CHAPTER 3

Collection and Selection of Efficient Nematophagous Fungi Against Root-knot Nematode in Thailand

3.1 Introduction

Root-knot nematodes *(Meloidogyne* spp.) are worm-like animals. They have a wide host range, and cause problems in many annual and perennial crops. Affected plants have an unthrifty appearance and often show symptoms of stunting, wilting or yellowing. Below ground, lumps or galls ranging in size from 1 to 10 mm in diameter develop all over the roots (Queensland Government, 2012). Control strategies for root-knot nematodes should be based on density reduction in soil by through sustainable and eco-friendly approaches. However, biological control including improvement of bio-agent establishment is a current challenge.

Nematophagous fungi are natural enemies of nematodes. They are abundantly found in soils rich in organic material. These fungi contain three main groups including the nematode trapping and the endoparasitic fungi that attack vermiform-living nematodes by using specialized structures and the egg- and cyst-parasitic fungi that attack these stages with their hyphal tips. The specialized trapping structures include branches, knobs and constricting rings (Nordbring-Hertz *et al.*, 2006). Accordingly, these fungi became of interest as bio-control agents against plant- and animal-parasitic nematodes. Persmark *et al*. (1996) showed that many nematophagous fungi have been found most frequently in the rhizospheres of plants which occur most commonly in the upper 30 cm of soil while below 40 cm few are encountered. In addition, the densities of these fungi and nematodes showed similar seasonal dynamics with optima during the late summer and autumn months, but were not correlated to each other. Many soils contain 10-15 different species of nematophagous fungi. Nevertheless, these fungi have more complex relationship with their nematode hosts, since they also showed an ability to live saprophytically habitats (Ming-He *et al*., 2006).

The objectives of this chapter were as follows:

1. To observe and isolate nemathophagous fungi from infected root-knot nematode in vegetable plantations of Thailand.

2. To select the efficient nematophagous fungi against root-knot nematodes, *Meloidogyne incognita*.

3.2 Materials and methods

3.2.1 Survey and collection of soil samplings

Infected root-knot nematode plantations in northern Thailand especially the most susceptible host plants e.g. lettuce, tomato, chili, tobacco, beetroot, cucumber and cabbage were surveyed and collected.

Soil samples of 500 g were randomly collected from rhizospheres of infected root knot nematode and adjacent areas at the depth of 10 cm. Soil was sampled with a narrow-bladed shovel or trowel and collected following the soil sampling pattern indicated in Figure 3.1. For each sampling, the soil was gently mixed and placed in a labeled plastic bag or container (Michael & John, 2006). The samples were kept at 4ºC until processed for isolation of nematophagous fungi. The number of soil samples depended on the size of the damaged areas (Table 3.1).

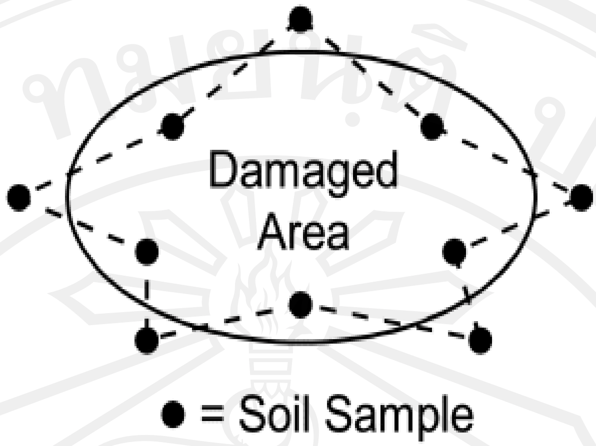

Figure 3.1 Sampling pattern for damaged area or infected patch in a crop

Source: Michael & John (2006)

Table 3.1 Number of soil samples/area required

Area	**Number of soil cores/sample**
< 500 m^2	8 - 10
500 m^2 - 0.5 ha	25 - 35
0.5 ha - 2.5 ha	50 - 60

Source: Michael & John (2006)

3.2.2 Isolation of nematophagous fungi

A simple method of obtaining nematophagous fungi is called *soil sprinkling technique*, where approximately 1 g of each sampling soil was sprinkled on the surface of water agar (WA) in 9 cm-diameter Petri dishes (3 replications) containing antibiotics (0.05% streptomycin sulphate and 0.01% chloramphenicol) together with a suspension of root-knot nematode eggs (*Meloidogyne incognita*) from lettuce added as bait. Developmental stage and mature stage of egg mass from galled root were extracted following "preparation of root-knot nematode egg suspension (Sobita & Anamika, 2011)" and each suspension of 100 eggs was placed onto medium. The Petri dishes were incubated at room temperature (25-30ºC) for nematophagous fungi observation after 3 and 5 days. The egg-masses and second stage juveniles (J2) colonizing fungi were preliminarily observed in Petri dishes under a microscope at low magnification for the presence of trapped nematodes, trapping organs and conidia. Pure cultures of the fungi were made by single spore isolation technique and incubated at 25± 10ºC for growth and sporulation. All fungal isolates were selected for subsequent experiment "3.2.3 *In vitro* predacity of nematophagous fungi against *Meloidogyne* spp.".

Preparation of root-knot nematode egg suspension

Egg sacs from *Meloidogyne incognita* galled roots of lettuce were picked up individually and shaken in an electric blender containing distilled water for 40 second. After addition of 500 ml of 1% sodium hypochlorite, the blender was operated at maximum speed for 40 seconds. The suspension of eggs was poured through 100 and 400 mesh sieves over a container. The material retained on 100 mesh sieve was discarded while that retained on 400 mesh sieve was washed several times with distilled water and collected in a beaker. Eggs were recovered by repeated sieving and rinsing before keeping in suspension (McClure *et al*., 1973; Sobita & Anamika, 2011) in order for the second stage juveniles (J2) to hatch after 2-3 days.

