CHAPTER 5

Study on Physiological Factors and Antagonistic Potential Related to Growth and Sporulationof Nematophagous Fungi

5.1 Introduction

Biotic and abiotic factors are interrelated to create a system or more precisely, an ecosystem, a community of living and nonliving things considered as a unit. Biotic factors are living factors in plants, animals, fungi, protista and bacteria, etc. The association of organisms may affect each other in an environment (Biology online, 2008a). Abiotic factors are non-living chemical or physiological factors in the environment and ecology. They are known as “density independent factors”. Abiotic factors may be grouped into (1) climatic factors such as sunlight, humidity, temperature and atmosphere (2) edaphic factors like the nature and type of the soil and geology of the land and (3) social factors, i.e. land use, water resources (Biology online, 2008b).

The capacity of soil ecosystem to prevent or reduce the spread of a pathogen, parasite, or other deleterious agent in soils is called antagonistic potential. It includes suppressiveness, fungistasis, antiphytopathogenic potential and biological buffering. In addition, management of antagonists in the soil requires an understanding not only ofthe intricate interrelationships between host-parasite and parasite-antagonist, but also of the interactions among these relationships, crop production practices and abiotic factors (Richard, 1992). The interaction of rhizosphere organisms and their physiological factors influence growth and sporulation. Each creature generally reacts

in different ways related to their survival and settlement characterizations. For instance, Nagesh *et al*. (2007) reported four isolates of *Pochonia chamydosporia* had morphological and molecular (with respect to the ß-tubulin gene) similarity, but differed significantly in their preferences for pH and temperatures for spore germination, mycelial growth, time taken for apparent completion of sporulation and spore production on a corn meal agar medium. Moreover, they preferred near neutral pH (6.5-7.7) and moderate temperatures (25-35^{o}C) for important features. Dhawan *et al*. (2004) studied the influence of abiotic and biotic factors on growth of three nematophagous fungi namely, *Paecilomyces lilacinus, Arthrobotrys oligospora* and *Pochonia chlamydosporia.* It was found that all fungi grew well in sandy loam soil and 25°C was found to be the optimum temperature for their growth. Growth of these fungi was excellent on chickpea pod waste and least on farmyard manure substrate.

Dong-Geun *et al*. (2002) compared the effects of biological and chemical soil factors on the distribution of nematophagous fungi. Stepwise logistic regression analysis indicated that the presence of endoparasites in soil was associated with pH, while net-forming species including *Arthrobotrys oligospora* as representative species were largely independent of soil elements, although generally they were isolated from soils with high phosphate. Constricting ring and adhesive knob-forming predators were influenced by pH and K+ concentration, while species with adhesive hyphae were associated with Mg++ concentration and more frequently isolated from poor soils containing low organic matter. *Arthrobotrys conoides, A. arthrobotryoides* and *Monacrosporium thaumasium* were affected by pH and Ca++ concentration. pH which was positively correlated with Ca++ concentration, appears to be one of the most important elements determining the presence of nematophagous fungi in soil.

The objectives of this chapter were as follows:

1. To study some physiological factors which affect the growth and sporulation of nematophagous fungi, *in vitro*.

2. To select the optimal culture conditions for supporting growth, sporulation and habitation of selected nematophagous fungi.

5.2 Materials and methods

5.2.1 *In vitro* study of physiological factors for growth and sporulation of selected nematophagous fungi

Physiological factors consisting of media, temperature, light, pH and pesticide were designed to measure growth and sporulation of eight nematophagous fungi from Chapter 3 which showed a high effectiveness to damage root knot nematodes *(Meloidogyne incognita*), which were *Arthrobotrys oligospora* isolate DLO1-001, *Arthrobotrys oligospora* isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, *Arthrobotrys thaumasium* isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003. All fungi were cultured on potato dextrose agar (PDA) and incubated for 7 days at 25°C. The purified fungi were used in this experiment

The physiological factors in this study were as follows:

(1) Six culture media include potato dextrose agar (PDA), ½PDA, corn meal agar (CMA), V-8 juice agar (VA), potato carrot agar (PCA) and yeast extract-peptone-glycerol (YPG) were examined at room temperature (27±3ºC). A fungal culture agar plug (5 mm-diameter) from the colony edge of each fungus isolate were

placed in the middle of 9 mm-diameter Petri dishes. Three Petri dishes of each isolate of each treatment were used as replicates. The experiment was done by two factors factorial in Completely Randomized Design (CRD). 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 and Factor B represented culture media where B1 = PDA, B2 = ½PDA, B3 = CMA, B4 = VA, B5 = PCA and B6 = YPG.

Colony diameters (fungal growth) were measured at 3, 5, 7 and 10 days. For sporulation, five colonized fungal agar plugs (0.4-cm-diameter) were removed from each plate after 10 days incubation was gently washed with 10 ml of sterile distilled water containing 0.05% Tween 80 and the suspensions collected were designated as stock suspensions of each isolate. The concentration of conidia in each was counted using a haemocytometer (Liu & Chen, 2002).

The data were analyzed by analysis of variance (ANOVA) “Two factors factorial in Completely Randomized Design (CRD) by 8×6 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

(2) Five temperature regimes include 15, 20, 25, 30 and 35^{o}C were selected to compare growth and sporulation of fungi cultured on potato dextrose agar (PDA). The colony edge of each fungal inoculum was placed in the middle of 9 mm-diameter Petri dishes and incubated at different temperatures. Three Petri dishes of each isolate of each treatment were used as replicates. Data collections of fungal growth and

sporulation were performed similarly as above-mentioned in the study of media factor.

The experiment was done by two factors factorial in Completely Randomized Design (CRD). 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 and Factor B represented temperature regimes where B1 = 15^{o}C, B2 = 20^{o}C, B3 = 25^{o}C, B4 = 30^{o}C and B5 = 35^{o}C.

The data were analyzed by analysis of variance (ANOVA) "Two factors factorial in Completely Randomized Design (CRD) by 8×5 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

(3) Four different light conditions comprised of 24 hour light, 12 hour light and 12 hour dark, 24 hour dark and 24 hour black light (NUV) were used to compare the growth and sporulation of fungi cultured. Generation of cool day light source was by a fluorescent lamp which had 36 Watt, color temperature 6,200 K and 2,600 lumen. Generation of black light was by a black light blue lamp "GE F40BLB GE" which had 40 Watt. The fungal culture inoculum of each isolate was inoculated on potato dextrose agar (PDA) and maintained under different regimes of light at room temperature (27±3ºC). Three Petri dishes of each isolate of each treatment were used as replicates. Data collections of fungal growth and sporulation were performed similarly study of media factor.

The experiment was done by two factors factorial in Completely Randomized Design (CRD). 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 and Factor B represented light conditions where B1 = 24 hour light, B2 = 12 hour light and 12 hour dark, B3 = 24 hour dark and B4 = 24 hour black light (NUV).

The data were analyzed by analysis of variance (ANOVA) "Two factors factorial in Completely Randomized Design (CRD) by 8×4 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

(4) pH levels in the range of 3-10 were used to examine growth and sporulation of selected fungi at room temperature (27±3ºC). A hydrogen chloride (HCl) and sodium hydroxide (NaOH) buffer was used to establish a range of pH values. Pre-test of HCl or NaOH quantity to achieve the required final pH level were recorded. PDA was amended with appropriate volumes of the recorded buffer. The amended media were poured into 9 cm sterilized Petri dishes, under aseptic conditions and allowed to cool. The fungal culture inoculum of each isolate was inoculated and incubated at 25±1°C. Three Petri dishes of each isolate of each treatment were used as replicates. Data collections of fungal growth and sporulation were performed similarly as above-mentioned in the study of media factor.

The experiment was done by two factors factorial in Completely Randomized Design (CRD). 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 and Factor B represented pH levels where B1 = pH level 3, B2 = pH level 4, B3 = pH level 5, B4 = pH level 6, B5 = pH level 7, B6 = pH level 8, B7 = pH level 9 and B8 = pH level 10.

The data were analyzed by analysis of variance (ANOVA) "Two factors factorial in Completely Randomized Design (CRD) by 8×8 factorial arrangement and Duncan's New Multiple Range Test (DMRT) for comparison of the means of each treatment.

5.2.2 Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi

The *in vitro* morphological sensitivity of fungal isolates to 14 different pesticides was assessed as the result of chemical pollution during pest management or toxic accumulation in soil. In addition, agricultural pesticides also influenced on antagonistic potential, survival and settlement characterizations in rhizosphere of nematophagous fungi. The pesticides with their trade names and recommended rates included

(1) Six insecticides, dazomet (Basamid-G 98 % GR®, 2,450 ppm a.i.), dinotefuran (Starkle-G 1% GR®, 40 ppm a.i.), lambda-cyhalothrin (Karate 2.5 % W/V CS®, 62.5 ppm a.i.), methomyl (Lannate 40 % SP®, 700 ppm a.i.), carbaryl (Sevin 85 % WP®, 2,975 ppm a.i.) and chlorpyrifos (Lorsban 40 % W/V EC®, 1,500 ppm a.i.).

Tree factors factorial in Completely Randomized Design (CRD) was design to observe the effect. 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. Factor B represented insecticide types where B1 = dazomet, B2 = dinotefuran, B3 = lambda-cyhalothrin, B4 = methomyl, B5 = carbaryl and B6 = chlorpyrifos. Factor C represented concentrations of insecticide where C1 = 2x of the recommended rate, C2 = the recommended rate, C3 = 1/2x of the recommended rate and C4 =1/3x of the recommended rate.

(2) Five fungicides, quintozene mixed with etridiazole (Terraclor Super X 30% W/V EC®, 900 ppm a.i.), fosetyl aluminium (Aliette 80 WG®, 8,000 ppm a.i.), metalaxyl-M mixed with mancozeb, (Ridomil Gold MZ 65 WG®, 1,700 ppm a.i.), toclofos methyl (Rizolex 50 % WP®, 1000 ppm a.i.) and propamocarb hydrochloride (Previcur - N 72.2 % W/V SL®, 722 ppm a.i.).

The experiment was done by tree factors factorial in Completely Randomized Design (CRD). 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. Factor B represented fungicide types where B1 = quintozene mixed with etridiazole, B2 = fosetyl aluminium, B3 = metalaxyl-M mixed with mancozeb, B4 = toclofos methyl

and B5 = propamocarb hydrochloride. Factor C represented concentrations of fungicide where C1 = 2x of the recommended rate, C2 = the recommended rate, C3 = 1/2x of the recommended rate and C4 =1/3x of the recommended rate.

(3) Three herbicides, paraquat dichloride (paraquat 27.6 % W/V SL®, 1,725 ppm a.i.), glyphosate-isopropylammonium (Glyphosate 48 % W/V SL®, 3000 ppm a.i.) and oxyfluorfen (Goal 2 E 23.5 % W/V EC®, 587.5 ppm a.i.).

This experiment was designed by tree factors factorial in Completely Randomized Design (CRD). 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. Factor B represented herbicide types where B1 = paraquat dichloride, B2 = glyphosate-isopropylammonium and B3 = oxyfluorfen. Factor C represented concentrations of herbicide where C1 = 2x of the recommended rate, C2 = the recommended rate, C3 = 1/2x of the recommended rate and C4 =1/3x of the recommended rate.

Each pesticide was tested at four recommended rate. A stock solution of each chemical was prepared in sterilized distilled water and appropriate quantities were added under aseptic conditions into 250 ml flasks, containing PDA, to achieve the required final concentrations. The amended media were poured into 9-cm-diameter sterilized Petri dishes, under aseptic conditions and allowed to cool. Petri dishes containing non-amended medium served as the control. A fungal culture agar plug (5-mm-diameter) from the colony edge of each fungal isolate was placed in the middle of the Petri dishes. Three Petri dishes of each isolate of each treatment were used as

replicates. The inoculated Petri dishes were incubated at room temperature (27±3ºC) followed by Goltapeh *et al*., 2008.

Data collection: The percentage of fungal growth inhibition was calculated and analyzed for statistical comparison following equation (Vincent, 1974): Percentage of fungal growth inhibition (PGI) = (C-T)/C×100 where; C = Growth of the fungus in control and T = Growth of the fungus in treatment. The resulting measurements of fungal sporulation were performed similarly as the study of media factor.

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

5.3 Result

5.3.1 *In vitro* study of physiological factors for growth and sporulation of selected nematophagous fungi

The effect of six media including potato dextrose agar (PDA), ½PDA, corn meal agar (CMA), V-8 juice agar (V8), potato carrot agar (PCA) and yeast extract-peptone-glycerol (YPG) on the radial growth (3, 5, 7 and 10 days) and sporulation (10 days) of eight nematophagous fungi at room temperature (27±3 ºC) are indicated in Tables 5.1-5.2 and Figures 5.1-5.2. All analysis of variance table by factorial treatment effects and interaction of fungal growth clearly indicated that media and fungal isolates affected growth and sporulation as well as an interaction of media and fungal isolates ($P<0.01$) which showed in Tables 1-5 of appendix.

CMA medium showed significantly promoted the growth of most nematophagous fungi; *Arthrobotrys oligospora* isolate DLO1-001 and MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, and MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003. Almost all fungi grew rapidly on this medium reaching 9 cm in diameter after 7 days at 27°C especially fungal isolate of *Arthrobotrys*. PCA media were second for suitable nematophagous fungi culture whereas YPG caused fluffy and bushy hyphae so it was poor quality for growth media, except *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 showed nearby response of mycelial growth on all media (Table 5.1).

A general culture medium for sporulation of selected nematophagous fungi was PCA. The results showed PCA induced the higest sporulation of *Arthrobotrys oligospora* isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003. However, variations were noted among the isolates. For example, V-8 juice agar (VA) was the best medium for inducing sporulation of *Arthrobotrys oligospora* isolate DLO1-001 and *A. thaumasium* isolate MPI1-003, but *A. musiformis* isolate MSO1-001 required YPG (Table 5.2). Each medium caused different growth, colony characterization and sporulation of each fungus, but genus *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 presented a similar pattern in vegetative and reproductive stage on all media (Figure 5.2).

