CHAPTER 7

Control of Root-knot Nematodes by Biological Agent (Nematophagous Fungi) in Greenhouse and Field Experiments

7.1 Introduction

Scientists have been using competent nematophagous fungi for controlling many plant parasitic nematodes and promoting environmental friendly achievement. The principal research has observed the growth and sporulation culture techniques including their appropriate application to decrease the population of nematode as well as to permit a fungal establishment in the complex soil. In addition, the field application, the formulation for delivery, the most appropriate farm management practices to enhance biological control and the education of farmers on the use of the technology were also addressed (Cook, 1994).

Many protocols accomplished induction of fungi by identifying desirous nutrients, signal of increasing antagonistic population (host) and sporulation factors that led to the production of virulent, long-lasting conidia composed of the fungal parasitic stage. The parasitic nematophagous fungi parasitized and killed nematodes. This occurred through the balance of nature and buffering capacity of soil biodiversity (Sobita & Anamika, 2011). Nevertheless, the capturing efficiency of the predacious fungi may be influenced by the environmental condition and nature of the soil (Jaffee *et al*., 2001).Some nematophagous fungi such as *Arthobotrys oligospora*, *Pochonia chlamydosporia*, *P. rubescens*, *Nematoctonus robustus* and *Drechslerella dactyloides* have the potential to multiply rapidly and colonized the rhizospere and plant root as a

probable survival strategy. These probably induce plant defense reactions and causing higher chance to parasitize nematodes and to decrease their succeeding spread and root infection (Jean & Kishan, 2011). Moreover, many records showed this fungal group implicated with plant growth factors as *Pochonia chlamydosporia* (Pc123) perhaps managing of root-knot nematode infestations and promoting growth of both roots and shoots of tomato plants compared with non-inoculated (control) plants (Escudero & Lopez-Llorca, 2012).

Kumar & Singh (2011a) studied the effect of *Dactylaria brochopaga* (isolate D) on the management of wheat root-knot disease. The results showed 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 *Meloidogyne graminicola* 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 heightened 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.

In this study lettuce was selected as indicator plant of *Meloidogyne* root galling instead of tomatoes which takes 90 days to obtain results. Lettuce galling increased dramatically after 42 days, with 70-80% galling being reached on the 45th day. These results confirm that lettuce can function as a rapid indicator of nematode galling and will shorten the screening time from 90 days to 45 days (Laura, 2003).

The objectives of this chapter were as follows:

1. To evaluate the efficiency of competent nematophagous fungi to enhance seedling growth and decrease root-knot nematode-infected plants in greenhouse.

2. To compare the capability of competent nematophagous fungi for controlling root-knot nematodes in field.

7.2 Materials and methods

7.2.1 The effect of nematophagous fungi on plant growth of lettuce seedling period under greenhouse conditions

Biomass culture of each nematophagous fungus (8 isolates) included *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 was inoculated to crushed corn grain, an optimal solid substrate media selecting from previous experiments (Chapter 6) and incubated in supporting conidial production state.

Two seedling media were selected to examine in this experiment. Medium 1(rich nutrient) was composed of peat moss. Medium 2 (poor nutrient) was composed of well decomposed cow dung manure and coconut husk at ratio 1:1. Five percent of the completed medium weight (g) was fungal biomass. The biomass and medium were amended together by hand before transferring the mixture to a tray and planting one head lettuce seed per a hole. The conidial concentration of each fungus in the mixture was $\times 10^6$ cfu/g.

The study of nematophagous fungi-amended seedling on plant growth of head lettuce seedling period under greenhouse conditions was divided into eighteen treatments referring eight nematophagous fungi and two seedling media. Three seedling trays of each treatment served as replications. The medium without amendment of fungal biomass served as the control. The experiment was done by two factors factorial in Randomized Completely Block Design (RCBD). Factor A epresented isolates of nematophagus fungi, A1 = *A. oligospora* isolate DLO1-001, A2 = *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 Factor B represented seedling media where B1 = formulation 1 and B2 = formulation 2.

Mass Preparation of nematophagous fungi on crushed corn grain media:

One hundred twenty-five gram of boiled corn grains were contained in polypropylene bags, sealed with a bottle neck and cotton. They were sterilized by autoclaving at 15 pound/inch^2 at 121^{o}C for 25 minutes. Fungal agar plug of each fungus was transferred to the sterilized corn grain. It was incubated at 30^{o}C, 24 hour light (inducing growth period) following 12 hour light (sporulation) for 14 days.

Data collection: Emergence percentage of all lettuce seedlings was observed at 7 and 14 days after planting. Ten seedlings of each treatment were randomized to measure the plant height, root length, fresh weight of shoot and dry weight of shoot 30 days after planting.

Statistical analysis: The data were analyzed by analysis of variance (ANOVA) “Two factors factorial in Randomized Completely Block Design (RCBD) by 9×2 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

7.2.2 The ability of nematophagous fungi-amended seedling application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

Nematophagous fungi-amended seedling application; *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003, from 7.2.1 experiment was continually observed for their capacity to reduce root galled disease of *Meloidogyne incognita*. Head lettuce seedlings of each treatment were planted in root-knot infested soil which contained in 5-inch-diameter plastic pots. One hundred of second stage juveniles (J2) of *M. incognita* per 1,000 g moisture soil were used for inoculums. The well amended inoculums of root-knot infected soil collecting from lettuce plantation at Mae Sapok Royal Project Foundation, Chiang Mai province, Thailand was estimated the density of J2 *M. incognita* in soil applying Xing & Westphal (2012) and amended to achieve the required final population.

Measuring the density of J2 root-knot nematode in soil:

Species of root-knot nematode on galled roots of lettuce was identified to confirm *Meloidogyne incognita*. The root-knot infested soil were collected and

amended before measuring the population of J2 *M. incognita*. One thousand gram of root-knot infested soil was mixed with water. The suspension of soil was poured through 100, 200 and 400 mesh sieves over a container. The material retained on 100 and 200 mesh sieve was discarded while that retained on 400 mesh sieves was washed two times and collected in a beaker. To measure the population of root-knot nematodes, the Baermann funnel method was operated followed by numerated the present of J2 *M. incognita*.

This experiment was divided into eighteen treatments referring two seedling media and eight nematophagous fungi. The treatments of each seedling medium without nematophagous fungi were used as the control. Ten pots of each treatment were used as replicates. The experiment was done by two factors factorial in Randomized Completely Block Design (RCBD). Factor A represented isolates of nematophagus fungi, A1 = *A. oligospora* isolate DLO1-001, A2 = *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 Factor B represented seedling media where B1 = formulation 1 and B2 = formulation 2.

Data collection: After 60 days transplantation, ten pots were measured for plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant and percentage of galled reduction.

Statistical analysis:

The data were analyzed by analysis of variance (ANOVA) “Two factors factorial in Randomized Completely Block Design (RCBD) by 9×2 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

7.2.3 The ability of biological agent (nematophagous fungi) application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

The biological agent of eight nematophagous fungi; *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003, was tested to observed the efficiency of root-knot disease reduction on lettuce. Each biological agent was amended with well decomposed cow dung manure (CDM). Five percent of a completed mixture weight calling biological fungicide was fungal biomass culture growing on crushed corn grain media, and then it was uniformly amended in root-knot infested soil before filled in the pots (5 inch diameter of bottom size). Each pot was contained 500 grams of amended soil. The conidial concentration of each fungus in amended soil was $\times 10^6$ cfu/g. Root-knot infested soil having approximately 1,000 second stage juveniles (J2) of *Meloidogyne incognita* per 1,000 g moist soil was used for this experiment. Preparation of them was described as above-mentioned in “Measuring the density of J2 root-knot nematode in soil”.

This experiment was divided into eighteen treatments referring biological agent of eight nematophagous fungi and compared into two applications of fermented

method by incubating the treatment pots in moisture condition and without fermented method of biological fungicides before 7 days transplantation. Ten pots of each treatment were used as replicates. The treatments of CDM with fermented method and CDM without fermented method were used as control. In addition, head lettuce and baby cos lettuce were selected in this study. The experiment was done by two factors factorial in Randomized Completely Block Design (RCBD). Factor A represented isolates of nematophagus fungi, A1 = *A. oligospora* isolate DLO1-001, A2 = *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 Factor B represented method applications where B1 = fermented method and B2 = without fermented method.