Single spore isolation technique

Conidia of nematophagous fungi were picked up with the sterilized fine needle and dragged lightly across in Petri dishes containing water agar medium. Well separated spores were located under stereoscopic microscope (100×). A disc of agar containing a single spore was cut and transferred into a Petri dish containing corn meal agar medium (CMA). Several single spores of each fungus were transferred in separate Petri dishes, and incubated at 25± 10ºC for growth and sporulation. After 4-5 days of incubation, spores of fungi were transferred aseptically into Petri dishes containing CMA. The cultures were maintained on CMA at 25±10°C by sub-culturing at regular intervals (Sobita & Anamika, 2011).

3.2.3 *In vitro* predacity of nematophagous fungi against *Meloidogyne* spp.

Predacity of nematophagous fungi against *Meloidogyne incognita* was tested by the method described by Jersys *et al.* (2009). Cultures of each nematophagous fungus selected from previous procedure (3.2.2 Isolation of nematophagous fungi) were grown in a 1:10 corn meal agar (CMA) medium containing antibiotics as previously indicated. Second stage juveniles (J2) were isolated from lettuce root galls, (Sobita & Anamika, 2011), washed 5 times with sterilize distilled water and two drops of water containing 100 J2 of *M. incognita* was immediately inoculated into each Petri dish (9 cm-diameter). Three Petri dishes served as replicates. On the other hand, 100 eggs of *M. incognita* were inoculated by each culture of egg parasitic fungi and then kept at 25°C. Observations on trapping structures and trapped nematodes were taken at 3, 5 and 7 days under a stereoscopic microscope at 100×. Verification of the formation of predaceous structures and capturing of nematodes were recorded and percentages calculated. Thereafter, the competent fungal isolates, which can either abundantly or immediately capture, kill and digest nematodes, were selected for subsequent experiments (Chapter 4).

3.3 Results

3.3.1 Survey and collection of soil samplings

Forty-five soil samplings were collected from both of infected root-knot nematode plantations and areas rich in organic matter during April 2007 to September 2008 in Thailand. The samplings were taken from vegetable crop regions in Chiang Mai (36 samplings), Chiang Rai (two samplings), Nakorn Sawan (one sampling) and Mae Hong Son (six samplings). Ninety percent of collected areas were highlands except for three areas of Chiang Mai and one in Nakornsawan. Table 3.2 and Figure 3.2 showed the isolate codes and sampled locations. The most of root-knot nematode-infested areas were sandy loam soils on which are cultivated such susceptible hosts as carrot, lettuce, cabbage, cucumber, tobacco, tomato, chili, beetroot, egg plant, artichokes and fruit tree while areas rich in organic matter were combinations of clay-containing composts or manures.

Allocations of isolate coding were dependent on area type and location as shown in the diagram below.

JDI1-001

JD at the first and second abbreviation refers to “sampling location”

I / O at the third abbreviation refers to “area type” which divided into

(1) Infected root-knot nematode plantations: I

(2) Areas rich in organic matter: O

1 at the fourth abbreviation refers to “plot number”

001 at the last three-letter abbreviation refers to “sequence of fungal isolate”

Table 3.2 List of soil samplings and their items from infected root-knot nematode plantations and areas rich in organic matter

No.	Isolate code	Collection date (month/year)	Plant Host	Location
1.	JDI1-	08/2007	Tobacco	Plot 1, Jadeemaekuey, T. Maejadeemai, A. Sansai, Chiang Mai
2.	JDI2-	08/2007	Tobacco	Plot 2, Jadeemaekuey, T. Maejadeemai, A. Sansai, Chiang Mai
3.	JDO1-	08/2007	Tobacco	Plot 1, Jadeemaekuey, T. Maejadeemai, A. Sansai, Chiang Mai
4.	LLI1-	08/2007	Tobacco	Plot 1, Lom luang, T. Maejadeemai, A. Sansai, Chiang Mai
5.	LLI2-	08/2007	Tobacco	Plot 2, Lom luang, T. Maejadeemai, A. Sansai, Chiang Mai
6.	LLO1-	08/2007	Tobacco	Plot 1, Lom luang, T. Maejadeemai, A. Sansai, Chiang Mai
7.	MJI1-	08/2007	Tobacco	Plot 1, Mae jo, T. Maejadeemai, A. Sansai, Chiang Mai
8.	MJI2-	08/2007	Tobacco	Plot 2, Mae jo, T. Maejadeemai, A. Sansai, Chiang Mai
9.	MJO1-	08/2007	Tobacco	Plot 1, Mae jo, T. Maejadeemai, A. Sansai, Chiang Mai
10.	MTI1-	09/2008	Lettuce	Plot 1, Mae tho, T. Laolee, A. Hot, Chiang Mai
11	MTI2-	09/2008	Lettuce	Plot 2, Mae tho, T. Laolee, A. Hot, Chiang Mai
12.	MTO1-	09/2008	Cabbage	Plot 1, Mae tho, T. Laolee, A. Hot, Chiang Mai
13.	MSI1-	09/2008	Tomato	Plot 1, Mae sariang, T. Aompai, A. Hot, Chiang Mai
14.	MSI2-	09/2008	Tomato	Plot 2, Mae sariang, T. Aompai, A. Hot, Chiang Mai
15.	MSO1-	09/2008	Chili	Plot 1, Mae sariang, T. Aompai, A. Hot, Chiang Mai
16.	MHI1-	12/2007	Beetroot	Plot 1, Mae hae, T. Mae vin, A. Mae wang, Chiang Mai
17.	MHI2-	12/2007	Artichokes	Plot 2, Mae hae, T. Mae vin, A. Mae wang, Chiang Mai
18.	MHO1 -	12/2007	Beetroot	Plot 1, Mae hae, T. Mae vin, A. Mae wang, Chiang Mai
19.	PKI1-	01/2008	Tomato	Plot 1, Pa kluai, T. Mae vin, A. Mae wang, Chiang Mai
20.	PKI2-	01/2008	Tomato	Plot 2, Pa kluai, T. Mae vin, A. Mae wang, Chiang Mai
21.	PKO1-	01/2008	Tomato	Plot 1, Pa kluai, T. Mae vin, A. Mae wang, Chiang Mai
22.	MPI1-	02/2008	Cabbage	Plot 1, Mae phae, T. Samoeng nuea, A. Samoeng nuea, Chiang Mai
23.	MPI2-	02/2008	Cabbage	Plot 2, Mae phae, T. Samoeng nuea, A. Samoeng, Chiang Mai