Table 5.1 Effect of six media on growth of nematophagous fungi at 3, 5, 7 and 10 days after incubation

Fungal isolates	Colony diameter on media (cm)[1/]																							
	3 days						5 days						7 days						10 days					
	PDA	½PDA	CMA	VA	PCA	YPG	PDA	½PDA	CMA	VA	PCA	YPG	PDA	½PDA	CMA	VA	PCA	YPG	PDA	½PDA	CMA	VA	PCA	YPG
DLO1-001	3.50	3.36	4.36	3.36	4.20	3.46	6.83	6.96	8.83	6.56	6.80	6.30	9.00	9.00	9.00	9.00	9.00	7.70	9.00	9.00	9.00	9.00	9.00	9.00
A. oligospora	fg[2/]	f-i	b	f-i	bc	f-h	ij	h-j	a	k	j	lm	a	a	a	a	a	d	a	a	a	a	a	a
MTI2-001	3.86	3.80	4.03	3.30	3.33	2.86	7.43	7.23	8.50	7.06	6.10	5.40	9.00	9.00	9.00	9.00	9.00	6.26	9.00	9.00	9.00	9.00	9.00	6.66
A. oligospora	de	e	cd	h-j	g-i	k	de	fg	b	gh	n	o	a	a	a	a	a	g	a	a	a	a	a	e
API3-001	2.90	3.26	4.73	2.36	3.53	2.36	5.56	7.53	8.63	4.73	7.23	4.33	8.50	9.00	9.00	6.90	9.00	6.40	9.00	9.00	9.00	9.00	9.00	8.10
A. conoides	k	ij	a	lm	f	lm	o	d	b	q	fg	r	c	a	a	e	a	g	a	a	a	a	a	c
JDI1-001	3.33	3.50	3.40	3.16	2.93	2.06	6.96	7.03	7.60	6.16	6.06	4.40	8.83	9.00	9.00	8.73	9.00	6.66	9.00	9.00	9.00	9.00	9.00	9.00
A. thaumasium	g-j	fg	f-i	j	k	n	h-j	h	cd	mn	n	r	b	a	a	b	a	f	a	a	a	a	a	a
MPI1-003	3.23	3.26	3.33	3.30	2.90	1.20	6.83	7.33	7.76	7.00	6.20	3.36	9.00	9.00	9.00	9.00	9.00	5.43	9.00	9.00	9.00	9.00	9.00	7.60
A. thaumasium	ij	ij	g-j	h-j	k	r	ij	ef	c	hi	l-n	t	a	a	a	a	a	h	a	a	a	a	a	d
MSO1-001	2.50	2.26	4.36	3.53	2.83	2.46	3.73	5.06	7.06	6.36	5.53	4.76	4.46	6.93	9.00	8.83	7.56	6.66	5.16	8.36	9.00	9.00	9.00	9.00
A. musiformis	l	m	b	f	K	l	s	P	gh	l	o	q	i	e	a	b	d	f	h	b	a	a	a	a
WJI1-003	1.73	1.60	1.73	1.56	1.63	1.56	2.93	2.93	3.03	2.63	2.83	3.13	4.00	3.90	4.00	3.43	3.73	4.26	5.36	5.00	5.36	4.43	4.93	5.70
Paecilomyces sp.	o	o-q	o	o-q	op	pq	vw	vw	uv	y	wx	u	k	k	k	n	l	j	g	i	g	m	ij	f
KJO1-003	1.56	1.53	1.63	1.43	1.50	1.53	2.86	2.66	2.63	2.56	2.63	2.63	3.66	3.56	3.56	3.46	3.43	3.46	4.86	4.73	4.76	4.46	4.53	4.83
Pochonia sp.	o-q	pq	op	q	pq	pq	vw	xy	y	y	y	y	lm	mn	mn	n	n	n	jk	l	kl	m	m	j-l
CV %[3/]	2.88						1.53						1.01						0.68					

[1/] Mean of colony diameter of each fungus 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%.

Table 5.2 Effect of six media on sporulation of nematophagous fungi at 10 days after incubation

Fungal isolates	Sporulation on media (×10^4 spore/ml)[1/]					
	potato dextrose agar (PDA)	**½potato dextrose agar (½PDA)**	**corn meal agar (CMA)**	**V-8 juice agar (VA)**	**potato carrot agar (PCA)**	**Yeast extract-peptone-glycerol (YPG)**
DLO1-001	6.03	29.03	23.66	141.67	16.86	40.00
A. oligospora	m[2/]	i	j	ef	k	g
MTI2-001	0.00	0.43	1.93	1.63	2.86	0.36
A. oligospora	v	t-v	q-t	r-u	p-r	uv
API3-001	0.00	0.00	0.00	0.53	2.06	0.53
A. conoides	v	v	v	t-v	q-s	t-v
JDI1-001	5.83	16.66	6.20	4.83	31.56	0.33
A. thaumasium	mn	k	m	m-o	hi	uv
MPI1-003	5.06	6.30	1.60	9.03	5.63	3.96
A. thaumasium	m-o	m	r-u	l	mn	n-p
MSO1-001	0.76	0.66	0.50	1.90	0.66	4.90
A. musiformis	s-v	s-v	t-v	q-t	s-v	m-o
WJI1-003	410.00	131.6)	384.33	154.00	542.33	258.33
Paecilomyces sp.	b	f	b	e	a	cd
KJO1-003	280.67	3.563	36.66	0.00	476.00	234.67
Pochonia sp.	c	o-q	gh	v	a	d
CV%[3/]	1.89					

[1/] Mean of conidia of each fungus 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%.

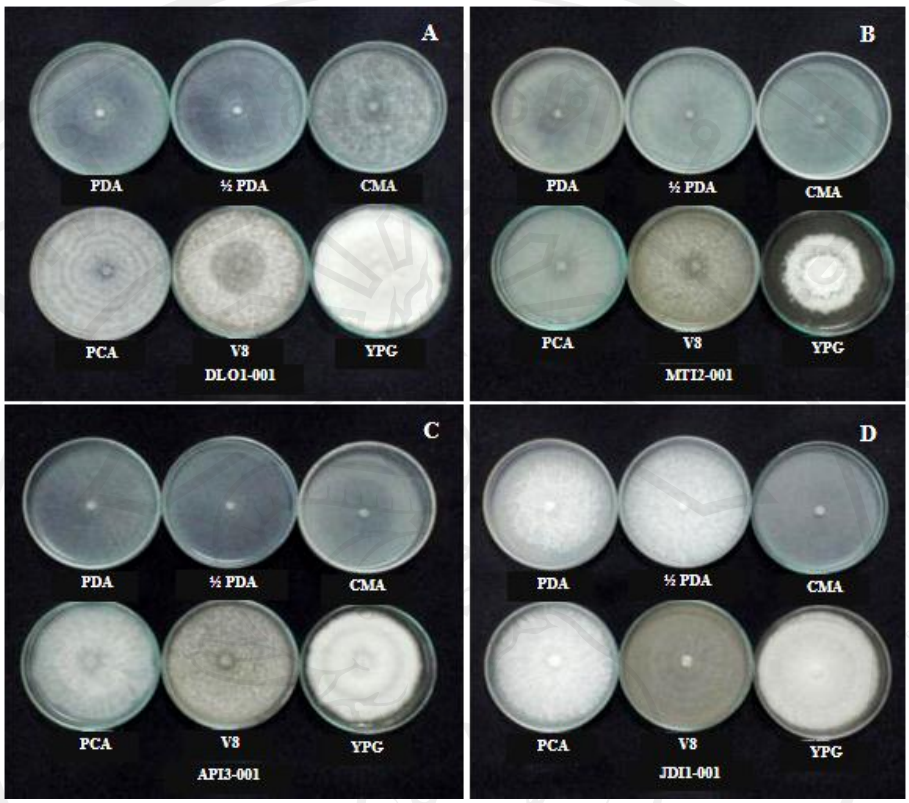

Figure 5.1 Colony characterizations of four nematophagous fungi on media 10 days after incubation. First row show PDA, ½ PDA, CMA and second row show PCA, V8 and YPG, respectively. (A) *Arthrobotrys oligospora* isolate DLO1-001 (B) *Arthrobotrys oligospora* isolate MTI2-001 (C) *Arthrobotrys conoides* isolate API3-001 and (D) *Arthrobotrys thaumasium* isolate JDI1-001.

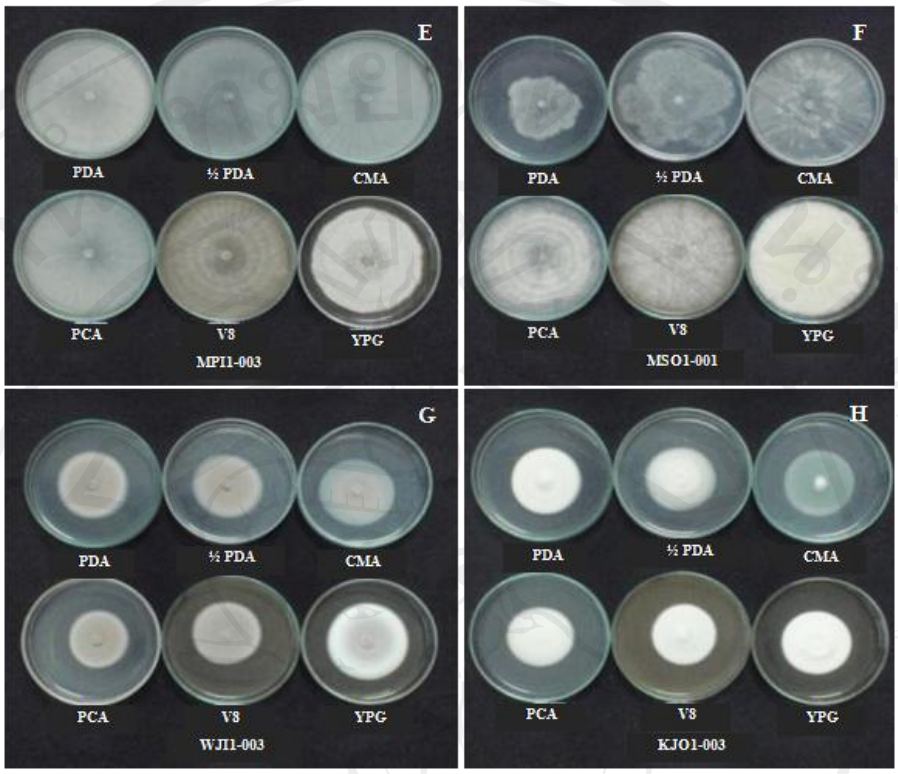

Figure 5.2 Colony characterizations of four nematophagous fungi on media 10 days after incubation. First row show PDA, ½ PDA, CMA and second row show PCA, V8 and YPG, respectively. (E) *Arthrobotrys thaumasium* isolate MPI1-003 (F) *Arthrobotrys musiformis* isolate MSO1-001 (G) *Paecilomyces* sp. isolate WJI1-003 and (H) *Pochonia* sp. isolate KJO1-003.

The result of different temperature influences to growth (3, 5, 7 and 10 days) and sporulation (10 days) of fungi incubating on potato dextrose agar (PDA) at 15, 20, 25, 30 and 35^{o}C are presented in Tables 5.3-5.4 and Figures 5.3-5.4. The analysis of variance table by factorial treatment effects and interaction of fungal growth indicated significant statistics ($P<0.01$) which showed in Tables 6-10 of appendix. Temperature and interaction of temperature and fungal isolate had an impact on growth and sporulation.

There was unidirectional feed back from all fungal isolates; *Arthrobotrys oligospora* isolate DLO1-001, *Arthrobotrys oligospora* isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, *Arthrobotrys thaumasium* isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003. Incubating at 30 ^{o}C was appropriate condition for vegetative stage following 25^{o}C, 20^{o}C and 15^{o}C, respectively; nevertheless, 35^{o}C causing slow growth of most fungi, except *Pochonia* sp. isolate KJO1-003. Moreover, this trial showed incubation of 25^{o}C and 30^{o}C stimulating massive sporulation.

Table 5.3 Effect of five temperatures on growth of nematophagous fungi at 3, 5, 7 and 10 days after incubation

Fungal isolates	Colony diameter on temperatures (cm)[1/]																			
	3 days					5 days					7 days					10 days				
	15˚C	20˚C	25˚C	30˚C	35˚C	15˚C	20˚C	25˚C	30˚C	35˚C	15˚C	20˚C	25˚C	30˚C	35˚C	15˚C	20˚C	25˚C	30˚C	35˚C
DLO1-001	2.05	2.53	3.08	3.30	0.88	4.33	5.71	6.75	6.43	0.71	5.71	7.53	8.80	8.85	1.01	8.86	9.00	9.00	9.00	1.00
A. oligospora	f-h[2/]	e	b	a	p-r	gh	d	a	ab	u	j	e	ab	a	v	a	a	a	a	n
MTI2-001	1.10	1.80	2.06	2.10	0.70	3.28	4.26	5.35	4.80	0.71	5.00	6.30	7.40	7.11	0.78	7.86	7.86	9.00	8.80	0.71
A. oligospora	l-o	ij	f-h	f-h	r	l	g-i	e	f	u	l	hi	ef	fg	v	c	c	a	ab	n
API3-001	1.83	2.11	2.51	1.90	0.93	3.81	4.51	5.81	5.25	1.16	5.33	6.53	8.03	7.03	1.81	7.70	8.53	9.00	9.00	1.51
A. conoides	i	fg	e	hi	o-q	j	fg	d	e	st	k	h	d	g	u	c	b	a	a	m
JDI1-001	1.20	1.53	2.13	2.75	2.63	3.13	3.98	5.25	6.16	4.70	4.63	5.33	7.53	8.43	4.26	7.23	8.80	8.76	8.96	6.06
A. thaumasium	lm	k	fg	cd	de	l	ij	e	bc	f	m	k	e	c	n	d	ab	ab	a	e
MPI1-003	1.16	1.61	2.13	2.60	0.88	2.71	4.18	5.23	5.85	0.95	4.35	6.16	8.35	8.5	0.96	7.06	8.73	9.00	9.00	0.90
A. thaumasium	l-n	jk	fg	de	p-r	m	hi	e	cd	tu	mn	i	cd	bc	v	d	ab	a	a	n
MSO1-001	1.80	1.96	2.21	2.88	0.78	3.48	3.70	4.53	5.26	2.73	4.36	5.03	6.61	7.65	3.41	6.26	7.20	9.00	9.00	4.26
A. musiformis	ij	g-i	f	bc	qr	kl	jk	fg	e	m	mn	kl	h	e	op	e	d	a	a	g
WJI1-003	0.78	1.03	1.06	1.18	0.96	1.46	1.65	2.23	2.61	1.76	2.05	2.23	3.00	3.51	2.00	2.63	3.00	3.95	5.01	2.90
Paecilomyces sp.	qr	l-p	l-p	lm	n-q	q-s	p-r	o	mn	pq	s-u	st	qr	op	tu	kl	j	gh	f	jk
KJO1-003	0.90	1.01	1.23	1.48	1.03	1.43	1.80	2.30	2.80	1.80	1.78	2.33	2.90	3.65	3.30	2.33	3.01	3.93	5.00	3.60
Pochonia sp.	o-r	m-p	l	k	l-p	rs	p	no	m	p	u	s	r	o	pq	l	j	h	f	i
CV%[3/]	5.90					4.07					3.01					2.34				

[1/] Mean of colony diameter of each fungus 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%.