Data collection: Ten plants of each treatment were used to measure the plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant and percentage of galled reduction after 60 days transplantation.

Statistical analysis: The data were analyzed by analysis of variance (ANOVA) “Two factors factorial in Randomized Completely Block Design (RCBD) by 9×2 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

7.2.4 The ability of nematophagous fungi-amended seedling application against root-knot of lettuce caused by *Meloidogyne 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, Thailand was observed and assessed. A selected area was harrowed and divided into five plots.

Nematophagous fungi-amended seedling application; *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003, from 7.2.1 experiment were transplanted in the field with a high density of *Meloidogyne incognita* root-knot; 1,000 J2 per 1,000 g moist soil. Before planting, 200 g of cow dung manure were grounded per head lettuce seedling.

This experiment was divided into eighteen treatments referring two seedling media (medium 1 and medium 2) and eight nematophagous fungi. The treatments of each seedling medium without nematophagous fungi were used as the control. Ten seedlings of each treatment were planted as a replication into five plots. The experiment was done by two factors factorial in Randomized Completely Block Design (RCBD). Factor A represented isolates of nematophagus fungi, A1 = *A. oligospora* isolate DLO1-001, A2 = *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 Factor B represented seedling media where B1 = formulation 1 and B2 = formulation 2.

Data collection: Ten plants of each treatment were used to measure the plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant and percentage of galled reduction after 60 days transplantation.

Statistical analysis: The data were analyzed by analysis of variance (ANOVA) "Two factors factorial in Randomized Completely Block Design (RCBD) by 9×2 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

7.2.5 The efficiency of bio-formulations of nematophagous fungi against root-knot of lettuce caused by *Meloidogyne incognita* in the field

Application of bio-formulations of selected nematophagous fungi was varied and compared on promotion of plant growth and reduction of root-knot gall disease. Another root-knot infested area of lettuce plantation at Mae Sapok Royal Project Foundation was harrowed and divided into five plots. Three competent nematophagous fungi; *Arthrobotrys oligospora* isolate MTI2-001 (formulation 1), *Arthrobotrys conoides* 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 reducing a number of galls per plant, resulting from 7.2.1-7.2.4 experiments.

Sporulation of each fungus was multiplied to a concentration of $\times 10^6$ cfu/g by the multiplied ingredient (kg) consisted of 40% well decomposed cow dung manure (CDM), 30% paddy husk, 20% powder of ash and 5% corn bran. The mixture was incubated in 75% RH, and obscure light at 21 days and mixed thoroughly (Mensin, 2006). The usage of the completed mixture, calling biological fungicide was 200

grams per head lettuce seedlings and was grounded before transplantation of head lettuce.

This experiment was divided into ten treatments referring application of bio-fungicide formulations. Fumigation with dazomet (Basamid-G 98 % GR®, 2,450 ppm a.i.; chemical control), *Paecilomyces* commercial bio-pesticide and non-treated soil were used as controls. Ten head lettuce seedlings of each treatment were served as a replication in each plot. In addition, data were also obtained from two areas which were applied chemical fertilizer (Area 1) and without chemical fertilizer (Area 2) during planted period. The treatments of each area were as follows:

T1 Bio-fungicide formulation 1

T2 Bio-fungicide formulation 2

T3 Bio-fungicide formulation 3

T4 *Paecilomyces* commercial bio-fungicide (control)

T5 Bio-fungicide formulation 1 amended formulation 2 ratio 1:1

T6 Bio-fungicide formulation 1 amended formulation 3 ratio 1:1

T7 Bio-fungicide formulation 2 amended formulation 3 ratio 1:1

T8 Bio-fungicide formulation 1 amended formulation 2 and formulation 3 ratio 1:1:1

T9 Soil fumigation with dazomet (control)

T10 Non-treated control

Data collection: Ten plants of each treatment were used to measure. The data of plant height, root length, fresh weight of shoot, dry weight of shoot, number of galls per plant on a scale of 0 - 100 (Barker, 1985) by counting and percentage of

galled reduction were recorded at the end of harvest, 60 days after transplantation (Hassan, 2010).

Statistical analysis: The data were analyzed by analysis of variance (ANOVA) “Randomized Completely Block Design (RCBD)” and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

Percentage of total root system galled	**Score**
0 - 10	0
20 - 30	1
40 - 60	2
70 - 80	3
90 - 100	4

Source: Barker's galling index (1985).

7.3 Results

7.3.1 The effect of nematophagous fungi on plant growth of lettuce seedling period under greenhouse conditions

Analysis of variance by two factorial treatment effects and interaction of fungal biomass to seedling emergence indicated that all factors including fungal biomass, seedling medium types and their interaction affected head lettuce seedling emergence were highly significant. The statistical analysis data are displayed in Tables 40-41 of appendix.

The results of percentage of seedling emergence after amended fungal biomass and medium, 7 days after planting seed, showed the lowest seedling emergent (46.00%) on biomass of *Arthrobotrys musiformis* isolate MSO1-001 amended with Medium 2 followed by biomass of *Arthrobotrys thaumasium* isolate JDI1-001 amended with Medium 2 (54.00%) and biomass of *Arthrobotrys oligospora* isolate DLO1-001 amended with Medium 2 (56.67%), respectively. On the other hand, biomass of *Arthrobotrys conoides* isolate API3-001 amended with Medium 2 was the highest (95.33%) followed by non-treated control Medium 1 (92.00%).

This result also indicated that mixing of fungal biomass culture with Medium 1 may have retarded the seedling emergence in earliest stage (7 days). However 14 days after planting, most fungal biomasses amended medium had more than 90% of seedling emergence except biomass of *Arthrobotrys thaumasium* isolate MPI1-003 amended with Medium 1 and biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 2 which had 82.66% and 89.33% (Table7.3).

Table 7.1 The effect of nematophagous fungi amended media to seedling emergent at 7 and 14 days after planting

Fungal isolate	Percentage of head lettuce seedling emergence (%)[1/]			
	7 days		14 days	
	Medium 1	Medium 2	Medium 1	Medium 2
A. oligospora isolate DLO1-001	88.66 a[2/]	56.66 f	90.66 de	94.66 a-d
A. oligospora isolate MTI2-001	80.66 b	74.66 bc	95.33 a-c	91.33 c-e
A. conoides isolate API3-001	60.33 ef	95.33 a	96.00 ab	96.33 ab
A. thaumasium isolate JDI1-001	73.00 c	54.00 f	97.66 a	94.33 a-d
A. thaumasium isolate MPI1-003	66.00 de	88.66 a	82.66 f	95.00 a-c
A. musiformis isolate MSO1-001	88.66 a	46.00 g	93.00 b-e	93.33 b-d
Paecilomyces sp. isolate WJI1-003	89.00 a	68.00 cd	93.00 b-e	89.33 e
Pochonia sp. isolate KJO1-003	89.33 a	80.00 b	94.33 a-d	96.00 ab
Non-treated control	92.00 a	58.00 f	92.66 b-e	96.66 ab
CV %[3/]	3.94		1.69	

[1/] Mean of each treatment calculated from three replications
[2/] Means followed by the same letter are not significantly different by DMRT at P=0.01.
[3/] CV% = coefficient of variation 99%.

Analysis of variance by two factorial treatment effects and interaction of fungal biomass to head lettuce seedling growth indicated that all factors including fungal biomass, seedling medium types and their interaction affected seedling height, root length, fresh weight of shoot and dry weight of shoot at a high significance level (P=0.01). Application of Medium 2 to amend with fungal biomass was more effective than Medium 1. The statistical analysis data are displayed in Tables 42-45 of appendix.