Table 3.2 Continued

No.	Isolate code	Collection date (month/year)	Plant Host	Location
24.	MPO2-	02/2008	Cucumber	Plot 1, Mae phae, T. Samoeng nuea, A. Samoeng nuea, Chiang Mai
25.	KJI1-	04/2007	Egg plant	Plot 1, Khun jaey, T. Mae waen, A. Pround, Chiang Mai
26.	KJI2-	04/2007	Egg plant	Plot 2, Khun jaey, T. Mae waen, A. Pround, Chiang Mai
27.	KJO1-	04/2007	Egg plant	Plot 1, Khun j aey, T. Mae waen, A. Pround, Chiang Mai
28.	WJI1-	04/2007	Artichokes	Plot 1, Wat jan, T. Wat jun, A. Kalayaniwattana, Chiang Mai
29.	WJI2-	04/2007	Cabbage	Plot 2, Wat jan, T. Wat jun, A. Kalayaniwattana, Chiang Mai
30.	WJO1-	04/2007	Artichokes	Plot 1, Wat jan, T. Wat jun, A. Kalayaniwattana, Chiang Mai
31.	KNI1-	05/2007	Egg plant	Plot 1, Kae noi, T. Mueang na, A. Chiang dao, Chiang Mai
32.	KNI2-	05/2007	Egg plant	Plot 2, Kae noi, T. Mueang na, A. Chiang dao, Chiang Mai
33.	KNO1-	05/2007	Egg plant	Plot 1, Kae noi, T. Mueang na, A. Chiang dao, Chiang Mai
34.	PDI1-	04/2008	Fruit tree	Plot 1, Pang da, T. Samoeng tai, A. Samoeng, Chiang Mai
35.	PDI2-	04/2008	Fruit tree	Plot 2, Pang da, T. Samoeng tai, A. Samoeng, Chiang Mai
36.	PDO1-	04/2008	Fruit tree	Plot 1, Pang da, T. Samoeng tai, A. Samoeng, Chiang Mai
37.	MPlI1-	04/2007	Egg plant	Plot 1, Mae pun luang, T. Wiang, A.Wiang pa pao,Chiang Rai
38.	MPlO1-	04/2007	Egg plant	Plot 1, Mae pun luang, T. Wiang, A.Wiang pa pao,Chiang Rai
39.	WYI1-	04/2007	Carrot	Plot 1, Wang yang, T. Wang yang, A. Muang Nakhon Sawan, Nakhon Sawan
40.	API1-	09/2007	Lettuce	Plot 1, Aom pai, T. Pa pae, A. Hot, Mae Hong Son
41.	API2-	09/2007	Lettuce	Plot 2, Aom pai, T. Pa pae, A. Hot, Mae Hong Son
42.	API3-	09/2007	Lettuce	Plot 3, Aom pai, T. Pa pae, A. Hot, Mae Hong Son
43.	APO1-	09/2007	Tomato	Plot 1, Aom pai, T. Pa pae, A. Hot, Mae Hong Son
44.	APO2-	09/2007	Tomato	Plot 2, Aom pai, T. Pa pae, A. Hot, Mae Hong Son
45.	APO3-	09/2007	Tomato	Plot 3, Aom pai, T. Pa pae, A. Hot, Mae Hong Son