Table 5.4 Effect of five temperatures on sporulation of nematophagous fungi at 10 days after incubation

Fungal isolates	Sporulation on temperatures ($\times 10^4$ spore/ml)[1/]				
	15˚C	**20˚C**	**25˚C**	**30˚C**	**35˚C**
DLO1-001 *A. oligospora*	1.66 o-q[2/]	13.00 i	25.63 h	32.56 g	1.43 o-r
MTI2-001 *A. oligospora*	0.00 t	1.10 l-n	1.13 kl	1.11 lm	1.10 l-n
API3-001 *A. conoides*	0.00 t	2.16 m-o	3.44 kl	3.36 kl	0.63 r-t
JDI1-001 *A. thaumasium*	0.73 r-t	0.51 st	4.20 jk	1.06 p-s	0.53 st
MPI1-003 *A. thaumasium*	0.83 q-t	1.90 n-p	0.56 r-t	2.20 m-o	0.63 r-t
MSO1-001 *A. musiformis*	0.80 q-t	1.16 p-s	0.69 r-t	5.30 j	2.83 l-n
WJI1-003 *Paecilomyces* sp.	1122.00 d	3058.30 c	6786.30 a	4413.30 b	756.33 f
KJO1-003 *Pochonia* sp.	0.00 t	0.00 t	0.00 t	0.00 t	653.33 f
CV%[3/]	1.14				

[1/] Mean of conidia of each fungus 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%.

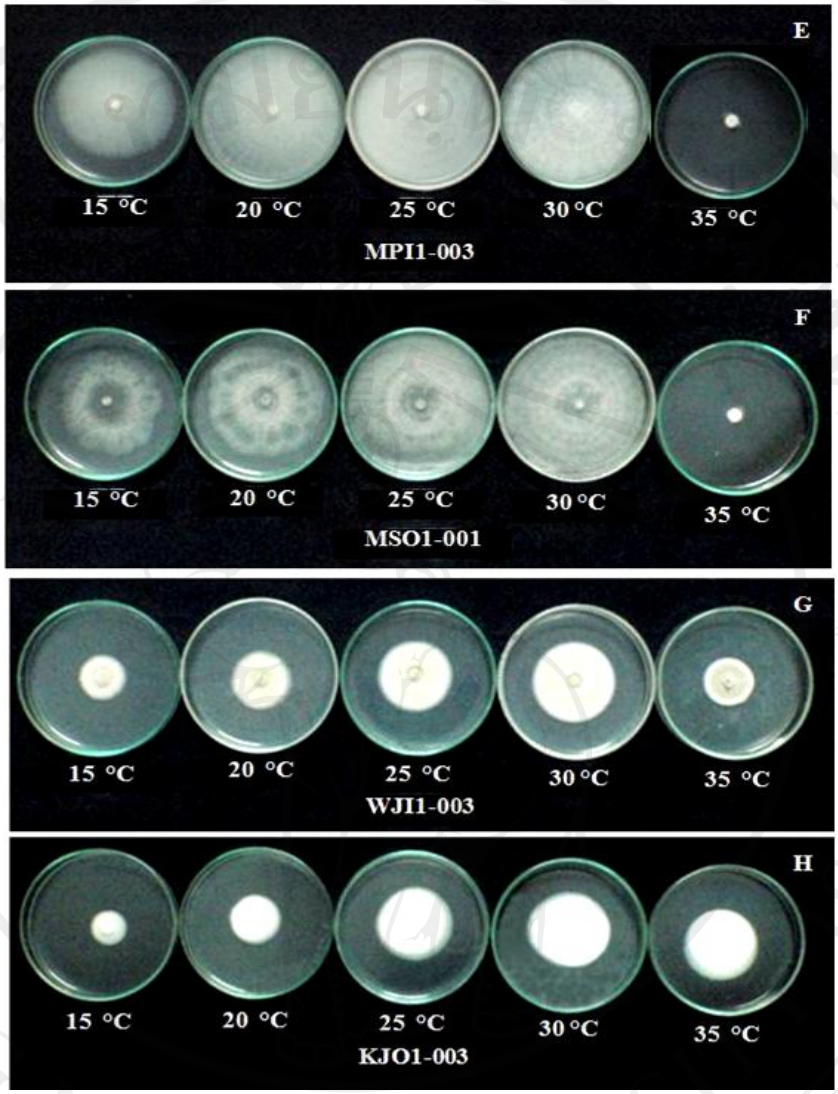

Figure 5.3 Colony characterizations of four nematophagous fungi comparing various temperatures on PDA 10 days after incubation. (A) *Arthrobotrys oligospora* isolate DLO1-001 (B) *Arthrobotrys oligospora* isolate MTI2-001 (C) *Arthrobotrys conoides* isolate API3-001 and (D) *Arthrobotrys thaumasium* isolate JDI1-001.

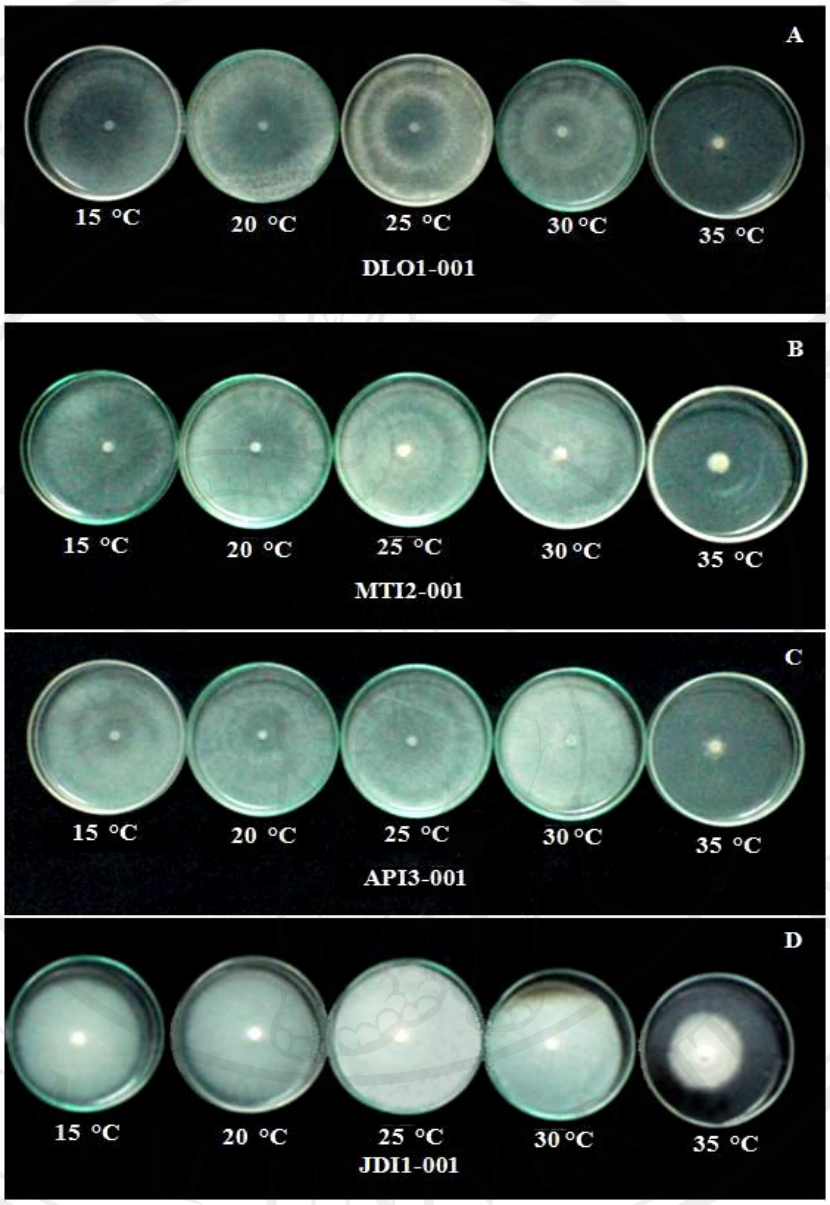

Figure 5.4 Colony characterizations of four nematophagous fungi comparing various temperatures on PDA 10 days after incubation. (E) *Arthrobotrys thaumasium* isolate MPI1-003 (F) *Arthrobotrys musiformis* isolate MSO1-001 (G) *Paecilomyces* sp. isolate WJI1-003 and (H) *Pochonia* sp. isolate KJO1-003.

The effect of four different light conditions comprised of 24 hour light, 12 hour light and 12 hour dark, 24 hour dark and 24 hour black light (NUV) on the growth (3, 5, 7 and 10 days) and sporulation (10 days) of fungi incubated on potato dextrose agar (PDA) at room temperature (27±3ºC) are indicated in Tables 5.5-5.6 and Figures 5.5-5.6. This experiment showed highly significant difference of statistical analysis. Both of light conditions and the interaction of light conditions and fungal isolate had an effect on fungal growth and sporulation (Tables 11-15 of appendix).

Incubation at 24 hour dark was a suitable condition for fungal colony growing because *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003 and *Arthrobotrys musiformis* isolate MSO1-001 reached 9 cm in growth as well as *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 produced a high level of growth after 10 day incubation. 12 hour light and 12 hour dark produced a moderate level of growth (Table 5.5).

Light condition may be a significant influence of sporulation because all fungi incubating in either 24 hour light or 12 hour light and 12 hour dark regime produced the highest number of conidia except *Paecilomyces* sp. isolate KJO1-003 which required 24 hour dark (Table 5.6).

Table 5.5 Effect of four light regimes on growth of nematophagous fungi at 3, 5, 7 and 10 days after incubation

Fungal isolates	Colony diameter on lights (cm)[1/]															
	3 days				5 days				7 days				10 days			
	24 L	12L/ 12D	24 D	24 NUV	24 L	12L/ 12D	24 D	24 NUV	24 L	12L/ 12D	24 D	24 NUV	24 L	12L/ 12D	24 D	24 NUV
DLO1-001	4.26	4.16	4.63	3.63	7.36	7.96	8.26	6.96	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. oligospora	bc[2/]	b-d	a	hi	d	b	a	ef	a	a	a	a	a	a	a	a
MTI2-001	4.16	3.76	4.13	3.93	7.73	6.93	7.60	7.10	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. oligospora	b-d	f-h	cd	ef	c	ef	c	e	a	a	a	a	a	a	a	a
API3-001	1.30	1.20	3.13	0.73	2.00	2.36	6.80	1.33	2.93	3.33	9.00	2.33	3.60	4.23	9.00	3.20
A. conoides	r	r	k	s	p	o	fg	q	n	m	a	o	i	h	a	j
JDI1-001	3.93	3.86	4.33	3.36	8.06	7.33	7.70	5.80	9.00	9.00	9.00	8.30	9.00	9.00	9.00	9.00
A. thaumasium	ef	e-g	b	j	b	d	c	i	a	a	a	e	a	a	a	a
MPI1-003	4.03	3.70	3.86	3.86	7.33	6.66	7.56	6.83	9.00	8.46	9.00	8.60	9.00	9.00	9.00	9.00
A. thaumasium	de	g-i	e-g	e-g	d	gh	c	fg	a	d	a	c	a	a	a	a
MSO1-001	1.93	3.16	3.53	2.66	4.03	5.96	6.53	5.30	6.16	7.90	8.83	6.30	7.53	9.00	9.00	7.26
A. musiformis	m	k	ij	l	k	i	h	j	h	f	b	g	b	a	a	c
WJI1-003	1.56	1.76	1.66	1.60	3.03	3.23	3.23	3.03	4.36	4.23	4.26	4.30	5.20	5.16	5.33	5.43
Paecilomyces sp.	q	m-p	o-q	pq	m	l	l	m	i	j	ij	ij	e	e	d	d
KJO1-003	1.86	1.80	1.80	1.73	2.96	2.96	3.06	2.76	4.03	3.90	4.03	3.96	5.00	4.83	4.90	4.86
Pochonia sp.	mn	m-o	m-o	n-q	m	m	lm	n	k	l	k	kl	f	g	fg	g
CV%[3/]	2.80				1.55				0.88				0.75			

[1/] Mean of colony diameter of each fungus 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%.

Table 5.6 Effect of four different light regimes on sporulation of nematophagous fungi at 10 days after incubation

Fungal isolates	Sporulation on lights (×10^4 spore/ml)[1/]			
	24 hour light	12 hour light and 12 hour dark	24 hour dark	24 hour black light (NUV)
DLO1-001	1.23	5.10	0.96	2.50
A. oligospora	i-k[2/]	g	i-k	hi
MTI2-001	1.43	2.10	0.83	1.00
A. oligospora	i-k	h-j	i-k	i-k
API3-001	0.00	0.00	0.00	0.00
A. conoides	k	k	k	k
JDI1-001	1.73	1.76	0.93	0.56
A. thaumasium	i-k	i-k	i-k	i-k
MPI1-003	0.63	0.00	0.00	0.43
A. thaumasium	i-k	k	k	i-k
MSO1-001	1.16	0.00	0.00	0.36
A. musiformis	i-k	k	k	jk
WJI1-003	488.67	271.33	254.00	169.67
Paecilomyces sp.	a	b	b	c
KJO1-003	52.00	4.23	86.00	20.00
Pochonia sp.	e	gh	d	f
CV%[3/]	2.83			

[1/] Mean of conidia of each fungus 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%.

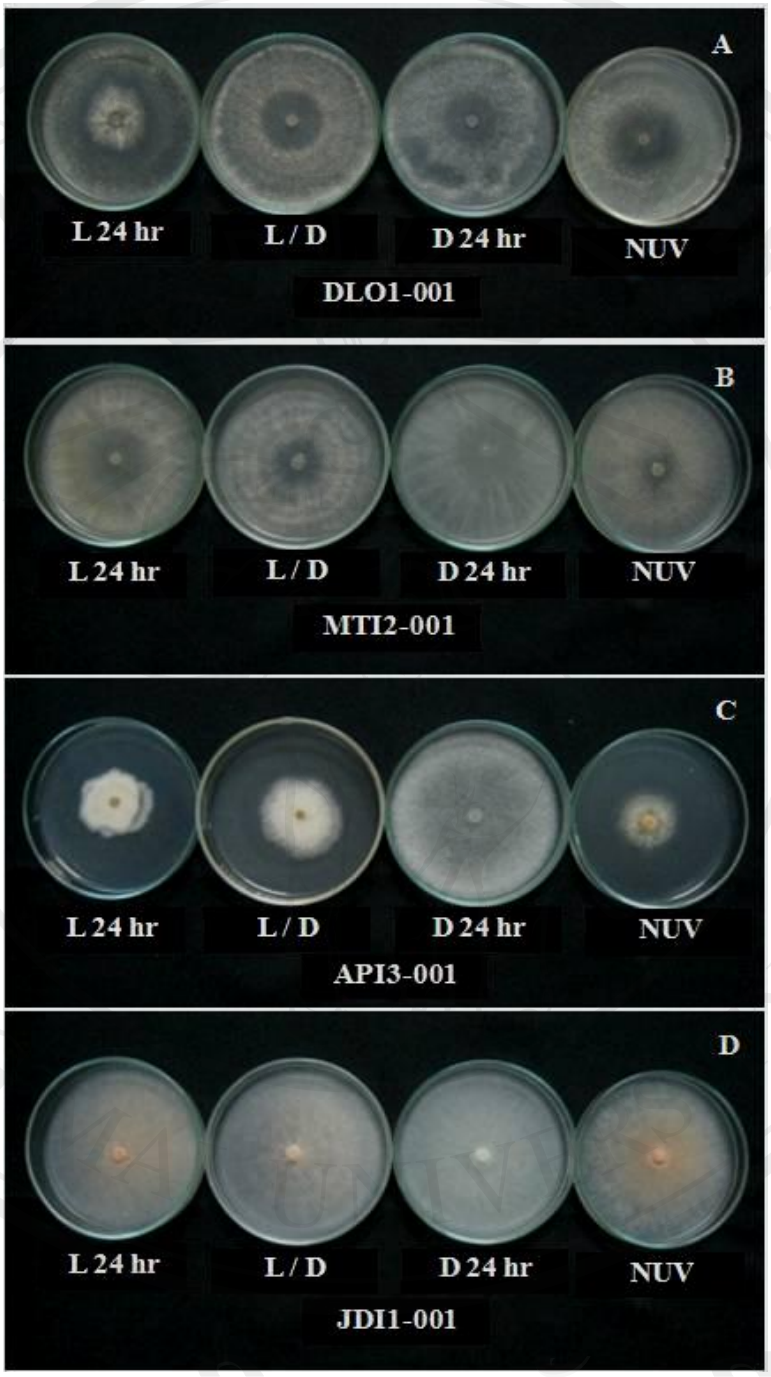

Figure 5.5 Colony characterizations of four nematophagous fungi comparing various light regimes on PDA 10 days after incubation. (A) *Arthrobotrys oligospora* isolate DLO1-001 (B) *Arthrobotrys oligospora* isolate MTI2-001 (C) *Arthrobotrys conoides* isolate API3-001 and (D) *Arthrobotrys thaumasium* isolate JDI1-001.