Head lettuce seedlings had greatest height (15.20 cm) by growing on non-treated control Medium 1 followed by biomass of *Pochonia* sp. isolate KJO1-003 amended with Medium 1(13.07 cm) and biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 1(12.84 cm). Biomass of *Arthrobotrys conoides* isolate API3-001 amended with Medium 1 supported the greatest root length followed by biomass of *A. conoides* isolate API3-001 amended with Medium 2; the root length were 14.51 cm and 11.49 cm, respectively which were significantly different by DMRT (P=0.01). In addition, biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 2 had the significantly greatest fresh weight of shoots (2.50 g) whereas the greatest dry weight of shoots were observed on biomass of *Paecilomyces* sp. isolate WJI1-003 amended with both media (0.21 g). The data are displayed in Table 7.2 and Figures 7.1 and 7.2.

Table 7.2 Effect of fungal biomass amended with medium on head lettuce seedling grow that 30 days after planting

Treatment		Measurement[1/]			
		Plant height (cm)	**Root length (cm)**	**Fresh weight of shoot (g)**	**Dry weight of shoot (g)**
Medium 1	*A. oligospora* isolate DLO1-001	11.46 c[2/]	6.83 h	0.41 l	0.07 gh
	A. oligospora isolate MTI2-001	11.50 c	11.12 b-d	0.46 l	0.11 d-f
	A. conoides isolate API3-001	8.57 d	14.51 a	0.19 m	0.04 h
	A. thaumasium isolate JDI1-001	12.84 b	6.97 h	0.59 k	0.18 ab
	A. thaumasium isolate MPI1-003	10.91 c	11.38 bc	0.22 m	0.08 f-h
	A. musiformis isolate MSO1-001	11.19 c	9.86 e-g	0.69 ij	0.17 bc
	Paecilomyces sp. isolate WJI1-003	12.84 b	9.17 g	0.95 gh	0.21 a
	Pochonia sp. isolate KJO1-003	13.07 b	10.16 d-f	0.87 h	0.16 b-d
	Non-treated control	15.20 a	6.36 h	0.61 jk	0.09 e-g
Medium 2	*A. oligospora* isolate DLO1-001	9.45 d	9.45 fg	0.75 i	0.11 d-f
	A. oligospora isolate MTI2-001	11.36 c	11.36 bc	1.16 f	0.13 c-f
	A. conoides isolate API3-001	11.49 c	11.49 b	1.49 d	0.14 b-d
	A. thaumasium isolate JDI1-001	9.25 d	9.25 fg	1.68 c	0.17 bc
	A. thaumasium isolate MPI1-003	10.48 c	10.48 c-e	1.26 e	0.14 b-d
	A. musiformis isolate MSO1-001	10.47 c	10.49 c-e	1.96 b	0.16 bc
	Paecilomyces sp. isolate WJI1-003	10.76 c	10.76 b-e	2.50 a	0.21 a
	Pochonia sp. isolate KJO1-003	9.19 g	9.19 g	1.73 c	0.14 b-d
	Non-treated control	10.78 c	10.78 b-e	0.99 g	0.14 b-d
	CV %[3/]	7.44	7.45	7.40	23.48

[1/] Mean of each treatment calculated from ten replications.
[2/] Means followed by the same letter are not significantly different by DMRT at P=0.01.
[3/] CV% = coefficient of variation 99%.

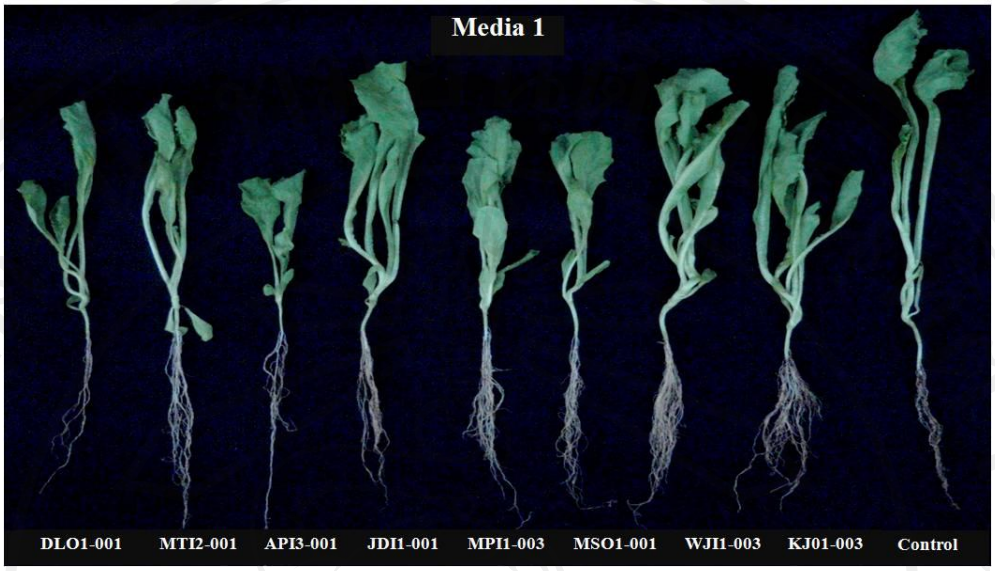

Figure 7.1 Characterizations of head lettuce seedling on different fungal biomasses amended with Medium 1

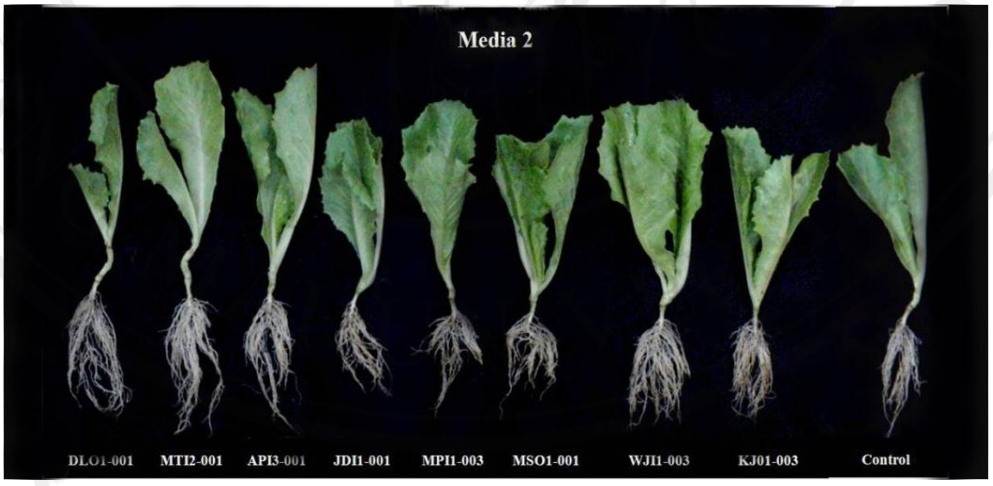

Figure 7.2 Characterizations of head lettuce seedling on different fungal biomasses amended with Medium 2

7.3.2 The ability of nematophagous fungi-amended seedling application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

Analysis of variance by two factorial treatment effects and interaction of different nematophagous fungi-amended seedling application to head lettuce height, root length, fresh weight of shoot and dry weight of shoot including the number of galls per root at 60 days after transplantation into pots containing root-knot nematodes were significantly different at P=0.01. The statistical analysis data are displayed in Tables 46-51 of appendix.

Biomass of *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001 and *Arthrobotrys thaumasium* isolate JDI1-001 amended with Medium 1 supported the highest of plant height of head lettuce seedling which are 30.80 cm, 30.30 cm and 30.50 cm and were not significantly different (P=0.01). A similar result on root length was seen with almost all of the fungal biomasses. Biomass of *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 amended with Medium 2 induced the highest shoot fresh weight which were 73.28 g and 72.82 g and were not significantly different (P=0.01). On the other hand, the lowest shoot fresh weight was observed on seedling transplanted in non-treated control Medium 1 followed by biomass of *Arthrobotrys musiformis* isolate MSO1-001 amended with Medium 1. The results of dry shoot weight of shoot were similar to shoot fresh weight. Biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 2 caused the highest dry weight 6.01 g and lowest dry weight 2.11 g on non-treated control Medium 1 and 2.