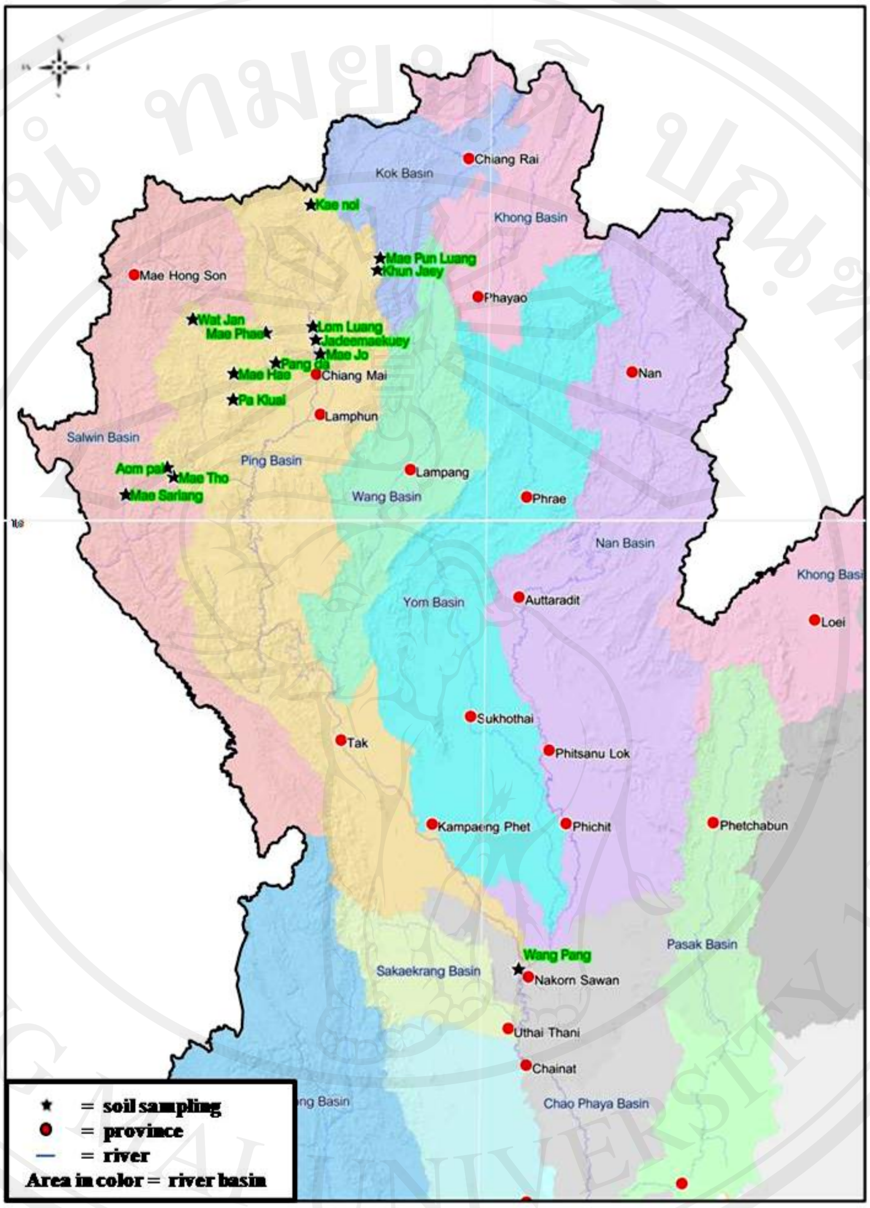

Figure 3.2 Location of soil samplings in river basin

3.3.2 Isolation of nematophagous fungi

One hundred and one nematophagous fungi classified as trapping fungi, five isolates as endoparasites and ten as egg parasites were isolated from forty-five soil samplings. The results showed that one hundred and three isolates were obtained from the Chiang Mai area, two from Chiang Rai, four from Nakhon Sawan and seven from Mae Hong Son. Preliminary examination of the isolates using "Key to the Nematode-Trapping Genera of Hyphomycetes and some Similar Genera" following Annemarthe (no date) enabled placement of the fungi into five genera: are *Arthrobotrys* sp., *Monacrosporium* sp. *Paecilomyces* sp. *Pochonia* sp and *Meristacrum* sp. The genus *Arthrobotrys* sp. was most commonly found (75%) followed by *Monacrosporium* sp. (12.93%), *Paecilomyces* sp. (7.76%), *Pochonia* sp. (0.86%) and *Meristacrum* sp. (3.45%) (Table 3.3).

The genus *Arthrobotrys* sp. formed either adhesive nets or constricting rings, depending on the species, to trap second stage juvenile (J2) of root-knot nematodes. Infective adhesive nets were plentifully found in this study. But, there were not similar characteristics such as size, amount and rapidity of structural formation observed among the isolates. Non-constricting ring, adhesive knobs (stalked knob) structures were formed by *Monacrosporium* sp. after the J2 were killed. Endoparsite *Meristacrum* sp. damaged J2 using adhesive spores. The genus *Paecilomyces* sp. attacked nematode eggs by their hyphal tips germinating from spores, thus they are called an egg parasite. Notably, nematophagous fungi obtained from infected root-knot nematode plantations represented 75% of the total vs. 25% found in areas rich in organic matter (Figure 3.3).

Table 3.3 List of nematophagous fungi isolating from soil plantations

No.	Isolate code	Plant Host	Province	Genera	Type of parasite	Infection structure
1.	JDI1-001	Tobacco	Chiang Mai	*Monacrosporium*	Trapping fungi	Non-constricting ring
2.	JDI2-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
3.	JDO1-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
4.	LLI1-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
5.	LLI1-002	Tobacco	Chiang Mai	*Monacrosporium*	Trapping fungi	Non-constricting ring
6.	LLI2-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
7.	LLO1-001	Tobacco	Chiang Mai	*Meristacrum*	Endoparasite	Adhesive spores
8.	MJI1-001	Tobacco	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
9.	MJI2-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
10.	MJO1-001	Tobacco	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
11	MTI1-001	Lettuce	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
12.	MTI1-002	Lettuce	Chiang Mai	*Meristacrum*	Endoparasite	Adhesive spores
13.	MTI1-003	Lettuce	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
14.	MTI1-004	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
15.	MTI1-005	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
16.	MTI1-006	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
17.	MTI1-007	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
18.	MTI1-008	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
19.	MTI1-009	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
20.	MTI1-0010	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
21.	MTI2-001	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
22.	MTI2-002	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
23.	MTI2-003	Lettuce	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
24.	MTO1-001	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
25.	MSI1-001	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
26.	MSI1-002	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
27.	MSI1-003	Tomato	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
28.	MSI2-001	Tomato	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
29.	MSI2-002	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
30.	MSI2-003	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
31.	MSI2-004	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
32.	MSO1-001	Chili	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
33.	MSO1-001	Chili	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
34.	MHI1-001	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
35.	MHI1-002	Beetroot	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
36.	MHI1-003	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
37.	MHI1-004	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
38.	MHI1-005	Beetroot	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
39.	MHI1-006	Beetroot	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
40.	MHI1-007	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
41.	MHI1-008	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
42.	MHI1-009	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
43.	MHI1-010	Beetroot	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
44.	MHI2-001	Artichokes	Chiang Mai	*Paecilomyces*	Eggparasite	Hyphal tips
45.	MHI2-002	Artichokes	Chiang Mai	*Paecilomyces*	Eggparasite	Hyphal tips

