WJI1-003 (formulation 3)
[5/]No. galls per plant counted from root system of each replication (plant) and averaged No. galls per each treatment (Hassan, 2010).
[6/]Barker's 0-5 root knot nematode gall rating scale was used (Barker, 1985).
[7/]Gall reduction (%) calculated from No. galls per root of each treatment compare with averaged No. galls per root of non-treated control treatment.

Root length was significantly increased by treatments 2 (*A. conoides* isolate API3-001), 3 (*Paecilomyces*sp. WJI1-003) and 1 (*A.oligospora* isolate MTI2-001 (formulation 1) + 3 and the commercial bio-control in Area 1 and by treatments 1, 1 + 2, and 1 + 2 + 3 in Area 2. Fresh weight of shoots was significantly increased to varying degrees by all treatments in Area 1 except 1 + 2 and all treatments in Area 2. The experimental biocontrols generally equaled or surpassed both commercial products in fresh weight production. Dry weight increases generally followed the same pattern.

As in experiment I, all experimental biocontrol treatments, as well as the commercial biocontrol and Dazomet significantly reduced the number of galls per plant and caused very dramatic gall reduction percentages ranging from 64-99% in both areas in experiment II. High coefficients of variation, 90% and 77% in Area 1 and 2, respectively, for the number of galls per plant would seem to make these findings less reliable. Root knot and other nematodes are often non-uniformly distributed in fields and are generally aggregated (Ferris,

1985; Noling, 2012). This non-uniform distribution may lead to high data variability in field research on this pathogen. The number of replications and/or subsamples used in these experiments may have been inadequate to overcome root knot data variability. Nevertheless, it is quite noteworthy that fungal isolates *A.oligospora* MTI2-001, *A. conoides* API3-001 and*Paecilomyces*sp.WJI1-003 consistently reduced root galls caused by *Meloidogyne incognita* in three commercial fields.

Our findings were similar to those of Niranjan and Singh (2011) who indicated that the application of a mass culture and a spore suspension of *Dactylariabrochopaga* with and without cow dung manure to soil infested with *M. graminicola* juveniles (root-knot disease of wheat) significantly improved plant height, root length, weights of shoots, roots, panicles and grains per hill compared to those in the control. Moreover, the fungus significantly reduced the number of root-knots, the number of egg masses, juveniles, and females per hill compared to the control.

Conclusion

This research demonstrated the effectiveness of nematophagous fungi recovered from Northern Thailand in increasing yield and reducing damage from the root knot nematode *Meloidogyne incognita* in lettuce grown in that region. Consistently effective biocontrols identified included *Arthrobotrysconoides*isolate API3-001, *A.oligospora* isolate DLO1-001, *Pochonia* sp.isolate KJO1-003 and *Paecilomyces*sp. isolate WJI1-003. Following confirmation of these results by large-scale field trials, these isolates may provide growers with alternatives to conventional nematicides.

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