Transplanting in biomass of *A. oligospora* isolate MTI2-001 and *A. conoides* isolate API3-001 amended with Medium 1 and biomass of *A. oligospora* isolate MTI2-001 amended with Medium 2 in root-knot infested soil reduced the number of

galls per root when compared with other fungal biomasses and the control. The number of gall per root was 12.90, 11.70 and 12.80 galls, respectively which were not significantly different (P=0.01). On the other hand, biomass of *Arthrobotrys thaumasium* isolate MPI1-003 amended with both media and biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 2 showed a great number of galls. The result of percentage of galled reduction indicated that nematophagous fungi-amended seedling application was not a suitable method to reduce root-knot disease (Table 7.3).

Table 7.3 Effect of nematophagous fungi-amended seedling application on head lettuce seedling growth at 60 days after transplantation in pots containing root-knot nematodes

Treatment		Measurement[1/]					
		Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Gall per root (gall)	Galled reduction (%)
Medium 1	*A. oligospora* isolate DLO1-001	30.80 a[2/]	14.20 ab	36.77 ef	3.20 ef	13.90 bc	56.83 a-c
	A. oligospora isolate MTI2-001	30.30 ab	14.10 ab	44.41 de	3.33 d-f	12.90 bc	59.93 a-c
	A. conoides isolate API3-001	28.50 a-d	13.80 ab	31.54 f	2.14 hi	11.70 bc	63.66 ab
	A. thaumasium isolate JDI1-001	30.50 ab	14.20 ab	30.01 f	2.23 g-i	17.00 a-c	47.20 a-c
	A. thaumasium isolate MPI1-003	29.40 a-c	13.30 ab	43.66 de	3.07 ef	35.50 a	-0.01c
	A. musiformis isolate MSO1-001	29.20 a-c	13.20 ab	28.56 f	1.64 i	14.30 a-c	55.59 a-c
	Paecilomyces sp. isolate WJI1-003	29.80 a-c	14.60 a	50.40 cd	3.55 de	17.70 a-c	45.03 a-c
	Pochonia sp. isolate KJO1-003	27.50 c-e	13.90 ab	30.82 f	2.31 gh	28.40 ab	11.80 bc
	Non-treated control	24.25 f	14.60 a	28.25 f	2.11 hi	32.2 ab	-
Medium 2	*A. oligospora* isolate DLO1-001	30.10 abc	12.80 a-c	61.15 bc	4.88 b	15.80 bc	50.93 a-c
	A. oligospora isolate MTI2-001	27.90 b-e	13.10 ab	68.41 ab	4.39 bc	12.80 bc	60.24 a-c
	A. conoides isolate API3-001	29.50 a-c	11.20 c	65.37 ab	3.86 cd	21.40 a-c	33.54 a-c
	A. thaumasium isolate JDI1-001	29.10 a-d	12.68 bc	50.39 cd	2.83 fg	19.20 a-c	40.37 a-c
	A. thaumasium isolate MPI1-003	29.00 a-d	13.30 ab	66.40 ab	3.33 d-f	31.40 a-c	2.48 bc
	A. musiformis isolate MSO1-001	25.60 ef	13.70 ab	53.16 cd	2.81 fg	19.10 a-c	40.68 a-c
	Paecilomyces sp. isolate WJI1-003	28.60 a-d	13.10 ab	73.28 a	6.01 a	32.50 ab	-0.93 bc
	Pochonia sp. isolate KJO1-003	26.50 d-f	13.20 ab	72.82 a	4.52 b	29.50 ab	8.38 bc
	Non-treated control	24.25 f	14.60 a	52.04 cd	2.11 hi	32.2 ab	-
	CV %[3/]	7.02	9.96	18.33	15.47	83.23[4/]	151.33

[1/] Mean of each treatment calculated from ten replications
[2/] Means followed by the same letter are not significantly different by DMRT at P=0.01.
[3/] CV% = coefficient of variation 99%.

7.3.3 The ability of biological agent (nematophagous fungi) application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

Analysis of variance by two factorial treatment effects and interaction of different biological agent (nematophagous fungi) application comparing fermented and without fermented method 7 days before transplantation on head lettuce height, root length, fresh weight of shoot and dry weight of shoot including the number of galls per root at 60 days were significantly different at P=0.01. The statistical analysis data are displayed in Tables 52-57 of appendix.

Biological agent *Arthrobotrys musiformis* isolate MSO1-001 with fermented method and *Paecilomyces* sp. isolate WJI1-003 without fermented method supported the highest plant height of head lettuce seedlings which were 28.19 and 28.22 cm, followed by biological agent *Paecilomyces* sp. isolate WJI1-003 with fermented method (26.92 cm) and was not significantly different (P=0.01). The highest root length was induced by biological agent *Arthrobotrys conoides* isolate API3-001 (16.39 cm) and *Arthrobotrys thaumasium* isolate MPI1-003 (16.01cm) with fermented method while the lowest was observed on *Arthrobotrys thaumasium* isolate JDI1-001 without fermentation (11.40 cm). The result of treatments supported fresh and dry weight of shoot were similar by application of biological agent *Paecilomyces* sp. isolate WJI1-003 with or without fermented method; fresh weight of shoot was 27.92 and 28.22 g whereas dry weight of shoot was 2.80 and 3.02 g, respectively (Table 7.4).

Almost all fungal biological agents with fermented method showed good growth and lower gall per root than without fermented method (1.20-7.00 galls) especially *A. oligospora* isolate MTI2-001 and *A. conoides* isolate API3-001.

Table 7.4 Effect of biological agent (nematophagous fungi) application compared fermented and without fermented method 7 days before transplantation on head lettuce growth at 60 days

Treatment		Measurement[1/]					
		Plant height (cm)	**Root length (cm)**	**Fresh weight of shoot (g)**	**Dry weight of shoot (g)**	**Gall per root (gall)**	**Galled reduction (%)**
Fermented method	*A. oligospora* isolate DLO1-001	22.76 c[2/]	13.30 b-e	19.05 e[2/]	1.68 d	4.60 de	90.47 a
	A. oligospora isolate MTI2-001	26.45 ab	14.90 ab	22.90 c-e	1.53 de	1.20 e	97.51 a
	A. conoides isolate API3-001	24.47 bc	16.39 a	23.72 b-d	2.38 b	1.30 e	97.30 a
	A. thaumasium isolate JDI1-001	24.34 bc	14.12 bc	19.98 de	1.55 de	5.20 de	89.23 a
	A. thaumasium isolate MPI1-003	24.28 bc	16.01 a	22.83 c-e	1.45 d-f	3.10 e	93.58 a
	A. musiformis isolate MSO1-001	28.19 a	14.19 bc	24.66 a-c	1.84 cd	6.10 de	87.37 ab
	Paecilomyces sp. isolate WJI1-003	26.92 ab	14.93 ab	27.92 ab	2.80 a	7.00 de	85.50 ab
	Pochonia sp. isolate KJO1-003	26.65 ab	12.34 c-e	28.70 a	2.09 bc	6.10 de	87.37 ab
	Non-treated control	22.15 c	13.90 b-d	24.67 a-c	1.77 cd	48.30 a	-
Without fermented method	*A. oligospora* isolate DLO1-001	22.74 c	14.10 bc	20.68 c-e	1.25 ef	15.50 b-d	67.90 bc
	A. oligospora isolate MTI2-001	23.80 bc	12.60 c-e	22.11 c-e	1.22 ef	8.10 c-e	83.23 ab
	A. conoides isolate API3-001	23.07 c	12.10 de	22.26 c-e	1.62 de	6.20 de	87.16 ab
	A. thaumasium isolate JDI1-001	24.35 bc	11.40 e	22.40 c-e	1.14 f	18.30 bc	62.11 cd
	A. thaumasium isolate MPI1-003	24.15 bc	12.10 de	22.91 c-e	1.46 d-f	18.90 bc	60.87 cd
	A. musiformis isolate MSO1-001	21.64 c	12.70 c-e	20.67 c-e	1.67 d	25.50 b	47.20 d
	Paecilomyces sp. isolate WJI1-003	28.22 a	13.30 b-e	28.22 a	3.02 a	24.20 b	49.89 cd
	Pochonia sp. isolate KJO1-003	24.25 bc	12.30 c-e	19.85 de	1.79 cd	20.80 b	56.93 cd
	Non-treated control	21.60 c	13.30 b-e	24.61 a-c	1.77 cd	48.30 a	-
	CV %[3/]	9.56	10.54	14.20	16.93	58.36	20.37

[1/] Mean of each treatment calculated from ten replications
[2/] Means followed by the same letter are not significantly different by DMRT at P=0.01.
[3/] CV% = coefficient of variation 99%.