Table 3.3 Continued

No.	Isolate code	Plant Host	Province	Genus	Taxonomic classification	Infection structure
46.	MHI2-003	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
47.	MHI2-004	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
48.	MHI2-005	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
49.	MHI2-006	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
50.	MHI2-007	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
51.	MHO1-001	Beetroot	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
52.	MHO1-002	Beetroot	Chiang Mai	*Meristacrum*	Endoparasite	Adhesive spores
53.	MHO1-003	Beetroot	Chiang Mai	*Paecilomyces*	Egg parasite	Adhesive spores
54.	PKI1-001	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
55.	PKI1-002	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
56.	PKI2-001	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
57.	PKI2-002	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
58.	PKO1-001	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
59.	PKO1-002	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
60.	PKO1-003	Tomato	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
61.	MPI1-001	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
62.	MPI1-002	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
63.	MPI1-003	Cabbage	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
64.	MPI1-004	Cabbage	Chiang Mai	*Monacrosporium*	Trapping fungi	Non-constricting ring
65.	MPI2-001	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
66.	MPI2-002	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
67.	MPI2-003	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
68.	MPI2-004	Cabbage	Chiang Mai	*Monacrosporium*	Trapping fungi	Adhesive knobs
69.	MPO2-001	Cucumber	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
70.	MPO2-002	Cucumber	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
71.	MPO2-003	Cucumber	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
72.	MPO2-004	Cucumber	Chiang Mai	*Monacrosporium*	Trapping fungi	Non-constricting ring
73.	KJI1-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
74.	KJI1-002	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
75.	KJI2-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
76.	KJI2-002	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
77.	KJI2-003	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
78.	KJO1-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
79.	KJO1-002	Egg plant	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
80.	KJO1-003	Egg plant	Chiang Mai	*Pochonia*	Egg parasite	Hyphal tips
81.	WJI1-001	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
82.	WJI1-002	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
83.	WJI1-003	Artichokes	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
84.	WJI2-001	Cabbage	Chiang Mai	*Paecilomyces*	Egg parasite	Hyphal tips
85.	WJI2-002	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
86.	WJI2-003	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
87.	WJI2-004	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
88.	WJI2-005	Cabbage	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
89.	WJO1-001	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
90.	WJO1-002	Artichokes	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets

Table 3.3 Continued

No.	Isolate code	Plant Host	Province	Genus	Taxonomic classification	Infection structure
91.	KNI1-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
92.	KNI1-002	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
93.	KNI2-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
94.	KNI2-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
95.	KNI2-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
96.	KNO1-001	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
97.	KNO1-002	Egg plant	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
98.	PDI1-001	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
99.	PDI2-001	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
100.	PDI2-002	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Constricting rings
101.	PDI2-003	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
102.	PDO1-001	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
103.	PDO1-002	Fruit tree	Chiang Mai	*Arthrobotrys*	Trapping fungi	Adhesive nets
104.	MPlI1-001	Egg plant	Chiang Rai	*Arthrobotrys*	Trapping fungi	Adhesive nets
105.	MPlO1-001	Egg plant	Chiang Rai	*Meristacrum*	Endoparasite	Adhesive spores
106.	WYI1-001	Carrot	Nakhon Sawan	*Arthrobotrys*	Trapping fungi	Adhesive nets
107.	WYI1-002	Carrot	Nakhon Sawan	*Monacrosporium*	Trapping fungi	Non-constricting ring
108.	WYI1-003	Carrot	Nakhon Sawan	*Monacrosporium*	Trapping fungi	Non-constricting ring
109.	WYI1-004	Carrot	Nakhon Sawan	*Monacrosporium*	Trapping fungi	Non-constricting ring
110.	API1-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets
111.	API2-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets
112.	API3-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets
113.	APO1-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets
114.	APO2-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Constricting rings
115.	APO2-002	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets
116.	API3-001	Lettuce	Mae Hong Son	*Arthrobotrys*	Trapping fungi	Adhesive nets