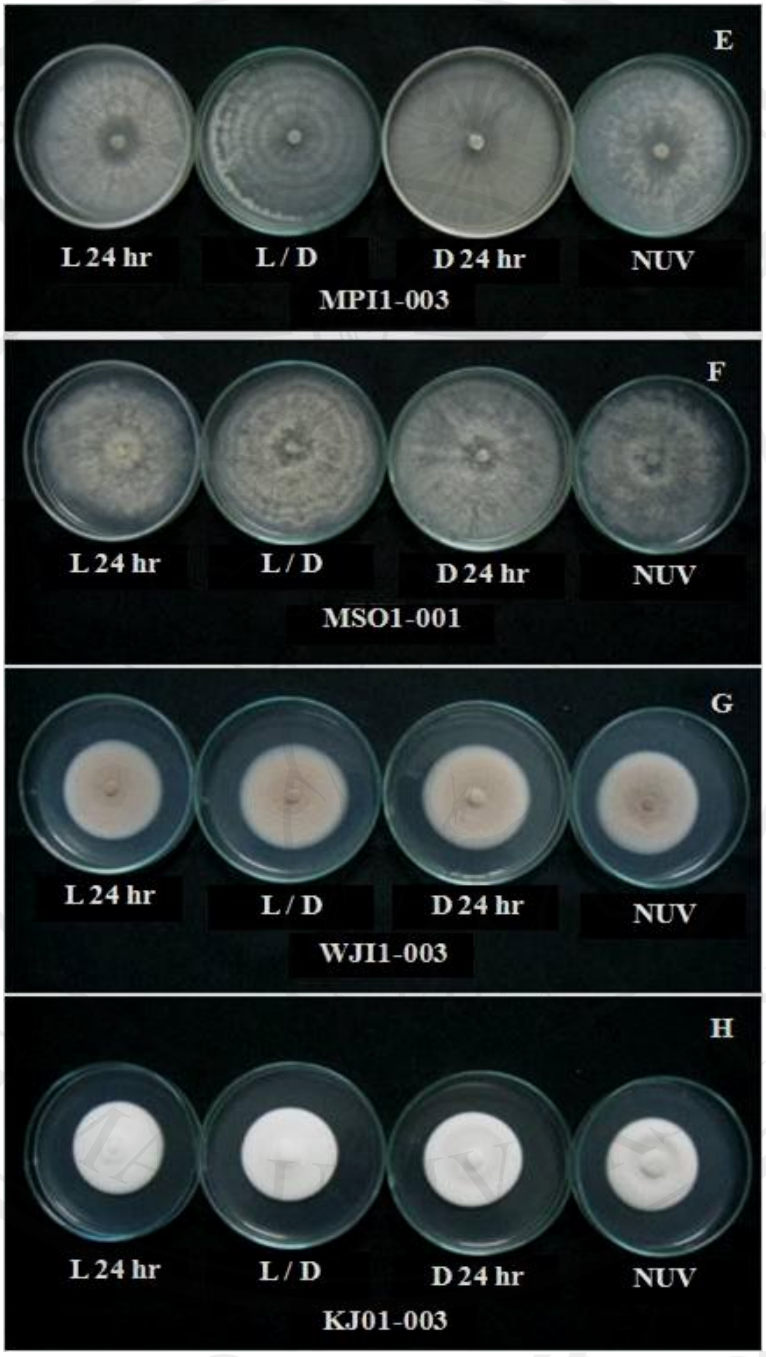

Figure 5.6 Colony characterizations of four nematophagous fungi comparing various light regimes on PDA 10 days after incubation. (E) *Arthrobotrys thaumasium* isolate MPI1-003 (F) *Arthrobotrys musiformis* isolate MSO1-001 (G) *Paecilomyces* sp. isolate WJI1-003 and (H) *Pochonia* sp. isolate KJO1-003.

The comparison of the effects of pH levels in the range of 3-10 on growth (3, 5, 7 and 10 days) and sporulation (10 days) of selected fungi at room temperature (27±3ºC) are presented in Tables 5.7-5.9 and Figures 5.7-5.8. The analysis of variance sources showed high significantly result of pH level and an interaction of pH level and fungal isolate effecting growth and sporulation (Tables 16-20 of appendix).

Generally, all fungi; *Arthrobotrys oligospora* isolate DLO1-001, *Arthrobotrys oligospora* isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, *Arthrobotrys thaumasium* isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 grew rapidly in pH 8 (alkaline state) and pH 7 (neutral state) followed by pH 9, but growth at pH 3 was noticeably reduced. Colony characterization of each fungus comparing pH 3-10 on PDA 10 days after incubation appeared similar. Most fungi were induced by pH 8 and pH 7 to produce the greatest number of conidia and highest level of mycelial development.

Table 5.7 Effect of pH on growth of nematophagous fungiat 3 and 5 days after incubation

Fungal isolates	Colony diameter on pH (cm)[1/]															
	3 days								5 days							
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
DLO1-001	2.63	4.65	4.56	4.73	5.53	5.43	5.26	5.00	5.16	7.50	7.60	7.63	8.86	8.56	8.43	8.06
A. oligospora	v[2/]	d-f	e-g	d	a	a	b	c	v	d-f	de	d	a	b	b	c
MTI2-001	1.83	3.41	3.33	3.13	4.20	4.60	4.40	3.91	3.16	6.28	5.75	5.73	7.00	7.46	7.43	6.48
A. oligospora	ab	rs	s	t	kl	d-g	h-j	m-o	abc	qr	st	t	kl	d-g	e-g	m-p
API3-001	2.40	3.76	3.63	4.16	4.46	4.40	4.33	3.80	4.03	6.50	6.43	7.10	7.63	7.53	7.20	6.43
A. conoides	w	op	pq	l	g-i	h-j	i-k	no	x	m-o	n-q	j-l	d	de	h-j	n-q
JDI1-001	2.56	3.40	3.33	3.76	4.33	3.96	3.96	3.50	4.30	6.13	6.36	7.13	7.46	7.20	7.00	6.30
A. thaumasium	v	rs	s	op	i-k	m	m	qr	w	r	o-q	i-k	d-g	h-j	kl	p-r
MPI1-003	2.38	3.43	3.10	3.90	3.93	3.86	3.30	2.93	3.66	5.90	5.53	5.93	6.16	6.16	5.75	5.20
A. thaumasium	wx	rs	t	m-o	mn	m-o	s	u	y	st	u	s	mn	mn	st	v
MSO1-001	2.23	4.26	4.66	4.70	4.90	5.16	4.56	4.53	4.06	6.26	6.66	7.30	7.10	7.33	6.93	6.66
A. musiformis	x	j-l	d-f	de	c	b	e-g	f-h	x	qr	m	g-i	j-l	f-h	l	m
WJI1-003	1.33	1.88	1.76	2.03	1.96	2.06	2.03	2.06	2.10	3.13	3.00	3.23	3.30	3.40	3.33	3.33
Paecilomyces sp.	f	z-b	b-d	yz	y-a	y	yz	y	g	bc	cd	z-b	z-b	z	za	za
KJO1-003	0.96	1.28	1.21	1.50	1.81	1.76	1.65	1.66	1.41	1.96	1.85	2.36	2.86	2.86	2.93	2.73
Pochonia sp.	g	f	f	e	a-c	b-d	de	cd	i	gh	h	f	de	de	d	e
CV%[3/]	2.18								1.60							

[1/] Mean of colony diameter of each fungus 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%.

Table 5.8 Effect of pH on growth of nematophagous fungi at 7 and 10 days after incubation

Fungal isolates	Colony diameter on pH (cm)[1/]															
	7 days								10 days							
	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
DLO1-001	7.06	8.93	8.73	8.80	9.00	9.00	9.00	9.00	9.00	9.00	8.93	9.00	9.00	9.00	9.00	9.00
A. oligospora	n[2/]	ab	b-g	a-f	a	a	a	a	a	a	a	a	a	a	a	a
MTI2-001	4.65	8.20	6.16	8.23	9.00	8.91	9.00	8.70	5.86	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. oligospora	st	kl	p	kl	a	a-c	a	c-h	i	a	a	a	a	a	a	a
API3-001	5.43	8.23	8.20	8.63	8.93	8.93	8.70	8.03	7.06	9.00	8.90	9.00	9.00	9.00	9.00	8.73
A. conoides	q	kl	kl	e-i	ab	ab	c-h	l	e	a	a	a	a	a	a	b
JDI1-001	6.10	8.53	8.50	9.00	9.00	9.00	8.90	8.66	7.63	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. thaumasium	p	g-i	hi	a	a	a	a-c	d-i	d	a	a	a	a	a	a	a
MPI1-003	5.06	8.26	7.73	8.03	8.60	8.70	8.13	7.20	6.33	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. thaumasium	r	jk	m	l	f-i	c-h	kl	n	g	a	a	a	a	a	a	a
MSO1-001	6.53	8.20	8.46	8.86	8.76	9.00	8.83	8.633	8.23	9.00	9.00	9.00	9.00	9.00	9.00	9.00
A. musiformis	o	kl	ij	a-d	b-f	a	a-e	e-i	c	a	a	a	a	a	a	a
WJI1-003	2.88	4.36	4.10	4.53	4.73	4.80	4.76	4.76	3.88	5.78	5.55	6.16	6.36	6.46	6.36	6.40
Paecilomyces sp.	x	u	v	tu	st	s	s	s	o	ij	l	h	fg	f	fg	fg
KJO1-003	1.85	2.48	2.41	3.13	4.00	4.03	4.06	3.96	2.38	3.33	3.16	4.35	5.36	5.50	5.68	5.56
Pochonia sp.	z	y	y	w	v	v	v	v	r	p	q	n	m	l	jk	kl
CV%[3/]	1.45								0.76							

[1/] Mean of colony diameter of each fungus 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%.

Table 5.9 Effect of pH on sporulation of nematophagous fungi at 10 days after incubation

Fungal isolates	Sporulation on pH (×10^4 spore/ml)[1/]							
	pH 3	**pH 4**	**pH 5**	**pH 6**	**pH 7**	**pH 8**	**pH 9**	**pH 10**
DLO1-001	1.36	0.46	0.63	0.76	0.63	0.90	2.90	0.83
A. oligospora	k-m[2/]	lm	lm	lm	lm	lm	jk	lm
MTI2-001	0.00	0.00	0.50	1.16	0.43	4.26	0.93	0.80
A. oligospora	m	m	lm	k-m	lm	ij	lm	lm
API3-001	0.63	4.16	1.60	1.00	1.00	0.56	0.00	0.00
A. conoides	lm	ij	k-m	m	m	lm	m	m
JDI1-001	0.00	1.50	0.60	0.00	0.56	0.56	0.66	0.00
A. thaumasium	m	k-m	lm	m	lm	lm	lm	m
MPI1-003	0.00	0.00	0.00	0.60	0.00	0.50	0.80	0.53
A. thaumasium	m	m	m	lm	m	lm	lm	lm
MSO1-001	0.53	0.56	1.66	0.00	1.33	0.00	0.00	0.70
A. musiformis	lm	lm	kl	m	k-m	m	m	lm
WJI1-003	1472.00	527.33	719.67	715.67	1740.70	361.00	110.67	412.67
Paecilomyces sp.	b	d	c	c	a	e	g	e
KJO1-003	0.00	1.36	0.00	6.66	0.00	218.33	11.33	0.00
Pochonia sp.	m	k-m	m	i	m	f	h	m
CV%[3/]	2.73							

[1/] Mean of conidia of each fungus 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%.

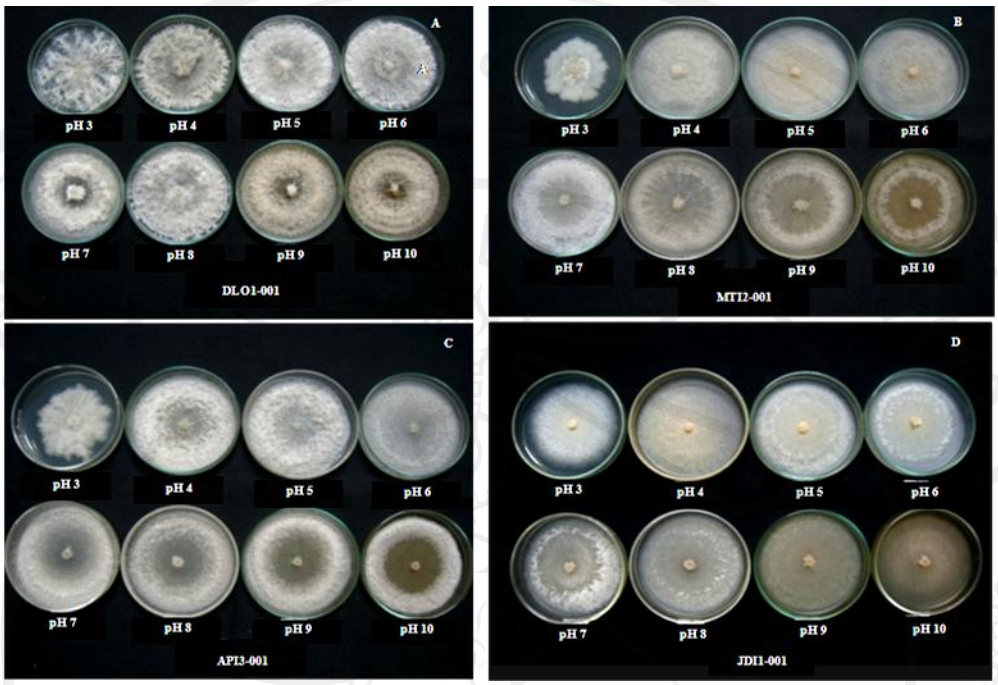

Figure 5.7 Colony characterizations of four nematophagous fungi comparing pH 3-10 on PDA 10 days after incubation. (A) *Arthrobotrys oligospora* isolate DLO1-001 (B) *Arthrobotrys oligospora* isolate MTI2-001 (C) *Arthrobotrys conoides* isolate API3-001 and (D) *Arthrobotrys thaumasium* isolate JDI1-001.