Analysis of variance by two factorial treatment effects and interaction of different biological agent (nematophagous fungi) application comparing fermented and without fermented method before 7 days transplantation on baby cos lettuce height, root length, fresh weight of shoot and dry weight of shoot include a number of galls per root at 60 days were significantly different at P=0.01. The statistical analysis data are displayed in Tables 58-63 of appendix.

The highest plant height, root length, fresh weight of shoot and dry weight of shoot of baby cos lettuce were observed on biological agents *Paecilomyces* sp. isolate WJI1-003 and *Arthrobotrys musiformis* isolate MSO1-001 with fermented method and were not significantly different (P=0.01). The plant height, root length, fresh weight of shoot and dry weight of shoot supported by WJI1-003 with fermented method were 31.30 cm, 17.40 cm, 50.89 g and 3.76 g, respectively. A similar result was seen with isolate *A. musiformis* isolate MSO1-001 which was 30.85 cm, 17.05 cm, 47.56 g and 3.88 g. Moreover, this study showed that applying WJI1-003 without fermented method also induced fresh and dry weight of shoot of baby cos lettuce.

All biological agent (nematophagous fungi) application with fermented method reduced a number of galls per root which were not significantly different (P=0.01) when contrasted with non-treated control. On the other hand, non-treated control, biological agent *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate MPI1-003 and *A. musiformis* isolate MSO1-001 without fermented method showed the highest galls per root and they were not significantly different: 22.80, 28.60, 28.50 and 18.50 galls, respectively (Table 7.5 and Figures 7.3-7.4).

Table 7.5 Effect of biological agent (nematophagous fungi) application comparing fermented and without fermented method 7 days before transplantation on baby cos lettuce growth at 60 days

Treatment		Measurement[1/]					
		Plant height (cm)	**Root length (cm)**	**Fresh weight of shoot (g)**	**Dry weight of shoot (g)**	**Gall per root (gall)**	**% galled reduction**
Fermented method	*A. oligospora* isolate DLO1-001	27.00 c-g	14.70 a-d	27.61 gh	2.16 e	1.90 b	91.66 ab
	A. oligospora isolate MTI2-001	27.00 c-g	14.70 a-d	32.19 d-h	2.16 e	1.40 b	93.85 ab
	A. conoides isolate API3-001	29.20 a-d	14.30 b-d	37.73 c-g	2.60 c-e	1.0 b	95.61 a
	A. thaumasium isolate JDI1-001	25.70 d-g	14.70 a-d	33.94 d-h	2.23 e	1.80 b	92.10 ab
	A. thaumasium isolate MPI1-003	29.90 a-c	15.60 a-d	34.80 d-h	2.48 de	2.00 b	91.22 ab
	A. musiformis isolate MSO1-001	30.85 ab	17.05 a-c	47.56 a-c	3.88 a	2.00 b	91.22 ab
	Paecilomyces sp. isolate WJI1-003	31.30 a	17.40 a	50.89 a	3.76 a	3.20 b	85.96 ab
	Pochonia sp. isolate KJO1-003	31.20 ab	14.85 a-d	41.94 a-e	3.01 b-d	2.50 b	89.03 ab
	Non-treated control	24.29 g	13.2 d	32.00 e-h	3.26 a-c	22.80 a	-
Without fermented method	*A. oligospora* isolate DLO1-001	27.70 b-g	15.00 a-d	30.21 f-h	2.49 de	15.20 ab	33.33 a-c
	A. oligospora isolate MTI2-001	25.60 e-g	14.60 a-d	33.26 d-h	2.41 de	2.60 b	88.59 ab
	A. conoides isolate API3-001	27.80 b-f	15.30 a-d	42.75 a-d	2.68 b-e	28.60 a	-25.43 c
	A. thaumasium isolate JDI1-001	27.30 c-g	14.00 ce	37.88 c-g	2.66 b-e	15.70 ab	31.13 a-c
	A. thaumasium isolate MPI1-003	27.90 a-f	17.00 a-c	25.42 h	2.00 e	28.50 a	-25.00 c
	A. musiformis isolate MSO1-001	25.60 e-g	17.30 ab	28.05 gh	2.01 e	18.50 ab	18.85 bc
	Paecilomyces sp. isolate WJI1-003	29.10 a-e	15.70 a-d	48.48 ab	3.35 ab	14.40 ab	36.84 a-c
	Pochonia sp. isolate KJO1-003	27.80 b-f	16.50 a-c	40.63 b-f	2.52 de	15.60 ab	31.57 a-c
	Non-treated control	24.5 fg	13.2 d	32.79 d-h	3.37 ab	22.80 a	-
	CV %[3/]	9.43	14.50	21.31	20.31	118.99	101.34

[1/] Mean of each treatment calculated from ten replications
[2/] Means followed by the same letter are not significantly different by DMRT at P=0.01.
[3/] CV% = coefficient of variation 99%.

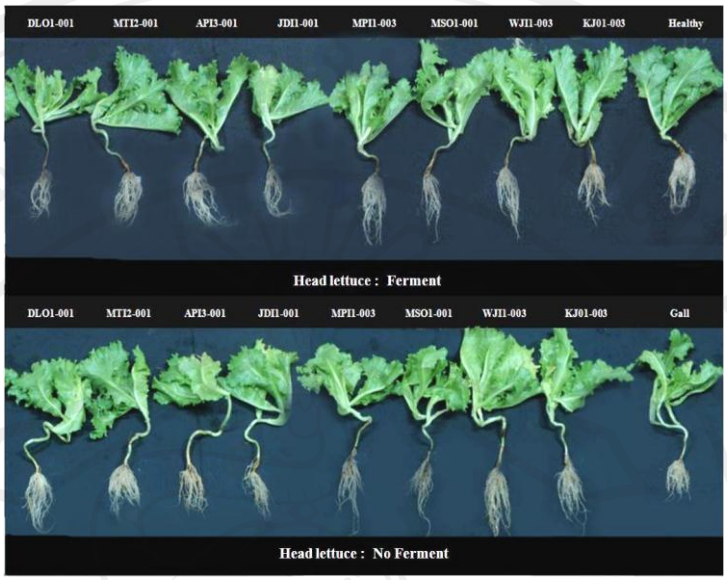

Figure 7.3 Characterizations of head lettuce seedling growth at 60 days after application of the fungal biological agents comparing fermented and without fermented method 7 days before transplantation

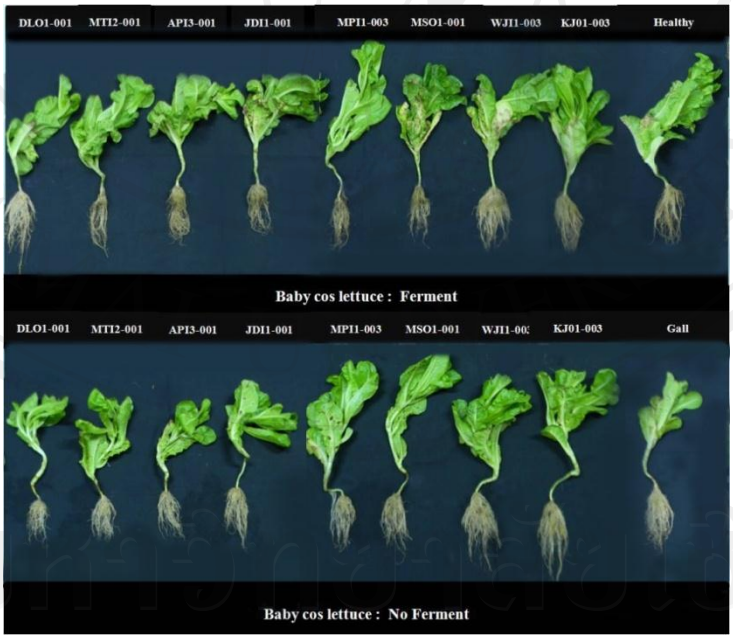

Figure 7.4 Characterizations of baby cos lettuce seedling growth at 60 days after application of the fungal biological agents comparing fermented and without fermented method 7 days before transplantation

7.3.4 The ability of nematophagous fungi-amended seedling application against root-knot of lettuce caused by *Meloidogyne incognita* in the field

Analysis of variance by two factorial treatment effects and interaction of different nematophagous fungi-amended seedling application comparing two seedling media at 60 days after transplantation in root-knot nematode infested area on head lettuce height, root length, fresh weight of shoot and dry weight of shoot including the number of galls per root were significantly different at P=0.01. The statistical analysis data are displayed in Tables 64-69 of appendix.