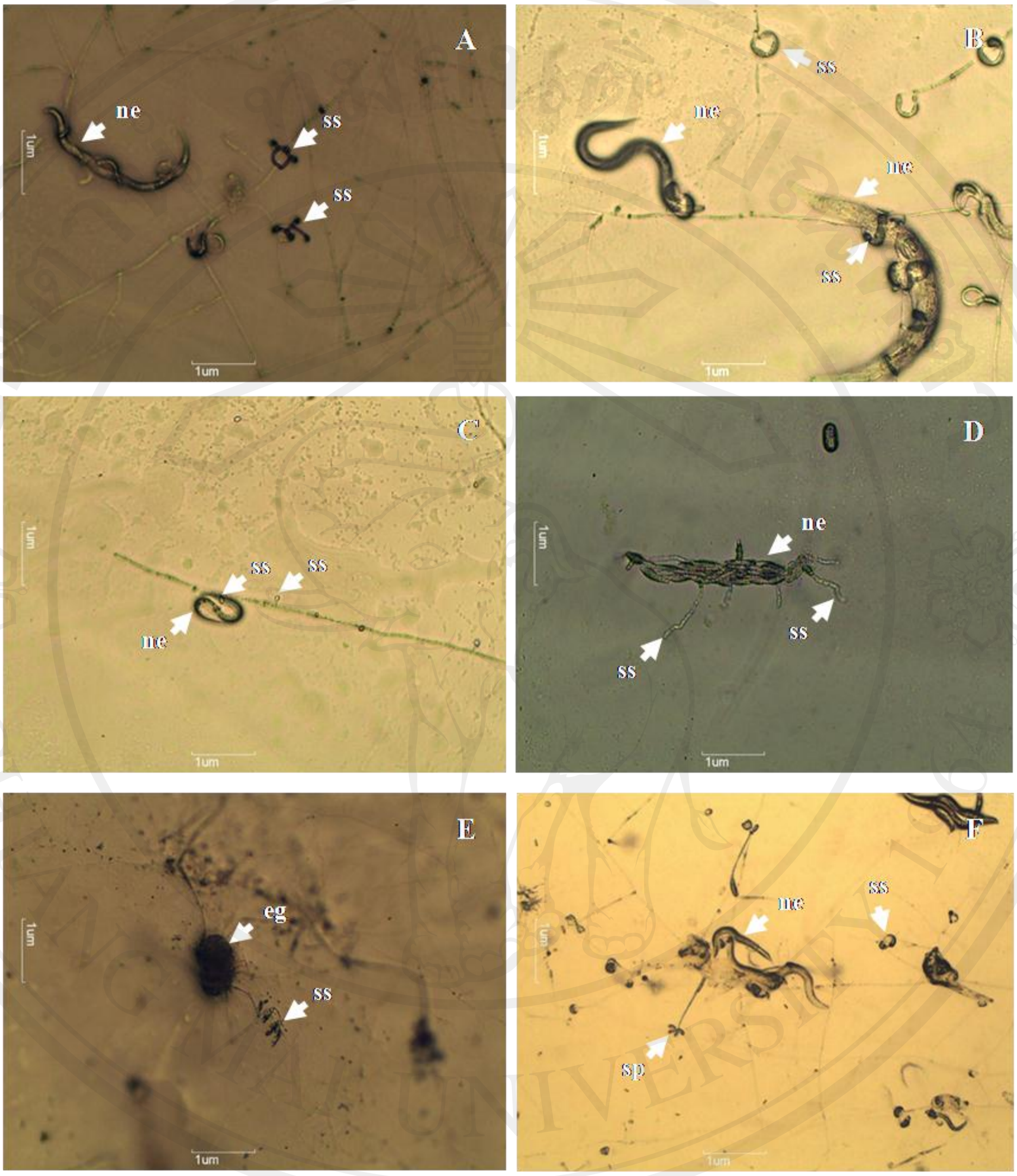

Figure 3.3 Characteristics of captured nematodes (ne) or egg (eg) by special structures (ss) of some nemathophagous fungi; A. adhesive nets of *Arthrobotrys* sp. B. constricting rings of *Arthrobotrys* sp. C. adhesive knobs of *Monacrosporium* sp. D. adhesive spores of *Meristacrum* sp. E. hyphal tips of *Paecilomyces* sp. F. sporulation (sp) of *Arthrobotrys* sp.

3.3.3 *In vitro* predacity of nematophagous fungi against *Meloidogyne* spp.

Results after inoculation of root-knot nematodes as bait on cultures of collected nematophagous fungi demonstrated an increasing percentage of damaged nematodes between 3 to 7 days. But, the capacity to capture and kill nematodes varied by each fungal isolate. A few fungal isolates showed quickness in capturing nematodes including *Monacrosporium* sp. isolate JDI1-001, *Arthrobotrys* sp. isolate MTI2-001, *Arthrobotrys* sp. isolate MJI2-001 and *Arthrobotrys* sp. isolate KNO1-001. The destructive process was initiated by most isolates after 5 days.

Seven fungal isolates, *Monacrosporium* sp. isolate JDI1-001, *Arthrobotrys* sp. isolate MTI2-001, *Arthrobotrys* sp. isolate MSO1-001, *Monacrosporium* sp. isolate MPI1-003, *Pochonia* sp. isolate KJO1-003, *Paecilomyces* sp. isolate WJI1-003 and *Arthrobotrys* sp. isolate API3-001, were selected for subsequent experiment "study and classification of fungal species" because of their destructive capacities against root-knot nematodes. After 7 days, *Monacrosporium* sp. isolate JDI1-001 damaged 100% of second stage juveniles (J2) of root-knot nematodes as *Arthrobotrys* sp. isolate MTI2-001. Moreover, *Arthrobotrys* sp. isolate MSO1-001, *Monacrosporium* sp. isolate MPI1-003 and *Arthrobotrys* sp. isolate API3-001 damaged J2 at 91.2%, 90.1% and 90.0%, respectively while *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 attacked 70.1 and 75.1% of eggs, respectively (Table 3.4).

Table 3.4 Percentage of nematophagous fungi to kill root-knot nematodes

No.	Isolate code	Genera	Percentage of damaged nematodes (%)[1/]		
			3 days	5 days	7 days
1.	**JDI1-001**	***Monacrosporium* sp.**	**9.0**	**83.0**	**100.0**
2.	JDI2-001	*Arthrobotrys* sp.	0.0	17.1	34.7
3.	JDO1-001	*Arthrobotrys* sp.	0.0	24.7	48.2
4.	LLI1-001	*Arthrobotrys* sp.	0.0	6.4	31.1
5.	LLI1-002	*Monacrosporium* sp.	4.8	18.7	52.8
6.	LLI2-001	*Arthrobotrys* sp.	6.1	28.1	58.2
7.	LLO1-001	*Meristacrum* sp.	0.0	2.4	12.5
8.	MJI1-001	*Monacrosporium* sp.	0.0	37.1	56.3
9.	MJI2-001	*Arthrobotrys* sp.	5.0	29.6	69.7
10.	MJO1-001	*Arthrobotrys* sp.	0.0	3.1	8.0
11	MTI1-001	*Monacrosporium* sp.	0.0	23.2	34.7
12.	MTI1-002	*Meristacrum* sp.	0.0	3.0	9.4
13.	MTI1-003	*Monacrosporium* sp.	9.7	37.7	71.1
14.	MTI1-004	*Arthrobotrys* sp.	0.0	38.1	48.7
15.	MTI1-005	*Arthrobotrys* sp.	0.0	4.7	11.1
16.	MTI1-006	*Arthrobotrys* sp.	0.0	34.2	43.7
17.	MTI1-007	*Arthrobotrys* sp.	0.0	8.0	15.8
18.	MTI1-008	*Arthrobotrys* sp.	0.0	4.7	12.4
19.	MTI1-009	*Arthrobotrys* sp.	8.3	63.8	70.2
20.	MTI1-0010	*Arthrobotrys* sp.	0.0	33.8	42.1
21.	**MTI2-001**	***Arthrobotrys* sp.**	**15.0**	**86.2**	**100 .0**
22.	MTI2-002	*Arthrobotrys* sp.	3.7	21.1	67.5
23.	MTI2-003	*Arthrobotrys* sp.	0.0	18.8	37.9
24.	MTO1-001	*Arthrobotrys* sp.	0.0	29.2	35.7
25.	MSI1-001	*Arthrobotrys* sp.	0.0	4.2	9.4
26.	MSI1-002	*Arthrobotrys* sp.	0.0	7.3	13.2
27.	MSI1-003	*Paecilomyces* sp.[2/]	0.0	12.8	18.6
28.	MSI2-001	*Paecilomyces* sp.[2/]	0.0	15.1	27.8
29.	MSI2-002	*Arthrobotrys* sp.	0.0	5.2	8.7
30.	MSI2-003	*Arthrobotrys* sp.	0.0	26.3	37.4
31.	MSI2-004	*Arthrobotrys* sp.	0.0	42.1	48.0
32.	MSO1-001	*Arthrobotrys* sp.	0.0	25.8	38.5
33.	**MSO1-001**	***Arthrobotrys* sp.**	**0.0**	**70.0**	**91.2**
34.	MHI1-001	*Arthrobotrys* sp.	0.0	7.7	20.1
35.	MHI1-002	*Monacrosporium* sp.	0.0	20.2	31
36.	MHI1-003	*Arthrobotrys* sp.	0.0	10.0	17.8
37.	MHI1-004	*Arthrobotrys* sp.	0.0	24.2	40.4
38.	MHI1-005	*Monacrosporium* sp.	0.0	27.7	38.8
39.	MHI1-006	*Paecilomyces* sp.[2/]	0.0	4.2	9.2
40.	MHI1-007	*Arthrobotrys* sp.	0.0	4.1	9.9
41.	MHI1-008	*Arthrobotrys* sp.	0.0	11.3	17.7
42.	MHI1-009	*Arthrobotrys* sp.	0.0	4.2	21.8
43.	MHI1-010	*Monacrosporium* sp.	0.0	7.4	27.0
44.	MHI2-001	*Paecilomyces* sp.[2/]	0.0	6.1	12.2
45.	MHI2-002	*Paecilomyces* sp.[2/]	0.0	7.8	13.4