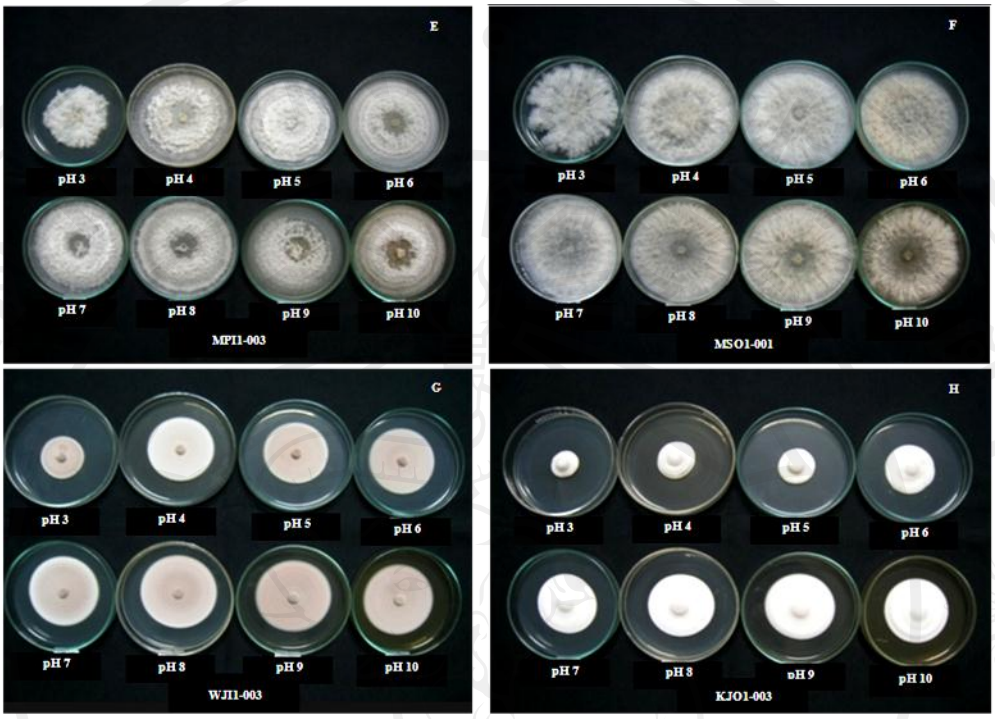

Figure 5.8 Colony characterizations of four nematophagous fungi comparing pH 3-10 on PDA 10 days after incubation. (E) *Arthrobotrys thaumasium* isolate MPI1-003 (F) *Arthrobotrys musiformis* isolate MSO1-001 (G) *Paecilomyces* sp. isolate WJI1-003 and (H) *Pochonia* sp. isolate KJO1-003.

5.3.2 Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi

The effects of six insecticides including dazomet (Basamid-G 98 % GR®), dinotefuran (Starkle-G 1% GR®), lambda-cyhalothrin (Karate 2.5 % W/V CS®), methomyl (Lannate 40 % SP®), carbaryl (Sevin 85 % WP®), chlorpyrifos (Losban 40 % W/V EC®) on growth (3, 5, 7 and 10 days) and sporulation (10 days) of nematophagous fungi are shown in Table 5.10-5.14. Insecticide, rate, fungal isolate and their interactions affected fungal vegetative and reproductive stage; these values were significantly different ($P<0.01$) which showed in Tables 21-25 of appendix.

All insecticides at all rates (2x, 1x, 1/2x and 1/3x the recommended rate) affected the development of all fungi; *Arthrobotrys oligospora* isolate DLO1-001, *Arthrobotrys oligospora* isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, *Arthrobotrys thaumasium* isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003.

Dazomet (2,450 ppm a.i.), carbaryl (2,975 ppm a.i.) and chlorpyrifos (1,500 ppm a.i.), rates which are normally used in soil caused high radial mycelia growth inhibition. On the other hand lambda-cyhalothrin (62.5 ppm a.i.) and methomyl (700 ppm a.i.) had low-high effect on growth and appearance which caused low inhibition in genus *Arthrobotrys* but moderately high inhibition in *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003.

Arthrobotrys thaumasium isolate JDI1-001 was the first isolate showing a slow or balky growth response at 3 days after inoculation (Table 5.10). Growth of most fungi except *Paecilomyces* sp. isolate WJI1-003 was inhibited 100% by

dazomet at all rates. Moreover, *P. lilacinus* isolate WJI1-003, *A. thaumasium* isolate JDI1-001 and *A. musiformis* isolate MSO1-001 were completely inhibited by dinotefuran treatment after 5 days. At 10 days after incubation, carbaryl usage caused 60-100 % inhibition of all fungal isolates especially *A. conoides* isolate API3-001. On the other hand, the 1x, 1/2x and 1/3x of the recommended rate of methomyl had low effect on *A. oligospora* isolate DLO1-001 and isolate MTI2-001.

Sporulation of most fungi was correlated with growth so non production of conidia was detected in many cases such as with all fungi at all rates of dazomet, five fungal isolates except *A. conoides* isolate API3-001, *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 at all rates of dinotefuran, seven fungal isolates except *Pochonia* sp. isolate KJO1-003 at all rates of carbaryl and chlorpyrifos. However, the number of conidia of sporulating fungi was lower than control except as a result of lambda-cyhalothrin affecting *Paecilomyces* sp. isolate WJI1-003 at 1/2x and 1/3x of the recommended rates.

Table 5.10 Effect of various insecticides on growth of nematophagous fungi on potato dextrose agar at 3 days after incubation

Fungal isolates	Growth inhibition of insecticides(%)[1/]																							
	Dazomet (2,450 ppm a.i.)				Dinotefuran (40 ppm a.i.)				Lambda-cyhalothrin (62.5 ppm a.i.)				Methomyl (700 ppm a.i.)				Carbaryl (2,975 ppm a.i.)				Chlorpyrifos (1,500 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	0.00 f[2/]	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
MTI2-001 *A. oligospora*	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
API3-001 *A. conoides*	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
JDI1-001 *A. thaumasium*	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	56.25 c	54.16 c	31.25 e	54.16 c	66.66 b	47.91 d	33.33 e	33.33 e	100 a	100 a	100 a	100 a	100 a	64.58 b	47.91 d	33.33 e
MPI1-003 *A. thaumasium*	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
MSO1-001 *A. musiformis*	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
WJI1-003 *Paecilomyces* sp.	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
KJO1-003 *Pochonia* sp.	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
CV%[3/]	17.75																							

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

Table 5.11 Effect of various insecticides on growth of nematophagous fungi on potato dextrose agar at 5 days after incubation

Fungal isolates	Growth inhibition of insecticides(%)[1/]																							
	Dazomet (2,450 ppm a.i.)				Dinotefuran (40 ppm a.i.)				Lambda-cyhalothrin (62.5 ppm a.i.)				Methomyl (700 ppm a.i.)				Carbaryl (2,975 ppm a.i.)				Chlorpyrifos (1,500 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	100.00 A	100.00 A	87.28 E-H	78.61 MN	69.94 R-T	57.22 e-g	56.65 e-g	34.68 uv	35.84 t-v	37.57 s-u	16.76 AB	1.73 LM	2.31 LM	5.20 J-L	68.21 R-W	64.74 W-a	65.32 V-Z	65.32 V-Z	89.60 C-E	86.13 E-I	74.57 O-Q	54.91 f-i
MTI2-001 *A. oligospora*	100.00 A	100.00 A	100.00 A	100.00 A	66.35 U-X	73.58 O-Q	74.84 OP	54.09 g-j	48.11 l-n	16.35 A-D	11.64 F-H	11.63 F-H	37.11 s-u	9.12 G-I	3.46 K-M	1.26 M	86.16 E-I	86.48 E-H	86.79 E-H	85.85 F-J	100.00 A	88.05 D-G	75.69 NO	71.07 Q-S
API3-001 *A. conoides*	100.00 A	100.00 A	100.00 A	100.00 A	66.67 T-X	64.10 X-b	57.05 e-g	45.51 m-p	51.92 i-k	33.33 v	28.85 w	22.44 yz	52.56 h-j	16.67 A-C	10.90 F-H	4.49 K-M	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	92.63 BC	89.10 C-F	85.90 F-I
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	57.14 e-g	40.48 q-s	33.33 v	55.95 f-h	69.05 R-U	58.33 d-f	54.76 g-i	39.29 r-t	69.05 R-U	67.86 S-W	66.67 T-X	65.48 V-Y	100.00 A	69.05 R-U	47.62 mn	36.31 t-v
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	75.88 NO	83.83 H-K	78.82 L-M	82.65 I-K	63.53 X-b	54.41 g-j	51.18 j-l	52.94 h-j	55.29 f-i	38.83 r-t	27.06 wx	28.23 w	83.82 H-J	82.35 J-K	83.83 H-J	83.83 H-J	100.00 A	85.00 G-J	80.59 K-M	75.79 NO
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	65.44 V-Y	54.41 g-i	37.50 s-u	25.73 w-y	59.56 c-e	38.97 r-t	42.28 p-r	25.74 w-y	84.19 H-J	91.55 B-D	86.76 E-H	88.97 D-F	93.38 B	82.35 J-L	66.54 T-X	65.08 W-Z
WJI1-003 *Paecilomyces* sp.	48.61 k-l	45.83 m-o	45.83 m-o	37.50 s-u	100.00 A	100.00 A	100.00 A	100.00 A	27.78 wx	16.67 A-C	19.44 zA	15.28 B-E	27.78 wx	18.05 AB	12.50 E-G	13.19 C-F	68.75 R-V	65.28 V-Z	62.50 Y-c	62.50 Y-c	71.53 P-R	61.11 b-d	48.61 k-l	43.06 0-q
KJO1-003 *Pochonia* sp.	100.00 A	100.00 A	100.00 A	100.00 A	45.00 n-p	48.75 k-m	46.25 m-o	36.25 t-v	24.38 xy	13.13 D-F	9.37 G-I	6.25 I-J	38.75 r-t	10.00 F-H	8.75 HI	8.13 H-J	61.88 Z-c	62.50 Y-c	62.50 Y-c	62.50 Y-c	66.25 U-X	61.25 a-d	53.75 g-j	46.25 m-o
CV%[3/]	2.62																							

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

Table 5.12 Effect of various insecticides on growth of nematophagous fungi on potato dextrose agar at 7 days after incubation

Fungal isolates	Growth inhibition of insecticides(%)[1/]																							
	Dazomet (2,450 ppm a.i.)				Dinotefuran (40 ppm a.i.)				Lambda-cyhalothrin (62.5 ppm a.i.)				Methomyl (700 ppm a.i.)				Carbaryl (2,975 ppm a.i.)				Chlorpyrifos (1,500 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	100.00 A	100.00 A	76.51 R-T	74.62 S-U	65.53 b-i	54.55 m-o	59.47 kl	40.15 v-x	28.60 EF	29.17 D-F	26.14 F-H	-1.14 R	0.38 R	1.89 R	71.97 U-W	71.97 U-W	74.62 S-U	76.14 R-T	92.05 C-E	87.12 G-J	80.68 OP	67.80 Z-f
MTI2-001 *A. oligospora*	100.00 A	100.00 A	100.00 A	100.00 A	62.27 i-k	61.82 jk	68.64 W-c	39.09 w-y	54.31 m-o	24.09 H	10.00 N-P	7.72 O-Q	43.41 t-v	11.81 MN	10.91 NO	11.59 MN	87.73 G-I	89.99 D-G	88.86 E-H	86.82 G-K	92.27 CD	91.36 D-F	77.73 P-S	75.68 R-T
API3-001 *A. conoides*	100.00 A	100.00 A	100.00 A	100.00 A	56.27 l-n	56.70 l-n	48.05 pq	43.29 t-v	62.77 h-k	48.91 p	32.03 B-D	28.13 FG	56.49 l-n	29.43 D-F	14.72 K-M	0.00 R	100.00 A	100.00 A	100.00 A	100.00 A	96.75 AB	88.09 F-I	81.82 NO	80.52 O-Q
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	57.04 l-n	35.55 zA	25.18 GH	36.67 y-A	37.41 x-A	28.88 D-F	27.04 F-H	14.81 K-M	77.22 Q-T	76.11 R-T	78.14 P-R	81.85 NO	95.37 BC	83.70 K-O	72.03 UV	68.52 X-d
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	67.97 Z-f	71.48 U-Y	68.16 Y-e	69.14 V-a	65.23 d-i	44.14 s-u	43.94 s-u	35.16 AB	53.91 no	35.55 zA	34.76 A-C	31.64 C-E	86.13 H-L	86.13 H-L	85.94 H-M	87.11 G-J	100.00 A	88.28 F-I	84.37 J-N	82.61 M-O
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	63.33 h-j	57.61 lm	47.61 p-r	38.57 x-z	64.76 f-j	46.18 p-t	45.95 p-t	34.76 A-C	86.18 H-L	88.57 F-I	85.23 I-M	87.14 G-J	91.43 D-F	82.86 L-O	71.66 U-X	69.52 V-Z
WJI1-003 *Paecilomyces* sp.	42.20 u-w	42.20 u-w	42.20 u-w	28.44 E-G	100.00 A	100.00 A	100.00 A	100.00 A	24.77 H	19.26 I	16.97 I-K	17.89 I-K	29.35 D-F	18.34 IJ	15.59 J-L	14.68 K-M	67.43 Z-g	68.80 V-b	66.05 a-h	68.80 V-b	74.31 TU	65.13 e-j	47.70 p-r	46.79 p-s
KJO1-003 *Pochonia* sp.	100.00 A	100.00 A	100.00 A	100.00 A	45.00 q-u	44.58 r-u	42.08 u-w	34.16 A-C	24.16 H	13.33 L-N	10.83 N-P	10.41 N-P	47.70 p-r	14.58 K-M	6.66 Q	7.50 PQ	64.16 g-j	63.33 h-j	63.33 h-j	65.41 c-i	75.41 R-T	68.33 X-e	63.75 h-j	52.50 o
CV%[3/]	2.46																							

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

Table 5.13 Effect of various insecticides on growth of nematophagous fungion potato dextrose agar at 10 days after incubation