The results of fungal biomass amended with seedling before transplantation in a root-knot nematode infested area which omitted any chemicals indicated fungal biomasses with Media 1 supported plant height of head lettuces higher than Media 2. On the other hand, fungal biomasses with Media 2 induced root length of head lettuces longer than Media 1.

Biomass of *Paecilomyces lilacinus* isolate WJI1-003 amended with Medium 1 supported the highest plant height (17.50 cm) followed by *Pochonia* sp. isolate KJO1-003, *Arthrobotrys thaumasium* isolate JDI1-001 and *Arthrobotrys musiformis* isolate MSO1-001 which were 16.50, 16.10 and 16.07 cm, respectively and they were not significantly different by DMRT (P=0.01). This study also observed that applying biomass of *Paecilomyces* sp. isolate WJI1-003 with Medium 1 induced fresh and dry weight of shoot which was 233.18 and 10.91 g followed by *Paecilomyces* sp. isolate WJI1-003 with Medium 2 and they were significantly different.

All nematophagous fungi-amended seedling application could not reduce the number of galls per root when compared with non-treated control by DMRT (P=0.01), but the best biomass was increased by *Arthrobotrys conoides* isolate API3-

001 amended with Medium 2 (11.60 galls) followed by biomass of *Paecilomyces* sp. isolate WJI1-003 amended with Medium 1 (13.50 galls) and *Arthrobotrys oligospora* isolate MTI2-001 amended with Medium 1 (13.60 galls). The data showed in Table 7.6 and Figures 7.5-7.6. In generally, this application showed a poor efficiency to reduce root-knot nematodes when contrasted to biological agent (nematophagous fungi) application with fermented method.

Table 7.6 Effect of nematophagous fungi-amended seedling application compared two seedling media on head lettuce growth at 60 days after transplantation in root-knot nematode infested area

Treatment		Measurement[1/]					
		Plant height (cm)	**Root length (cm)**	**Fresh weight of shoot (g)**	**Dry weight of shoot (g)**	**Gall per root (gall)**	**% galled reduction**
Medium 1	*A. oligospora* isolate DLO1-001	13.68 d[2/]	9.35 e	149.89 c	9.19 bc	24.60 bc	39.11 bc
	A. oligospora isolate MTI2-001	14.25 cd	9.30 e	104.82 f-h	6.38 e-g	13.60 bc	66.33 ab
	A. conoides isolate API3-001	15.14 b-d	9.15 e	78.68 i	5.08 h-j	20.70 bc	48.76 a-c
	A. thaumasium isolate JDI1-001	16.07 a-c	8.80 e	94.69 hi	4.62 ij	24.70 bc	38.86 bc
	A. thaumasium isolate MPI1-003	14.64 b-d	8.15 e	79.90 i	4.15 j	21.10 bc	47.77 a-c
	A. musiformis isolate MSO1-001	16.10 a-c	8.60 e	75.35 i	4.83 ij	25.40 bc	37.12 bc
	Paecilomyces sp. isolate WJI1-003	17.50 a	8.35 e	233.18 a	10.91 a	13.50 bc	66.58 ab
	Pochonia sp. isolate KJO1-003	16.50 ab	8.10 e	130.17 c-e	8.06 cd	14.50 bc	64.10 ab
	Non-treated control	15.85 a-c	15.00 cd	115.63 d-h	5.67 f-i	40.40 a	-
Medium 2	*A. oligospora* isolate DLO1-001	9.17 e	15.35 b-d	132.25 c-e	8.11 cd	20.80 bc	48.51 a-c
	A. oligospora isolate MTI2-001	9.05 e	17.80 a	107.71 f-h	6.78 e-g	18.90 bc	53.21 a-c
	A. conoides isolate API3-001	8.30 e	15.95 b-d	112.67 e-h	6.37 ef	11.60 c	71.28 a
	A. thaumasium isolate JDI1-001	8.60 e	16.70 ab	100.88 gh	5.05 h-j	16.70 bc	58.66 a-c
	A. thaumasium isolate MPI1-003	8.00 e	15.65 b-d	124.96 d-f	6.34 e-g	17.10 bc	57.67 a-c
	A. musiformis isolate MSO1-001	8.85 e	15.01 cd	120.45 d-g	6.18 e-h	27.00 b	33.16 c
	Paecilomyces sp. isolate WJI1-003	9.03 e	14.65 d	182.93 b	9.70 b	24.20 bc	40.09 bc
	Pochonia sp. isolate KJO1-003	9.50 e	16.20 bc	136.31 cd	7.21 de	16.50 bc	59.15 a-c
	Non-treated control	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 at P=0.01.
[3/] CV% = coefficient of variation 99%

Figure 7.5 Head lettuce seedling grown in field application 7 days after transplantation in a root-knot nematode infested area during raining.

Figure 7.6 Characterizations of head lettuce after trimming of diseased leaves comparing fungal biomass applications at 60 days after transplantation in a root-knot nematode infested area.

7.3.5 The efficiency of bio-formulations of nematophagous fungi against root-knot of lettuce caused by *Meloidogyne incognita* in the field

Arthrobotrys oligospora isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001 and *Paecilomyces* sp. isolate WJI1-003 were selected for this examination of growth enhancement and gall reduction.

Analysis of variance by RCBD of different bio-fungicide formulations of nematophagous fungi on head lettuce height, root length, fresh weight of shoot and dry weight of shoot including the number of galls per root at 60 days after transplantation in root-knot nematodes infested area (Area 1) were significantly different at P=0.01. The statistical analysis data are displayed in Tables 70-75 of appendix.

Application of dazomate treatment supported the highest of plant height on head lettuce in Area 1 followed by bio-fungicide formulation No.1 amended with No.3 and *Paecilomyces* (control) and bio-fungicide formulation No.1, but they were not significantly different by DMRT (P=0.01). Fungal biomass included bio-fungicide formulation No.2, *Paecilomyces* (control), Bio-fungicide formulation No.3 and bio-fungicide formulation No.1 amended with No.3 induced higher root length than other which was 11.80, 11.80, 11.70 and 11.45 cm, respectively and they were not significantly different. The treatments caused a similar higher fresh and dry weight of shoot including bio-fungicide formulation No.2, bio-fungicide formulation No.3 and bio-fungicide formulation No.1 amended with No.3. The fresh shoot weight was 378.00, 323.00 and 379.00 g which were not significantly different. A similar result was observed with the dry shoot weight which was 11.49, 11.79 and 11.00 g, respectively.

The effect of a mixture of all biological agents (bio-fungicide formulation 1- 2 -3) and bio-fungicide formulation No.2 amended with No.3 to reduce a number of galls per root was the lowest (0.30 and 0.40 galls), but they were not significantly different from application of bio-fungicide formulation No.3 (3.00 galls), dazomet (3.10 galls) and bio-fungicide formulation No.1 (3.50 galls).