[1/] Calculated from three replications

[2/] Attacked eggs of root-knot nematodes

Table 3.4 Continued

No.	Isolate code	Genera	Percentage of damaged nematodes (%)[1/]		
			3 days	5 days	7 days
46.	MHI2-003	*Arthrobotrys* sp.	0.0	18.7	30.1
47.	MHI2-004	*Arthrobotrys* sp.	0.0	14.5	21.1
48.	MHI2-005	*Arthrobotrys* sp.	0.0	3.3	21.0
49.	MHI2-006	*Arthrobotrys* sp.	0.0	9.5	17.1
50.	MHI2-007	*Arthrobotrys* sp.	0.0	8.8	30.4
51.	MHO1-001	*Arthrobotrys* sp.	0.0	13.1	24.2
52.	MHO1-002	*Meristacrum* sp.	0.0	2.0	7.8
53.	MHO1-003	*Paecilomyces* sp.[2/]	0.0	7.7	11.8
54.	PKI1-001	*Arthrobotrys* sp.	0.0	3.0	14.4
55.	PKI1-002	*Arthrobotrys* sp.	0.0	8.0	15.7
56.	PKI2-001	*Arthrobotrys* sp.	0.0	3.2	7.8
57.	PKI2-002	*Arthrobotrys* sp.	0.0	4.7	11.2
58.	PKO1-001	*Arthrobotrys* sp.	0.0	9.8	13.3
59.	PKO1-002	*Arthrobotrys* sp.	0.0	4.4	21.0
60.	PKO1-003	*Arthrobotrys* sp.	0.0	9.8	13.7
61.	MPI1-001	*Arthrobotrys* sp.	0.0	14.1	21.3
62.	MPI1-002	*Arthrobotrys* sp.	0.0	10.7	18.5
63.	**MPI1-003**	***Monacrosporium* sp.**	**0.0**	**76.4**	**90.1**
64.	MPI1-004	*Monacrosporium* sp.	0.0	13.7	23.8
65.	MPI2-001	*Arthrobotrys* sp.	0.0	14.0	17.7
66.	MPI2-002	*Arthrobotrys* sp.	0.0	7.2	31.1
67.	MPI2-003	*Arthrobotrys* sp.	0.0	7.9	43.7
68.	MPI2-004	*Monacrosporium* sp.	0.0	4.0	21.3
69.	MPO2-001	*Arthrobotrys* sp.	0.0	9.8	18.4
70.	MPO2-002	*Arthrobotrys* sp.	0.0	3.8	7.7
71.	MPO2-003	*Arthrobotrys* sp.	0.0	20.1	34.8
72.	MPO2-004	*Monacrosporium* sp.	0.0	7.7	14.2
73.	KJI1-001	*Arthrobotrys* sp.	0.0	7.1	20.8
74.	KJI1-002	*Arthrobotrys* sp.	0.0	8.7	14.7
75.	KJI2-001	*Arthrobotrys* sp.	0.0	3.9	8.1
76.	KJI2-002	*Arthrobotrys* sp.	0.0	18.4	24.2
77.	KJI2-003	*Arthrobotrys* sp.	0.0	4.3	23.6
78.	KJO1-001	*Arthrobotrys* sp.	0.0	0.1	7.8
79.	KJO1-002	*Paecilomyces* sp.[2/]	0.0	8.0	42.7
80.	**KJO1-003**	***Pochonia* sp.[2/]**	**0.0**	**18.1**	**70.1**
81.	WJI1-001	*Arthrobotrys* sp.	0.0	2.3	18.3
82.	WJI1-002	*Arthrobotrys* sp.	0.0	4.6	11.1
83.	**WJI1-003**	***Paecilomyces* sp.[2/]**	**0.0**	**15.4**	**75.1**
84.	WJI2-001	*Paecilomyces* sp.[2/]	0.0	7.8	17.6
85.	WJI2-002	*Arthrobotrys* sp.	0.0	11.4.	37.4
86.	WJI2-003	*Arthrobotrys* sp.	0.0	14.5	28.8
87.	WJI2-004	*Arthrobotrys* sp.	0.0	9.9	29.6
88.	WJI2-005	*Arthrobotrys* sp.	0.0	12.4	37.1
89.	WJO1-001	*Arthrobotrys* sp.	3.6	20.3	48.3
90.	WJO1-002	*Arthrobotrys* sp.	0.0	21.1	30.7