Fungal isolates	Growth inhibition of insecticides (%)[1/]																							
	Dazomet (2,450 ppm a.i.)				Dinotefuran (40 ppm a.i.)				Lambda-cyhalothrin (62.5 ppm a.i.)				Methomyl (700 ppm a.i.)				Carbaryl (2,975 ppm a.i.)				Chlorpyrifos (1,500 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	100.00 A	100.00 A	62.59 W-Z	59.26 a-d	45.18 o-q	32.96 A	47.40 no	20.00 D-F	10.37 K-M	19.26 E-G	5.93 O-R	0.00 T	3.70 RS	0.00 T	64.07 V-Y	63.70 V-Y	64.07 V-Y	65.18 U-W	91.11 DE	85.18 GH	73.33 O-Q	54.44 h-j
MTI2-001 *A. oligospora*	100.00 A	100.00 A	100.00 A	100.00 A	58.15 b-f	55.18 g-i	67.41 S-U	32.59 A	46.30 o-q	17.03 GH	5.92 O-R	5.92 O-R	35.93 w-z	0.00 T	0.00 T	0.00 T	89.63 DE	90.37 DE	85.18 GH	81.85 I-K	91.48 CD	88.52 EF	74.81 NO	69.63 RS
API3-001 *A. conoides*	100.00 A	100.00 A	100.00 A	100.00 A	50.74 lm	45.18 o-q	39.63 uv	34.44 y-A	57.41 d-g	44.44 p-r	22.59 D	21.48 DE	49.63 l-n	12.96 I-K	10.37 K-M	4.07 Q-S	100.00 A	100.00 A	100.00 A	100.00 A	97.96 A	81.85 I-K	71.85 P-R	68.15 ST
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	38.15 vw	10.74 KL	4.81 P-R	6.67 O-Q	29.63 BC	4.81 P-R	7.41 N-P	0.00 T	65.92 T-V	62.59 W-Z	64.44 V-X	67.78 S-U	90.92 DE	74.44 N-P	59.26 a-d	52.22 j-l
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	100.00 A	100.00 A	55.92 f-i	60.37 Z-c	51.48 k-m	49.63 l-n	49.26 mn	19.63 E-G	14.81 HI	13.70 IJ	93.89 BC	86.67 FG	82.22 IJ	80.18 J-L	78.52 LM	79.26 KL	80.74 J-L	80.00 J-L	94.25 B	86.67 FG	82.22 IJ	80.18 J-L
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	62.22 X-Z	49.63 l-n	44.07 q-s	32.96 A	63.70 V-Y	47.04 n-p	44.07 q-s	36.30 w-y	83.70 HI	81.66 I-K	79.63 J-L	81.48 I-K	89.44 DE	78.33 LM	63.70 V-Y	58.89 b-e
WJI1-003 *Paecilomyces* sp.	33.57 zA	35.71 w-z	35.71 w-z	21.42 DE	100.00 A	100.00 A	100.00 A	100.00 A	17.85 FG	15.03 HI	13.57 IJ	15.00 HI	27.14 C	17.14 GH	13.57 IJ	7.85 M-O	63.57 V-Y	62.14 X-Z	56.42 e-h	60.71 Z-b	71.42 QR	57.85 c-f	53.57 i-k	41.42 s-u
KJO1-003 *Pochonia* sp.	100.00 A	100.00 A	100.00 A	100.00 A	37.74 v-x	39.73 t-v	35.10 x-A	29.80 B	19.86 EF	9.93 L-N	3.97 RS	3.97 RS	42.38 r-t	11.25 J-L	1.98 ST	3.97 RS	61.58 Y-a	62.25 X-Z	63.57 V-Y	63.57 V-Y	76.16 MN	65.89 T-V	62.25 X-Z	50.33 lm
CV%[3/]	2.65																							

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

Table 5.14 Effect of various insecticides on sporulation of nematophagous fungi on potato dextrose agar at 10 days after incubation

Fungal isolates	Sporulation of insecticides (×10^4 spore/ml)[1/]																							
	Dazomet (2,450 ppm a.i.)				Dinotefuran (40 ppm a.i.)				Lambda-cyhalothrin (62.5 ppm a.i.)				Methomyl (700 ppm a.i.)				Carbaryl (2,975 ppm a.i.)				Chlorpyrifos (1,500 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	0.00 k[2/]	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 jk	0.00 e-k	0.00 i-k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
MTI2-001 *A. oligospora*	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.96 d-j	2.83 X-a	1.73 b-e	3.86 V-X	1.66 b-f	3.73 V-Y	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
API3-001 *A. conoides*	0.00 k	0.00 k	0.00 k	0.00 k	0.53 i-k	0.93 d-j	1.36 d-i	1.76 b-d	0.00 k	0.63 h-k	1.50 c-h	1.60 c-g	0.45 i-k	0.69 g-k	0.68 g-k	0.77 f-k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
JDI1-001 *A. thaumasium*	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 UV	0.00 T	0.00 M	0.00 N	5.56 U	16.26 S	17.0 RS	18.56 R	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
MPI1-003 *A. thaumasium*	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 W-Z	0.00 V-Y	0.00 VW	0.00 UV	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
MSO1-001 *A. musiformis*	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	1.83 a-d	2.40 Z-c	0.28 jk	0.36 jk	0.40 jk	2.63 Y-b	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
WJI1-003 *Paecilomyces* sp.	0.00 k	0.00 k	0.00 k	0.00 k	1695 F	2400 E	2999 D	2584 E	4943 C	7099 B	7804 A	5186 C	63.66 I	821 G	889 G	827 G	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k	0.00 k
KJO1-003 *Pochonia* sp.	0.00 k	0.00 k	0.00 k	0.00 k	17.26 RS	25.93 P	52.80 JK	143.1 H	43.53 L	50.13 K	51.96 JK	52.63 JK	52.76 JK	51.56 JK	49.20 K	55.86 J	22.96 Q	32.33 N	27.86 OP	32.26 N	26.93 P	38.40 M	25.76 PQ	30.23 NO
CV%[3/]	1.39																							

[1/] Mean of conidia of each fungus 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%.

The effect of five fungicides including quintozene mixed with etridiazole (Terraclor Super X 30% W/V EC®), fosetylaluminium (Aliette 80 WG®), metalaxyl - M mixedwith mancozeb, (Ridomil Gold MZ 68 W/G®), toclofos methyl (Rizolex 50 % WP®), propamocarb hydrochloride (Previcur - N 72.2 % W/V SL®) on the growth (3, 5, 7 and 10 days) and sporulation (10 days) of selected fungi at room temperature (27±3ºC) are indicated in Tables 5.15-5.19. The analysis of variance table by factorial treatment effects and interaction of fungal growth inhibition on fungicides after inoculation presented significant sources affecting growth and sporulation which showed in Tables 26-30 of appendix. These sources were fungicide, usage rate, fungal isolate and all of their interaction.

At all examined times, metalaxyl mixed with mancozeb (1,700 ppm a.i.) caused almost complete inhibition of all fungal radial mycelial growth, with significance ($P<0.01$), at all tested concentrations consisting of 2x, 1x, 1/2x and 1/3x of recommended rate in comparison to non-treated treatment. Fosetylaluminium (8,000 ppm a.i.) caused 100% inhibition at all rates of five fungal isolates except *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003. Quintozene mixed with etridiazole (900 ppm a.i.) were the third fungicide to reduce fungal growth because it caused 100% inhibition of most fungi at 2x and 1x of recommended rate. All rates of toclofos methyl (1,000 ppm a.i.) inhibited *Arthrobotrys musiformis* isolate MSO1-001 more than other fungal isolates by 65-89% depending on the concentration. In addition, propamocarb hydrochloride containing 722 ppm a.i. had the least effect which showed zero percentage of growth inhibition at 10 days after incubation.

All fungicides at all concentrations affected sporulation by inhibiting or decreasing its appearance. Metalaxyl mixed with mancozeb caused non sporulation of all fungi at all rates as did fosetylaluminium except for *Paecilomyces* sp. isolate WJI1-003. Five fungi, *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and MPI1-003 could not produce conidia in the quintozene mixed with etridiazole treatment. Propamocarb hydrochloride, generally decreased sporulationin comparison to the control.

Table 5.15 Effect of various fungicides on growth inhibition of nematophagous fungi on potato dextrose agar at 3 days after incubation

Fungal isolates	Growth inhibition of fungicdes(%)[1/]																			
	Quintozene + etridizole (900 ppm a.i.)				Fosetylaluminium (8,000 ppm a.i.)				Metalaxyl -M +mancozep (1,700 ppm a.i.)				Toclofos methyl (1000 ppm a.i.)				Propamocarb hydrochloride (722 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	100.00 A	87.23 B	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	78.36 CD	50.70 M-P	30.49 VW	17.73 Za	1.42 gh	2.84 e-h	0.71 gh	21.98 YZ
MTI2-001 *A. oligospora*	100.00 A	100.00 A	100.00 A	100 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	79.34 C	71.19 GH	21.74 YZ	3.25 e-h	-6.52 i	22.82 XY
API3-001 *A. conoides*	100.00 A	100.00 A	100.00 A	100 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	48.38 OP	43.55 QR	32.26 UV	30.64 VW	33.87 UV	14.51 ab	-22.58 k	-12.90 j
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	87.5 B	76.38 C-F	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	80.55 C	72.20 F-H	69.44 HI	61.11 J	12.15 bc	1.39 gh	1.38 gh	27.08 WX
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	100.00 A	79.78 C	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	77.05 C-E	60.66 JK	46.44 PQ	35.52 TU	12.57 bc	3.82 e-g	3.82 e-g	7.10 de
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	100 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	9.70 cd	5.82 d-f	-0.97 h	27.18 W
WJI1-003 *Paecilomyces* sp.	100.00 A	100.00 A	66.66 I	56.66 KL	100.00 A	100.00 A	74.16 D-G	70.83 G-I	100.00 A	100.00 A	100.00 A	100.00 A	50.00 N-P	46.66 PQ	41.66 RS	39.16 ST	1.66 f-h	0.00 gh	3.33 e-h	0.00 gh
KJO1-003 *Pochonia* sp.	100.00 A	100.00 A	100.00 A	76.78 C-E	100.00 A	100.00 A	100.00 A	73.21 E-H	100.00 A	100.00 A	100.00 A	100.00 A	52.68 L-O	54.46 LM	52.68 L-O	53.57 L-N	0.89 gh	1.78 f-h	0.89 gh	2.67 f-h
CV%[3/]	2.85																			

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

Table 5.16 Effect of various fungicides on growth inhibition of nematophagous fungi on potato dextrose agar at 5 days after incubation

Fungal isolates	Growth inhibition of fungicdes(%)[1/]																			
	Quintozene + etridizole (900 ppm a.i.)				Fosetylaluminium (8,000 ppm a.i.)				Metalaxyl -M +mancozep (1,700 ppm a.i.)				Toclofos methyl (1000 ppm a.i.)				Propamocarb hydrochloride (722 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	86.55 CD	83.85 D-F	93.72 B	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	71.30 KL	43.05 R	26.45 VW	14.80 X	4.03 Z-b	4.03 Z-b	2.69 Z-c	23.76 W
MTI2-001 *A. oligospora*	100.00 A	100.00 A	89.31 C	88.62 C	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	72.41 KL	59.31 O	55.86 P	2.07 a-c	-5.52 h	-13.79 i	13.79 X
API3-001 *A. conoides*	100.00 A	100.00 A	100.00 A	77.78 HI	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	76.54 IJ	41.97 R	32.09 TU	30.86 U	13.57 X	14.81 X	-11.11 i	3.70 Z-b
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	82.68 E-G	72.29 KL	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	64.93 MN	51.95 Q	49.78 Q	38.52 S	3.46 Z-b	0.00 c-e	-3.46 f-h	24.24 W
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	100.00 A	76.83 IJ	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	65.85 M	50.00 Q	34.76 T	27.74 V	4.88 Y-a	-4.27 gh	-5.49 h	5.49 YZ
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	86.55 CD	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	85.48 DE	81.18 FG	100.00 A	12.36 X	7.52 Y	5.37 YZ	32.26 TU
WJI1-003 *Paecilomyces* sp.	100.00 A	82.47 E-G	49.48 Q	44.84 R	100.00 A	100.00 A	80.41 GH	74.22 JK	100.00 A	100.00 A	100.00 A	100.00 A	49.48 Q	44.84 R	42.27 R	38.14 S	1.03 b-d	-1.03 d-f	5.15 YZ	0.00 c-e
KJO1-003 *Pochonia* sp.	100.00 A	100.00 A	74.11 JK	72.35 KL	100.00 A	100.00 A	100.00 A	69.41 L	100.00 A	100.00 A	100.00 A	100.00 A	62.35 NO	62.35 NO	64.71 MN	61.18 O	0.00 c-e	1.18 b-d	-2.35 e-g	1.18 b-d
CV%[3/]	2.07																			

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

Table 5.17 Effect of various fungicides on growth inhibition of nematophagous fungi on potato dextrose agar at 7 days after incubation

Fungal isolates	Growth inhibition of fungicdes(%)[1/]																			
	Quintozene + etridizole (900 ppm a.i.)				Fosetylaluminium (8,000 ppm a.i.)				Metalaxyl -M +mancozep (1,700 ppm a.i.)				Toclofos methyl (1000 ppm a.i.)				Propamocarb hydrochloride (722 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	79.26 B-G	73.33 D-K	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	57.04 N-T	28.15 Y-a	11.85 b-g	2.59 e-g	0.00 fg	0.00 fg	0.00 fg	10.37 b-g
MTI2-001 *A. oligospora*	100.00 A	100.00 A	91.85 AB	83.26 B-E	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	81.50 B-F	63.44 I-P	52.86 O-V	43.17 T-X	16.74 a-d	0.88 fg	-1.32 fg	11.45 b-g
API3-001 *A. conoides*	60.97 J-Q	100.00 A	100.00 A	71.03 E-M	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	64.02 H-O	49.39 Q-W	43.90 S-X	39.02 V-Y	17.07 a-c	15.85 a-e	1.83 fg	26.83 Y-a
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	79.63 B-G	66.30 G-H	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	55.55 N-U	37.77 W-Y	28.15 Y-a	19.26 ab	0.00 fg	0.00 fg	0.00 fg	10.37 b-g
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	81.74 B-F	75.86 D-I	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	59.63 K-Q	44.42 R-X	33.46 X-Z	22.51 Z-b	9.94 b-g	2.23 e-g	0.61 fg	12.57 b-f
MSO1-001 *A. musiformis*	100.00 A	100.00 A	100.00 A	82.18 B-F	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	89.87 A-C	77.73 C-H	68.83 F-N	65.99 G-O	4.86 c-g	2.02 e-g	1.62 fg	27.73 Y-a
WJI1-003 *Paecilomyces* sp.	100.00 A	73.46 D-K	57.69 M-S	44.61 R-X	80.77 B-F	100.00 A	85.38 B-D	80.38 B-F	100.00 A	100.00 A	100.00 A	100.00 A	49.99 P-W	43.08 U-X	40.76 V-Y	36.92 W-Y	3.84 c-g	0.77 fg	1.54 fg	3.08 d-g
KJO1-003 *Pochonia* sp.	90.29 A-C	83.49 B-E	73.79 D-J	71.84 D-L	100.00 A	100.00 A	100.00 A	71.36 E-M	100.00 A	100.00 A	100.00 A	100.00 A	58.25 L-R	64.08 H-O	62.13 I-Q	62.14 I-Q	0.00 fg	0.00 fg	-1.94 g	0.97 fg
CV%[3/]	9.78																			