Overall of results may be summarized that all bio-formulations of nematophagous fungi in this study could apply to dissolve root-knot disease so that the optimal treatments to support growth and also reduce a number of galls per root were bio-fungicide formulation No.3 followed by bio-fungicide formulation No.2. On the other hand, application of bio-fungicide formulation No.1-2-3 and bio-fungicide formulation No. 2-3 were used for controlling a severe level of root-knot disease in field (Table 7.7 and Figures 7.7-7.8).

Table 7.7 Effect of various fungal biomass formulations on head lettuce growth at 60 days after transplantation in root-knot nematode infested area (Area 1)

Treatment	Measurement[1/]						
	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Gall per root (gall)	Scale of total root system galled[4/]	% galled reduction
Bio-fungicide formulation 1	21.00ab-[2/]	10.05 a-c	229.00 cd	7.74 bc	3.50 c	0	92.69 a
Bio-fungicide formulation 2	20.80 a-c	11.80 a	378.00 a	11.49 a	8.70 bc	0	81.83 ab
Bio-fungicide formulation 3	18.60 e	11.70 a	323.00 a	11.79 a	3.00 c	0	93.73 a
Bio-fungicide *Paecilomyces* (control)	21.00 ab	11.80 a	228.00 b	9.42 ab	7.10 bc	0	85.17 a
Bio-fungicide formulation 1 - 2	19.50 de	9.20 bc	174.00 bc	9.95 ab	1.90 c	0	96.03 a
Bio-fungicide formulation 1- 3	21.10 ab	11.45 ab	379.00 a	11.00 a	17.30 b	0	63.88 b
Bio-fungicide formulation 2-3	20.00 b-d	10.20 a-c	214.00 b	9.04 ab	0.40 c	0	99.16 a
Bio-fungicide formulation 1- 2 -3	19.80 cd	10.30 a-c	218.00 b	7.53 bc	0.30 c	0	99.37 a
Soil fumigation with dazomet (control)	21.90 a	9.95 a-c	319.00 a	8.95 ab	3.10 c	0	93.52 a
Non-treated control	16.80 f	8.30 c	133.00 c	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 at P=0.01.
[3/] CV% = coefficient of variation 99%.
[4/] Reference from Barker's galling index (1985).

Analysis of variance by RCBD of different bio-formulations of nematophagous fungi on head lettuce height, root length, fresh weight of shoot and dry weight of shoot including the number of galls per root at 60 days after transplantation in root-knot nematode infested area (Area 2) were significantly different at P=0.01. The statistical analysis data are displayed in Tables 76-80 of appendix.

The highest plant height of head lettuce in Area 2 (22.40 cm) was induced by bio-fungicide formulation No.1 amended with No.3 whereas root-knot infested soil treatment (non-treated control) showed the lowest height (19.60 cm). Bio-fungicide formulation No.3 and dazomet treatment was second to support the head lettuce height followed by bio-fungicide formulation No.2 and bio-fungicide formulation No.1 which were 22.20, 22.20, 21.80 and 21.70 cm, respectively and they were not significantly different by DMRT (P=0.01). The effect of different fungal biomass formulations on root length of head lettuce was between 9.00-11.70 cm. The amended ingredients treatment showed the highest support was application of all fungal bio-fungicide formulation (bio-fungicide formulation 1- 2 -3) which were 11.70. This trial indicated that the result of fresh and dry weight of shoot was not directly. Application of bio-fungicide formulation No.1 amended with No.3 induced the highest value (209.90 g) followed by bio-fungicide formulation No.2 (177.10 g). On the other hand, the highest dry weight of shoot (10.36 g) was observed with all fungal biological agent treatments followed by bio-fungicide formulation No.3 (9.07 g) which were not significantly different.

All reductions in root-knot nematode galling in this study were not significantly different by DMRT (P=0.01). They were grouped in a similar scale of

total root gall rating system (Barker's galling index, 1985) which were scale 0, but difference from control treatments; root-knot infested soil (scale 2). However, bio-fungicide formulation No.2 amended with No.3 showed the lowest number of galls per root followed the fumigation of dazomet which were 3.00 galls and 3.60 galls, respectively (Table 7.8 and Figures 7.7-7.8).

Overall result in this study (Area 2) may conclude that bio-formulations of nematophagous fungi were a suitable method to reduce the damage of galled root following the percentage of galled reduction was > 76.90 to 92.85.

Table 7.8 Effect of various fungal biomass formulations on head lettuce growth at 60 days after transplantation in root-knot nematode infested area (Area 2)

Treatment	Measurement[1/]						
	Plant height (cm)	Root length (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Gall per root (gall)	Scale of total root system galled[4/]	% galled reduction
Bio-fungicide formulation 1	21.70 ab[2/]	11.15 ab	94.60 d	4.51 df	5.90 b	0	85.94 a
Bio-fungicide formulation 2	21.80 ab	10.30 a-d	177.10 ab	5.81 cd	4.60 b	0	89.05 a
Bio-fungicide formulation 3	22.20 ab	9.45 b-d	152.00 bc	9.07 ab	6.70 b	0	84.04 a
Bio-fungicide *Paecilomyces* (control)	20.20 cd	10.25 a-d	166.40 ab	5.89 cd	5.89 b	0	86.19 a
Bio-fungicide formulation 1 - 2	19.60 d	11.10 ab	126.80 b-d	7.70 a-c	5.40 b	0	87.14 a
Bio-fungicide formulation 1- 3	22.40 a	9.00 d	209.90 a	7.09 b-d	4.40 b	0	89.52 a
Bio-fungicide formulation 2-3	21.30 a-c	9.60 b-d	102.20 cd	5.20 c-e	3.00 b	0	92.85 a
Bio-fungicide formulation 1- 2 -3	21.00 bc	11.70 a	132.40 b-d	10.36 a	9.70 b	0	76.90 a
Soil fumigation with dazomet (control)	22.20 ab	9.10 d	135.50 b-d	7.37 b-d	3.60 b	0	91.43 a
Non-treated soil (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 at P=0.01.
[3/] CV% = coefficient of variation 99%.
[4/] Reference from Barker's galling index (1985).

Figure 7.7 Characterizations of the tested plot of head lettuce and famer on Area 1 (left) and Area 2 (right)

Figure 7.8 Characterizations of head lettuce at 60 days after transplantation in root-knot nematodes infected area comparing various fungal biomass formulations.

7.4 Discussion

The component of seedling medium directly affected seedling emergence and growth. In this study, fungal biomass was amended to the growing medium to determine the effect (emergence and growth) and the potential for root-knot nematode management. The results showed almost all treatments were not inhibitory to seedling emergence; moreover, some fungi induced growth including *Arthrobotrys conoides* isolate API3-001, *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003. These results related to the interactions between fungi and plants. Bordallo *et al*. (2002); Nordbring-Hertz *et al*. (2006) stated that *Arthrobotrys oligospora*, *P. chlamydosporia* and other nematophagous fungi have the capacity to grow inter- and intracellularly in plant roots, but never enter vascular tissues. Pornthip *et al*. (2010) reported nematophagous fungi isolated from northern and central Thailand produced plant growth hormone including indole-3-acetic acid; IAA (a phytohormone called auxin) and hydroxamatesiderophore (iron chelating compounds or the strongest soluble Fe^{3+} binding agents). IAA is predominantly produced in cells of the apex (bud) and very young leaves of a plant (Wikipedia, 2012j) while iron is essential for almost all life, essential for processes such as respiration and DNA synthesis (Wikipedia, 2012k).

Notable, application of fungal biomass amended with Medium 2 (cow dung manure and coconut husk) showed a great advantage for seedlings than amending with Medium 1 (peat moss). This result correlated with the component of medium which affected fungal establishment and their expression. Wikipedia (2012l) stated that cow dung is a fecal matter and rich in minerals that provides food for a wide range of animal and fungal species while peat moss is used for increasing the soil's

capacity to hold water and nutrients. In addition, peat moss is absorptive and extremely acidic, inhibiting growth of bacteria and fungi (Wikipedia, 2012m).