[1/] Calculated from three replications

[2/] Attacked eggs of root-knot nematodes

Table 3.4 Continued

No.	Isolate code	Genus	Percentage of damaged nematodes (%)[1/]		
			3 days	5 days	7 days
91.	KNI1-001	*Arthrobotrys* sp.	0.0	10.7	14.4
92.	KNI1-002	*Arthrobotrys* sp.	0.0	23.1	37.0
93.	KNI2-001	*Arthrobotrys* sp.	0.0	14.2	40.0
94.	KNI2-001	*Arthrobotrys* sp.	0.0	9.5	13.1
95.	KNI2-001	*Arthrobotrys* sp.	5.0	37.9	60.5
96.	KNO1-001	*Arthrobotrys* sp.	12.0	25.1	67.6
97.	KNO1-002	*Arthrobotrys* sp.	0.0	16.0	30.3
98.	PDI1-001	*Arthrobotrys* sp.	0.0	20.3	39.4
99.	PDI2-001	*Arthrobotrys* sp.	0.0	7.1	19.9
100.	PDI2-002	*Arthrobotrys* sp.	0.0	30.0	40.1
101.	PDI2-003	*Arthrobotrys* sp.	0.0	21.2	47.1
102.	PDO1-001	*Arthrobotrys* sp.	0.0	30.1	37.3
103.	PDO1-002	*Arthrobotrys* sp.	0.0	11.3	20.1
104.	MPlI1-001	*Arthrobotrys* sp.	0.0	24.6	49.0
105.	MPlO1-001	*Meristacrum* sp.	0.0	0.0	7.6
106.	WYI1-001	*Arthrobotrys* sp.	0.0	34.1	47.8
107.	WYI1-002	*Monacrosporium* sp.	0.0	23.1	40.3
108.	WYI1-003	*Monacrosporium* sp.	9.7	28.7	58.4
109.	WYI1-004	*Monacrosporium* sp.	0.0	25.2	37.3
110.	API1-001	*Arthrobotrys* sp.	0.0	0.9	7.1
111.	API2-001	*Arthrobotrys* sp.	0.0	12.2	47.0
112.	API3-001	*Arthrobotrys* sp.	0.0	17.3	37.1
113.	APO1-001	*Arthrobotrys* sp.	0.0	18.7	57.7
114.	APO2-001	*Arthrobotrys* sp.	7.0	27.7	61.3
115.	APO2-002	*Arthrobotrys* sp.	0.0	18.4	39.1
116.	**API3-001**	***Arthrobotrys* sp.**	**0.0**	**75.1**	**90.0**

[1/] Calculated from three replications

[2/] Attacked eggs of root-knot nematodes

3.4 Discussion

The genera of nematophagous fungi which were collected from soil around infected root-knot nematode areas and areas rich in organic matter in this study, *Arthrobotrys* sp., *Monacrosporium* sp., *Paecilomyces* sp., *Meristacrum* sp. and *Pochonia* sp. including their taxonomic classification and infection structures were similar taxonomically and morphologically (infection structures) to those reported by Nordbring-Hertz *et al*. (2006). At 71 isolates *Arthrobotrys* sp. was the most commonly recovered genus (Wikipedia, 2012c). A larger number of the fungi were obtained from infected root-knot nematode plantations more than the areas rich in organic matter because presumably the presence of obligate fungal parasites was associated with high soil nematode densities (Gray, 2002). In addition, organic matter had a high level and diversity of soil macro-organisms such as nematodes, arthropods; termites, mites, soil insects etc. that possibly consume the fungi especially fungivorous mites. The results differed from the belief of Jerzy *et al*. (2009) who stated that higher densities of nematophagous fungal species are possibly due to increased input of organic debris. In *in vitro* predation experiments, some isolates of collected fungi which are nematode trapping species, endoparasites and egg parasites had low capacities to damage either second stage juveniles (J2) or eggs of root-knot nematodes. This phenomenon may be related to the level of their saprophytic or parasitic ability, i.e. whether they are obligate parasites or facultative predators. The ability to capture nematodes is connected to a specific developmental phase of the fungal mycelium (Nordbring-Hertz *et al*., 2006). Gray (2002) reported that the presence of predatory fungi was influenced by pH and moisture more than the other soil factors, while the presence of the conidia-forming endoparasites was influenced by organic matter. Moreover, some species are able to compete well saprophytically under low organic matter and low moisture, and when nutrients or moisture temporarily improve, they are able to maintain their competitive advantage, especially in a suitable host. Nordbring-Hertz *et al*. (2006) stated that nematodes are attracted to the mycelia of the fungi which induces trap formation and development of traps and spores by a contact communication: adhesion. This step may involve an interaction between a carbohydrate-binding protein (lectin) in the fungus and a carbohydrate receptor on the nematode. Recognition of the host is probably also important for the subsequent steps of the infection, including penetration of the nematode cuticle. Furthermore, Mariam (2008) reported root-knot nematode *Meloidogyne incognita* was the most susceptible to predation by *Monacrosporium eudermatum*, *Arthrobotrys conoides*, *A. candida* and *A. conoides* in soil and on water agar. *A. brochopaga* formed the highest number of structures, in response to the inoculation of its culture with nematodes, but was unable to capture any nematodes. Result found that the use of second stage juvenile (J2) and eggs of root-knot nematodes as baits for selecting virulent nematophagous fungi is an effective approach in that it allows estimation of superficial damage, traps or spores formation, growth and sporulation. Nevertheless, nutrient levels, physical habitats, competitive conditions and compounds secreted by the host nematode along with the interactions in biochemical, physiological or morphological response are primary factors involved in fungal parasitic ability.

3.5 Conclusion

A number of highly virulent nematophagous fungi were recovered from both infested nematode plantations, which had high densities of root-knot nematodes, and areas rich in organic matter possessing a high biodiversity. Effective nematophagous fungi were isolated and selected from forty-five soil samplings in four provinces of Thailand. Five isolates, *Monacrosporium* sp. isolate JDI1-001, *Arthrobotrys* sp. isolate MTI2-001, *Arthrobotrys* sp. isolate MSO1-001, *Monacrosporium* sp. isolate MPI1-003 and *Arthrobotrys* sp. isolate API3-001 damaged 90-100% second stage juveniles (J2) of root-knot nematodes using special infection structures while two isolates, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003, infected 70-75% of eggs by means of hyphal tips. All isolates were used for subsequent experiments (Chapter 4).