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

Table 5.18 Effect of various fungicides on growth inhibition of nematophagous fungi on potato dextrose agar at 10 days after incubation

Fungal Isolates	Growth inhibition of fungicdes(%)[1/]																			
	Quintozene + etridizole (900 ppm a.i.)				Fosetylaluminium (8,000 ppm a.i.)				Metalaxyl -M +mancozep (1,700 ppm a.i.)				Toclofos methyl (1000 ppm a.i.)				Propamocarb hydrochloride (722 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	100.00 A	62.96 LM	50.37 PQ	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	22.96 XY	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
MTI2-001 *A. oligospora*	100.00 A	100.00 A	81.11 E	72.22 J	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	60.37 N	38.15 T	22.22 Y	13.33 b	0.00 g	0.00 g	0.00 g	0.00 g
API3-001 *A. conoides*	100.00 A	100.00 A	78.89 F	69.07 K	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	50.37 PQ	42.96 R	24.81 X	34.81 U	15.55 a	16.29 a	24.81 X	11.85 b
JDI1-001 *A. thaumasium*	100.00 A	100.00 A	68.52 K	48.52 Q	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	19.26 Z	6.30 c	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g	0.00 g
MPI1-003 *A. thaumasium*	100.00 A	100.00 A	73.70 IJ	62.59 M	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	32.22 V	13.33 b	5.56 cd	2.22 ef	0.00 g	0.00 g	0.00 g	0.00 g
MSO1-001 *A. musiformis*	100.00 A	100.00 A	57.04 O	56.30 O	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	82.22 E	56.67 O	43.70 R	40.37 S	0.00 g	0.00 g	0.00 g	0.00 g
WJI1-003 *Paecilomyces* sp.	100.00 A	64.63 L	51.22 P	40.24 S	100.00 A	100.00 A	88.41 C	84.75 D	100.00 A	100.00 A	100.00 A	100.00 A	42.68 R	36.58 TU	34.76 U	29.88 W	3.66 de	1.22 fg	1.22 fg	1.22 fg
KJO1-003 *Pochonia* sp.	93.46 B	84.96 D	77.12 FG	75.16 HI	100.00 A	100.00 A	81.04 E	75.81 GH	100.00 A	100.00 A	100.00 A	100.00 A	60.12 N	58.16 O	58.16 O	57.51 O	1.96 ef	1.30 fg	1.96 ef	1.30 fg
CV%[3/]	1.48																			

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

Table 5.19 Effect of various fungicides on sporulation of nematophagous fungi on potato dextrose agar at 10 days after incubation

Fungal isolates	Sporulation of fungicdes ($\times 10^4$ spore/ml)[1/]																			
	Quintozene + etridizole (900 ppm a.i.)				Fosetylaluminium (8,000 ppm a.i.)				Metalaxyl -M +mancozep (1,700 ppm a.i.)				Toclofos methyl (1000 ppm a.i.)				Propamocarb hydrochloride (722 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.6	4.4	13.4	13.9	7.7	15.3	20.6	22.2
A. oligospora	f[2/]	f	f	f	f	f	f	f	f	f	f	f	X-c	U-Z	NO	NO	PQ	N	M	M
MTI2-001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	13.3	12.7
A. oligospora	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	a-d	NO	O
API3-001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.0
A. conoides	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	f	ef	e
JDI1-001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.5	0.5	5.3	4.9	3.9	6.9
A. thaumasium	f	f	f	f	f	f	f	f	f	f	f	f	f	ef	ef	ef	T-W	U-X	X-c	P-S
MPI1-003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.7	5.7	7.9	6.4	8.3
A. thaumasium	f	f	f	f	f	f	f	f	f	f	f	f	f	f	ef	ef	R-U	PQ	Q-T	P
MSO1-001	0.0	0.0	2.7	2.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	2.2	4.2	3.8	4.0	4.5	4.7
A. musiformis	f	f	cd	b-d	f	f	f	f	f	f	f	f	f	ef	d	V-a	X-c	W-b	U-Z	U-Y
WJI1-003	0.0	84.0	334.3	949.0	0.0	0.0	117.3	122.0	0.0	0.0	0.0	0.0	2400.0	2657.3	3220.7	4446.7	345.6	643.6	778.0	996.0
Paecilomyces sp.	f	J	H	E	f	f	I	I	f	f	f	f	D	C	B	A	H	G	F	E
KJO1-003	0.0	3.8	3.5	5..4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.4	43.4	84.7	87.0	3.5	3.3	4.5	7.2
Pochonia sp.	f	X-c	Y-d	S-V	f	f	f	f	f	f	f	f	L	K	J	J	Y-c	Z-d	U-Z	P-R
CV%[3/]	1.60																			

[1/] Mean of conidia of each fungus 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%.

The effect of three herbicides including paraquat dichloride (paraquat 27.6 %W/V SL®), glyphosate-isopropylammonium (Glyphosate 48% W/V SL®) and oxyfluorfen (Goal 2 E 23.5 % W/V EC®) on the growth (3, 5, 7 and 10 days) and sporulation (10 days) of selected fungi at room temperature (27±3ºC) are indicated in Tables 5.20-5.24 and Figure 5.9. Herbicide, usage rate, fungal isolate and all their interactions significantly affected the growth and sporulation at a high probability (Tables 31-35 of appendix).

All herbicides at serial concentrations caused abnormal developments. Radial mycelia of *Arthrobotrys oligospora* isolate DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001, and isolate MPI1-003 and *Arthrobotrys musiformis* isolate MSO1-001 had the highest sensitivity to paraquat dichloride (1,725 ppm a.i.). Their mycelia were inhibited by 100% at the 2x and 1x of recommended rate. Oxyfluorfen (587.5 ppm a.i.) exerted the second strongest effect among herbicides involving reduction of sporulation. Glyphosate-isopropylammonium (3,000 ppm a.i.) at the 2x caused highest reduction of *Arthrobotrys* spp. mycelial growth.

Non-sporulation of fungi was detected with at least 90% of all treatments except *Pochonia* sp. isolate KJO1-003 which only showed a decrease of conidial production (Table 5.24).

Table 5.20 Effect of various herbicides on growth inhibition of nematophagous fungi on potato dextrose agar at 3 days after incubation

Fungal isolates	Growth inhibition of herbicides(%)[1/]											
	Glyphosate-isopropylammonium (1,725 ppm a.i.)				Paraquat dichloride (3000 ppm a.i.)				Oxyfluorfen (587.5 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	0.00 m[2/]	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m
MTI2-001 *A. oligospora*	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m
API3-001 *A. conoides*	100.00 a	100.00 a	100.00 a	5.26 l	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
JDI1-001 *A. thaumasium*	100.00 a	66.87 de	31.25 ij	28.12 jk	100.00 a	100.00 a	45.62 f-h	23.75 k	90.62 b	71.25 d	67.50 de	50.00 f
MPI1-003 *A. thaumasium*	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m
MSO1-001 *A. musiformis*	100.00 a	43.83 gh	4.11 lm	1.37 lm	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	78.08 c	63.01 e	41.10 h
WJI1-003 *Paecilomyces* sp.	28.78 ij	6.06 l	3.03 lm	3.03 lm	100.00 a	100.00 a	68.18 d	42.42 h	50.00 f	48.48 fg	28.78 ij	33.33 i
KJO1-003 *Pochonia* sp.	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m	0.00 m
CV%[3/]	7.04											

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

Table 5.21 Effect of various herbicides on growth inhibition of nematophagous fungi on potato dextrose agar at 5 days after incubation

Fungal isolates	Growth inhibition of herbicdes(%)[1/]											
	Glyphosate-isopropylammonium (1,725 ppm a.i.)				Paraquat dichloride (3000 ppm a.i.)				Oxyfluorfen (587.5 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001	100.00	87.86	57.81	21.39	100.00	100.00	100.00	100.00	87.28	84.97	79.48	66.76
A. oligospora	A[2/]	B-D	N-Q	Za	A	A	A	A	B-D	CD	EF	H-J
MTI2-001	100.00	100.00	12.12	6.06	100.00	100.00	100.00	100.00	100.00	87.88	77.27	60.61
A. oligospora	A	A	c-e	fg	A	A	A	A	A	B-D	FG	L-O
API3-001	100.00	89.06	42.97	39.84	100.00	100.00	100.00	100.00	100.00	87.50	75.78	74.22
A. conoides	A	BC	VW	WX	A	A	A	A	A	B-D	FG	G
JDI1-001	100.00	74.42	48.84	40.05	100.00	100.00	36.43	17.31	88.89	68.47	56.07	54.01
A. thaumasium	A	G	TU	WX	A	A	X	ab	BC	HI	P-R	Q-S
MPI1-003	100.00	100.00	80.00	56.47	100.00	100.00	100.00	100.00	100.00	86.17	83.83	65.30
A. thaumasium	A	A	EF	O-R	A	A	A	A	A	B-D	DE	H-K
MSO1-001	100.00	53.03	9.85	15.15	100.00	100.00	100.00	100.00	90.53	76.51	62.88	60.98
A. musiformis	A	R-T	d-f	bc	A	A	A	A	B	FG	J-M	K-N
WJI1-003	31.25	7.81	6.25	3.12	100.00	79.69	64.06	22.66	59.37	51.56	45.31	40.62
Paecilomyces sp.	Y	ef	fg	gh	A	EF	I-M	Z	M-P	ST	UV	WX
KJO1-003	25.63	12.50	2.50	-0.63	100.00	77.50	68.75	55.63	78.13	62.50	55.63	48.75
Pochonia sp.	Z	cd	gh	h	A	FG	H	P-S	FG	J-M	P-S	TU
CV%[3/]	2.98											

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

Table 5.22 Effect of various herbicides on growth inhibition of nematophagous fungi on potato dextrose agar at 7 days after incubation

Fungal isolates	Growth inhibition of herbicdes(%)[1/]											
	Glyphosate-isopropylammonium (1,725 ppm a.i.)				Paraquat dichloride (3000 ppm a.i.)				Oxyfluorfen (587.5 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	90.87 BC	60.46 PQ	24.93 Y	100.00 A	100.00 A	100.00 A	100.00 A	91.63 BC	90.11 BC	85.17 DE	71.48 KL
MTI2-001 *A. oligospora*	100.00 A	75.61 H-J	20.73 Z	6.10 ef	100.00 A	100.00 A	100.00 A	100.00 A	78.05 GH	65.85 MN	56.10 RS	51.22 U-W
API3-001 *A. conoides*	100.00 A	73.61 I-K	20.45 Za	-22.72 j	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	76.36 G-J	62.27 N-Q	58.63 QR
JDI1-001 *A. thaumasium*	96.67 A	73.33 JK	41.48 X	38.52 X	100.00 A	100.00 A	26.67 Y	12.59 cd	87.96 CD	70.92 KL	48.89 VW	48.15 W
MPI1-003 *A. thaumasium*	100.00 A	91.86 B	80.09 FG	61.53 O-Q	100.00 A	100.00 A	100.00 A	100.00 A	100.00 A	82.80 EF	78.73 GH	64.48 NO
MSO1-001 *A. musiformis*	100.00 A	55.52 R-T	16.08 bc	16.08 bc	100.00 A	100.00 A	100.00 A	100.00 A	91.45 BC	76.38 G-J	64.32 NO	62.56 N-P
WJI1-003 *Paecilomyces* sp.	19.07 Z-b	5.20 f	0.57 gh	-3.47 i	100.00 A	77.45 G-I	51.44 U-W	16.76 ab	58.96 p-r	53.18 S-U	42.19 X	38.73 X
KJO1-003 *Pochonia* sp.	25.00 Y	9.16 de	4.16 fg	0.00 hi	85.83 DE	78.75 GH	69.16 LM	52.08 T-V	80.00 FG	68.33 LM	62.50 N-P	50.00 U-W
CV%[3/]	2.68											

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

Table 5.23 Effect of various herbicides on growth inhibition of nematophagous fungi on potato dextrose agar at 10 days after incubation

Fungal isolates	Growth inhibition of herbicdes(%)[1/]											
	Glyphosate-isopropylammonium (1,725 ppm a.i.)				Paraquat dichloride (3000 ppm a.i.)				Oxyfluorfen (587.5 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001 *A. oligospora*	100.00 A[2/]	90.00 CD	50.37 Q	12.96 YZ	100.00 A	100.00 A	100.00 A	100.00 A	91.85 C	85.74 D	78.52 E-G	60.37 K-N
MTI2-001 *A. oligospora*	100.00 A	72.30 I	60.81 K-N	55.41 OP	100.00 A	100.00 A	100.00 A	100.00 A	76.35 E-I	62.16 J-L	21.62 T-V	10.81 Za
API3-001 *A. conoides*	100.00 A	79.61 EF	24.12 TU	15.57 W-Z	100.00 A	100.00 A	100.00 A	100.00 A	85.09 D	73.90 G-I	61.18 J-N	59.21 L-O
JDI1-001 *A. thaumasium*	98.52 AB	64.26 JK	25.37 T	2.96 bc	100.00 A	100.00 A	4.81 bc	1.48 bc	77.22 E-I	43.89 R	18.15 V-W	14.81 X-Z
MPI1-003 *A. thaumasium*	100.00 A	92.59 C	75.93 E-I	61.48 J-M	100.00 A	100.00 A	100.00 A	100.00 A	93.70 BC	80.00 E	74.81 F-I	56.30 NO
MSO1-001 *A. musiformis*	100.00 A	50.58 PQ	17.57 V-Y	16.22 W-Y	100.00 A	100.00 A	100.00 A	100.00 A	85.13 D	73.17 HI	61.97 J-L	56.95 M-O
WJI1-003 *Paecilomyces* sp.	15.35 X-Z	0.79 c	0.79 c	3.15 bc	90.94 C	73.62 G-I	42.52 RS	12.99 YZ	60.63 K-N	76.38 E-I	44.88 R	38.58 S
KJO1-003 *Pochonia* sp.	20.39 U-W	5.92 ab	1.32 bc	1.32 bc	85.85 D	76.65 E-I	64.80 JK	42.11 RS	77.63 E-H	65.79 J	57.89 L-O	44.74 R
CV%[3/]	3.62											

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

Table 5.24 Effect of various herbicides on the sporulation of nematophagous fungi on potato dextrose agar at 10 days after incubation

Fungal isolates	Sporulation of herbicides($\times 10^4$ spore/ml)[1/]											
	Glyphosate-isopropylammonium (1,725 ppm a.i.)				Paraquat dichloride (3000 ppm a.i.)				Oxyfluorfen (587.5 ppm a.i.)			
	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x	2x	1x	1/2x	1/3x
DLO1-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. oligospora	K[2/]	K	K	K	K	K	K	K	K	K	K	K
MTI2-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. oligospora	K	K	K	K	K	K	K	K	K	K	K	K
API3-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. conoides	K	K	K	K	K	K	K	K	K	K	K	K
JDI1-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. thaumasium	K	K	K	K	K	K	K	K	K	K	K	K
MPI1-003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A. thaumasium	K	K	K	K	K	K	K	K	K	K	K	K
MSO1-001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	46.66	55.33	66.00	68.00
A. musiformis	K	K	K	K	K	K	K	K	F	CD	B	B
WJI1-003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	52.00	53.66	58.00	87.33
Paecilomyces sp.	K	K	K	K	K	K	K	K	E	DE	C	A
KJO1-003	46.66	55.33	66.00	68.00	52.00	53.66	58.00	87.33	27.66	36.00	40.33	43.66
Pochonia sp.	G	EF	D	D	F	F	E	C	J	I	H	G
CV%[3/]	0.86											

[1/] Mean of conidia of each fungus 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%..