The usability of fungal-amended seedlings to control root-knot nematodes in pot and field experiment showed a similar failures because of a starting fungal material was inadequate and secondary juvenile (J2) of root-knot nematode took a short time to infect root system. Therefore, the approach to decrease population of root-knot nematode should be as follows:(1) fermenting a competent fungal biomass amended with sporulated material At least 7 days before transplantation or (2) grinding the multiplied spore of competent fungi, which had difference method depended on specific requirements of each fungus include material and incubated condition (pH, light, time, RH etc.), followed by combining the ingredients (called biological agent) and rapidly transplant. This study indicated that the usability of fungal biological agent amended well decomposed cow dung manure by grinding and fermenting 7 days before transplantation in pot experiment mostly supported head lettuce and baby cos lettuce root length and fresh weight of shoot higher than root-knot infected soil treatment; moreover, they could reduce a number of galls per root than without fermented method especially MTI2-001 and API3-001 isolate.

The key concept of Nordbring-Hertz *et al*. (2011) stated isolation of more virulent strains, and the development of better formulations and fermentation techniques have significantly improved the efficiency of nematophagous fungi as biological control agents against plant- and animal parasitic nematodes. The previous data reaffirmed by this research that found application of bio-fungicide formulation No.2 (*Arthrobotrys conoides* isolate API3-001), bio-fungicide formulation No.3 (*Paecilomyces* sp. isolate WJI1-003) and bio-fungicide formulation No.1

(*Arthrobotrys oligospora* isolate MTI2-001) amended with No.3, which have different features as to inducing plant growth, especially fresh weigh of shoot, or reducing J2 of root-knot nematode or both features, showed greater ability to control galled disease of head lettuce and baby cos lettuce than dazomet fumigation. These results reached a similar conclusion to that of Niranjan & Singh (2011) who indicated that the application of a mass culture and a spore suspension of *Dactylaria brochopaga* 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 those in the control.

This study indicated that *Arthrobotrys oligospora* isolate MTI2-001 has a greater capacity to damage nematodes than to induce plant growth, *A. conoides* isolate API3-001 is able to damage nematodes similarly and also induce plant growth and *P. lilacinus* isolate WJI1-003 showed the highest plant growth induction capability but was not clear the ability to damage J2 root-knot nematodes. However, many researches showed a great number of nematode eggs were infected by *P. lilacinus* (Bordallo *et al*., 2002, Dhawan *et al*., 2004, Thakur & Devi, 2007, Diogo *et al*., 2009, Brand *et al*., 2010). The combination of fungal biological agents to induce plant growth and reduce galls per root correlated with their interaction. Fungi which have the characteristic of rapid growth and/or high sporulation would more effective than the lower-growing and/or poor conidial formation. For example, bio-fungicide formulation No.1 (isolate MTI2-001) amended with No.3 (isolate WJI1-003) supported growth of head lettuce, but damaged J2 of root-knot nematodes at medium

level. This occurrence caused by *Paecilomyces* sp. isolate WJI1-003 produced a great number of spores than *A. oligospora* isolate MTI2-001 as well as the result of morphological interaction among selected nematophagous fungi indicating that sporulation by *A. oligospora* isolate MTI2-001 was inhibited by *Paecilomyces* sp. isolate WJI1-003 at 62.89% when *A. oligospora* isolate MTI2-001 inhibited sporulation of *Paecilomyces* sp. isolate WJI1-003 at 32.57% (data not shown). Another assurance sampling was explained by application of bio-fungicide formulation No.2 (*A. conoides* isolate API3-001) amended with No.3 (*Paecilomyces* sp. isolate WJI1-003). The result indicated this treatment did not induce head lettuce growth, but only reduced the number of galls per root at a high level as was similarly shown in the *in vitro* interaction in which *A. conoides* isolate API3-001 was activated to sporulate by *Paecilomyces* sp. isolate WJI1-003 higher than the effect of *A. conoides* isolate API3-001 activated sporulation of *Paecilomyces* sp. isolate WJI1-003 (data not shown).

7.5 Conclusion

The effect of nematophagous fungi on plant growth of lettuce seedling period under greenhouse

The fungal biomass, medium type and their interaction showed different effects to head lettuce seedling emergence and their growth including height, root length, fresh weight and dry weight of shoot. This trial showed the mixing of fungal biomass with seedling medium promoted high seedling emergence greater than 90%. Amendment of Medium 2 with fungal biomass induced a higher growth of seedlings than Medium 1. Application of *Arthrobotrys conoides* isolate API3-001 biomass

supported a great root length (14.51 cm) and *Paecilomyces* sp. isolate WJI1-003 biomass induced high fresh (2.50 g) and dry weight of shoot (0.21 g).

The ability of nematophagous fungi-amended seedling application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

Application of nematophagous fungi-amended seedling to control root-knot nematode on head lettuce showed different effect. Major treatments supported head lettuce growth, except root length, higher than controls especially usability of *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 biomass amended with Medium 2 followed by biomass of *Arthrobotrys thaumasium* isolate MPI1-003 amended with Medium 2, biomass of *Arthrobotrys oligospora* isolate MTI2-001 and *Arthrobotrys conoides* isolate API3-001 amended with Medium 1. The number of galls indicated that this application was not a good means to reduce root-knot nematode disease.

The ability of biological agent (nematophagous fungi) application to control *Meloidogyne incognita* causing root-knot of lettuce in pot experiment

The compost of fungal biomass and well decomposed cow dung manure (CDM), called biological agent, comparing fermented and without fermented method 7 days before transplantation on head lettuce growth and root-knot nematode management at 60 days showed different results. Usability of fungal biological agent grounded and fermented before transplantation in pot experiment mostly supported head lettuce root length and fresh weight of shoot higher than non-treated control so that *Paecilomyces* sp. isolate WJI1-003 was the best biological agent. Moreover, usability of fungi with fermented method could reduce a number of galls per root than without fermented method especially *Arthrobotrys oligospora* isolate MTI2-001 (1.20

galls; 97.51 % of galled reduction) and *Arthrobotrys conoides* isolate API3-001 (1.30 galls; 97.30 % of galled reduction). Those results were similar with the tested results of baby cos lettuce. Therefore, *A. oligospora* isolate MTI2-001, *A. conoides* isolate API3-001 and *Paecilomyces* sp. isolate WJI1-003 would be selected for the next trial.

The ability of nematophagous fungi-amended seedling application against root-knot of lettuce caused by *Meloidogyne incognita* in the field

Application of different nematophagous fungi-amended seedling application comparing two seedling media in root-knot nematodes infected area on head lettuce showed difference results. Seedlings treated using amended fungal biomass with Medium 2 greatly supported all growth parameters except plant height. *Paecilomyces* sp. isolate WJI1-003 was the best biomass inducing fresh weight of shoot (233.18 g) and dry weight of shoot (10.91 g) contrasted with non-treated control (115.53, 5.67 g). However, the ability of nematophagous fungi-amended seedling application for gall reduction was poor.

The efficiency of bio-formulations of nematophagous fungi against root-knot of lettuce caused by *Meloidogyne incognita* in the field

The grinding of three bio-formulations of nematophagous fungi (No.1 *A. oligospora* isolate MTI2-001, No.2 *A. conoides* isolate API3-001 and No.3 *Paecilomyces* sp. isolate WJI1-003) in root-knot nematode infested area before transplantation showed different results. All fungal applications and dazomet fumigation promoted higher growth as plant height, root length, fresh weight of shoot and dry weight of shoot than non-treated control. Application of bio-fungicide formulation No.3 was the best to induce growth and showed a good tendency to reduce a number of galls per root on head lettuce (Area 1: applied chemical fertilizer)

so that bio-fungicide formulation No.1-2-3 showed the highest capacity on galled reduction followed by bio-fungicide formulation No.2 amended with No.3 and bio-fungicide formulation No.1 amended with No.2, but showed mediumry on growth. The results of another area (Area 2: without applied chemical fertilizer) was not clear; however, bio-fungicide formulation No.1 amended with No.3 was a good inducing plant height and fresh weight of shoot and could reduce the galled formation to scale 0 (Barker's galling index, 1985).