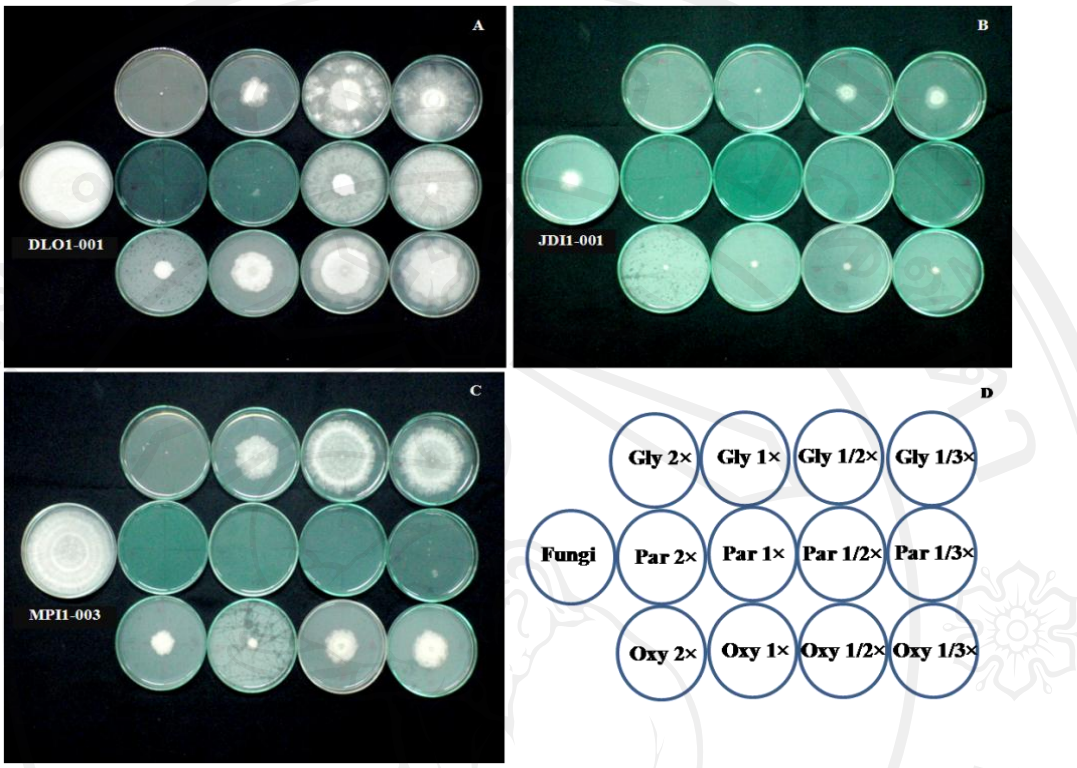

Figure 5.9 Sampling of colony characterizations of three nematophagous fungi comparing effect from herbicides on PDA after 10 days incubation. (A) *Arthrobotrys oligospora* isolate DLO1-001 (B) *Arthrobotrys thaumasium* isolate JDI1-001 (C) *Arthrobotrys thaumasium* isolate MPI1-003 and (D) Location of tested Petri dish, Gly= glyphosate-isopropylammonium, Par= paraquat dichloride, Oxy= oxyfluorfen, 2**x**= Double rate, 1**x**= Recommended rate, 1/2**x**= Half of recommended rate and 1/3**x**= One-third of recommended rate.

5.4 Discussion

It is important to understand how physical factors consisting of media, temperature, light, pH and pesticides (insecticide, fungicide and herbicide) including their interactions relate to microorganism development and establishment in soil ecosystem. The analysis of factorial treatment effects and their interactions with potential physiological factors for the growth and sporulation of nematophagous fungi in this study indicated significant difference at the highest probability. These results were in accord with those of Richard (1992) who found that the interaction of rhizosphere organisms and their physiological factors influenced growth and sporulation and may also be related to their survival and establishment characteristics.

Media generally contain a source of carbon, nitrogen and vitamins. Normally, each fungus utilizes different carbon sources and requires several specific elements for growth and reproduction. The requirements for growth are generally less stringent than for sporulation (General Mycology, 2012). In this research, the study of culture media for mass production found that corn meal agar media (CMA) and potato carrot agar (PCA) promoted the growth of most fungi while PCA and V-8 juice agar (VA) were good media for sporulation. This result was similar to those of Liu & Chen (2002) who reported that VA, CMA and potato dextrose agar (PDA) were good media for growth, in general, and malt extract agar, VA and yeast dextrose agar were good for sporulation of *Hirsutella rhossiliensis.* Nagesh *et al*. (2007) reported that CMA enhanced mycelial growthand sporulation of four isolates of *Pochonia chamydosporia*. Most fungi grew well on PDA, but in some cases it produced excessive mycelia growth and caused a lack of sporulation. CMA was a relatively weak medium compared with PDA, but it was an acceptable medium for soil

microorganisms because it had less easily digestible carbohydrate than PDA. In addition, water agar (WA) and PCA were weak media for retaining the cellulose production ability of cellulose-destroying fungi and spoilage fungi (General Mycology, 2012).

Temperature affects the adaptation, metabolism and development of living organisms. In agricultural soils in temperate climates, nematode trapping fungi followed a seasonal variation. Generally, the optimum temperature for growth and spore production of nematophagous fungi ranged between 24 -35^{o}C such as reports of Mostafa *et al*. (2012), Jerzy *et al*. (2009), Nagesh *et al*. (2007) and Dhawan *et al*. (2004). This research reached a similar conclusion that incubating eight nematophagous fungi at 30^{o}C was appropriate for their vegetative stage following 25^{o}C while 25^{o}C and 30^{o}C also stimulated massive sporulation. In any case, Sanyal *et al*. (2007) indicated significant growth of *Arthrobotrys oligospora* and *Duddingtonia flagrans* was recorded between 18–34°C and the optimum temperature observed for their growth was 26°C. Nagesh *et al*. (2005) demonstrated that the optimum temperatures for *A.oligospora* growth, sporulation, conidiospore germination and conidiospore production ranged were between 25 and 35°C. Gomez *et al.* (2003) reported two Cuban isolates of *A. oligospora* showed greatest and least growth at 25 and 32°C, respectively. In this study, *Pochonia* sp. isolate KJO1-003 showed similar mycelial growth at 35°C (3.60 cm) and 30°C (3.65 cm) and 35°C induced this fungus to produce the highest conidial production. Gao (2011) reported the optimized culture condition of *P. chlamydosporia* was 32°C in two-step cultivation.

Light regulates physiological processes such as morphogenesis, particularly sexual and asexual spore formation of soil microorganisms and affects fungal density.

In this research 24 h dark was a suitable regime for most fungal growth. All fungi incubated in 24 h light (artificial light) produced highest conidia followed by 12 h light and 12 h dark. Mostafa *et al*. (2012) stated the maximum growth of *Trichoderma harzianum* isolates T7, T8 and *Pochonia chlamydosporia* var. *chlamydosporia* was in darkness. Best growth of *T. harzianum* (T7) was in light and maximum growth of *A.oligospora* and *T. harzianum* T14 occurred in 12 h light and 12 h dark. Gao (2011) reported the best light condition for biomass yield of *P. chlamydosporia* was 24 h. This study similarly found that 24 h light was good for *P. chlamydosporia* and 24 h dark activated highest sporulation following 24 h light. Near-ultraviolet radiation (NUV), 400-300 nm, has a variety of effects on biological agents and mostly induction of conidial formation. In contrast, in this study use of NUV resulted in low or moderate growth and sporulation. Perhaps the heat generated by the NUV reduced the metabolism of treated fungi. In addition, NUV wave length may not be a suitable condition for nematophagous fungi which have established in soil and have little exposure to such light.

Most research reported the suitable pH for development of endoparasitic fungi or egg-parasitic fungi of nematode in soil and *in vitro* condition preferred near neutral pH (6.5-7.5) in accordance with the research of Mostafa *et al*. (2012), Nagesh *et al*. (2007) and Sanyal *et al*. (2007). Dong-Geun *et al*. (2002) indicated the presence of nematophagous fungi in soil was associated with pH and pH which, in general, was positively correlated with Ca++ concentration. However, soil elements Ca++, K+ and Mg+ influenced specific species of fungal predators. Ca++ caused loose soil structure and mostly related to organic debris appearing so the fungi should have higher density in soil rich in organic matter according to the research of Jerzy *et al*. (2009), also. The

results of the current research were similar in that all nematophagous fungi grew rapidly and produced the highest number of conidia *in vitro* at pH 8 (alkaline state). Whereas, Sanyal *et al*. (2007) stated that the optimal pH required for growth of *A.oligospora* and *Duddingtonia flagrans* was 6 and further acidic pHs (4 and 5) adversely affected their *in vitro* growth. In addition, Duponnois *et al.* (1995) reported *A.oligospora* grew better on an acidic medium (pH 5.6) than on a basic medium (pH 7.8). Ming *et al.* (2006) demonstrated the optimal pH range of mycelial growth of *P. chlamydosporia* was 5-6 with 6.0 giving the greatest biomass.

In worldwide agriculture, chemical products may detrimentally affect bio-control agents. Most pesticides including fungicides, insecticides and herbicides affect growth and development causing abnormalities of many organisms (Wikipedia, 2012i). Goltapeh *et al.* (2008) indicated commonly pesticides used as supplements in mushroom cultivation, carbendazim, mancozeb, diflubenzuron and malathion, inhibited radial mycelial growth of *A. oligospora* in Petri dishes. In this treatment, the analysis of data showed selected fungi responded to a group of pesticides differently. Priority of effect was fungicide phenylamide (metalaxyl mixed with mancozeb) followed by ethyl phosphonate (fosetyl aluminium), but a wide range of pesticide classes including a dithiocarbamate (dazomet), carbamate (carbaryl and chlorpyrifos), organophosphorus (fosetylaluminium), phenylamide (metalaxyl mixed with mancozeb), quintozene (quintozene mixed with etridiazole), bipyridylium (paraquat dichloride) and diphenyl ether (oxyfluorfen) especially in double and recommended rates were moderately or highly inhibitory of the growth and sporulation of most fungi. On the other hand, only low inhibition by pesticides was often observed in *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003. Both fungi

rapidly produce numerous small conidia and also have thickly velvety and cottony colonies, respectively, which may act to lessen pesticide inhibition compared to *Arthobotrys* which has a fuzzy or powdery texture. However, no record indicated *Paecilomyces* sp. and *Pochonia* sp. could release enzymes or metabolites to digest toxicant chemical substances. These results indicate that application of nematophagous fungi as bio-control agent against root-knot nematodes in chemical plantations should be timed with respect to conventional chemical application to avoid biocontrol inactivation. Furthermore, increasing the amount of agent formulation and adding a sporulation promoter may reduce the effect of pesticide usage.

The effects of agricultural abiotic factors consist of fertilizers, pesticides, pH, relative humidity, light and organic matters correlating with the rhizosphere ecosystem have many consequences on soil microorganism populations. The foregoing was based on routine growth of each fungus. However, the most important consideration for the mass *in vitro* culture process relating the establishment in soil ecology of nematophagous fungi was imitating the real-world soil ecology of the agricultural production site as closely as possible. In some cases, both of artificial pressure and activator such as adding of chemical products or organic materials (filter paper, wheat straw, rice, grains, leaves or dung) in/on the agar, cultural practice etc. was a good promoter for growth and spore production and thus it should be studied further.

5.5 Conclusion

The physical factors for growth and sporulation of eight nematophagous fungi examined in this study were media, temperature, light, pH and pesticides (insecticide, fungicide and herbicide). All factorial treatment effects and all ways of their interaction had an action on fungal vegetative and reproductive stages. These were highly significantly, different at 99% probability ($P<0.01$), depending on specific characterization of each fungal isolate. This research was designed for selecting an optimal condition of *in vitro* culture of nematophagous fungi and studied the effect of some importance influences related to a survival tendency of selected fungi in rhizoshere. The summery of all results consist of:

1) *In vitro* culture condition and some physical factor for fungal growth and reprodution

Media: Corn meal agar media (CMA) promoted the growth of most fungi. General culture media for sporulation were PCA.

Temperature: Incubating at 30^{o}C was appropriate for vegetative stage following 25^{o}C. In addition, 25^{o}C and 30^{o}C stimulated massive sporulation.

Light: 24 hour dark was suitable regime for fungal colony growing. Light condition was found to be a determinant of sporulation because six fungal isolates incubating in either 24 hour light or 12 hour light and 12 hour dark produced highest number of conidia.

pH: All fungi grew rapidly in pH 8 (alkaline state) and pH 7 (neutral state). Most fungi were activated by pH 8 and pH 7 to produce a great number of conidia.

2) Effect of agricultural pesticides in rhizoshere was found to have a detrimental influence on growth and sporulation of nematophagous fungi

Most of pesticides affected the development of all fungi based on serial of concentration, the 2x, 1x, 1/2x and 1/3x of the recommended rate respectively. Dazomet, carbaryl and chlorpyrifos which are commonly-used insecticides in soil caused high radial mycelial growth inhibition. Sporulation of all fungi was related to growth so non-production of conidia was detected in many cases. Priority of fungicide action on fungal radial mycelial growth and sporulation was metalaxyl mixed with mancozeb, fosetylaluminium, quintozene mixed with etridiazole, toclofos methyl and propamocarb hydrochloride. For herbicide, the most active in suppressing growth and sporulation in decreasing order were paraquat dichloride, oxyfluorfen and glyphosate-isopropylammonium. This result indicated that certain pesticides were potentially toxic to nematophagous fungi in the soil; therefore, their establishment and capacity to control plant-parasitic nematode population may be decreased by pesticide usage.