BIOLOGICAL CONTROL POTENTIALITY OF FUNGI ASSOCIATED WITH ROOT KNOT NEMATODES

(*Meloidogyne* spp.)

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CERTIFICATE

This is to certify that thesis entitled, **"BIOLOGICAL CONTROL POTENTIALITY OF FUNGI ASSOCIATED WITH ROOT KNOT NEMATODES (*Meloidogyne* spp.)"** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of *bona fide* research work carried out by **Sayada Nasrin Jahan**, **Registration No. 05-01567** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 20th September, 2012
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Dedicated to

My

Parents & Teachers
Who Laid the Foundation of My Success

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The Author

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ABSTRACT

A survey was conducted to determine the microflora on egg and egg masses of *Meloidogyne* spp. collected from galled plant roots in Bangladesh. A total of 69 isolates belonging to 15 genera were obtained from 42 galled root samples collected from nursery and agricultural field of Dhaka, Gazipur, Narayangonj, Sirajgonj, Natore, Jessore and Jhenaidah district. *Aspergillus* spp. was the most predominent species and accounted for 28.99% of the total collected fungal isolates. Other fungal species frequently encountered were *Penicillium* spp., (15.94%), *Colletotrichum* spp. (10.14%), *Fusarium* spp. (5.80%), *Monocillium indicum* (4.35%) and *Paecilomyces* spp. (2.90%), *Trichoderma* spp (2.90%) and *Thysanopharo* spp. (2.90%). A total of 50 fungal isolates were used for pathogenecity tests *in-vitro*. Most of the isolates parasitized eggs, inhibit egg hatching and killed juveniles of *Meloidogyne* spp., although the pathogenecity varied among fungal species and isolates. The average egg parasitism, egg hatch and juvenile mortality ranged from 0.22 to 47.56%, 14.28 to 79.64% and 2.01 to 45.73%, respectively for all isolate tested. Only 28% of the isolates had no ability to parasitize *Meloidogyne* spp. eggs. The highest parasitism with 47.56% eggs was observed in *Aspergillus fumigatus*. More than half fungal isolates were capable of killing *Meloidogyne* spp. juveniles comparing to natural juvenile mortality of 3.34% in sterile water. The lowest egg hatch rate (14.28%) was observed in *Paecilomyces variotii* and the highest juveniles mortality rate (45.73%) was observed in *Fusarium culmorum* after 7 days of incubation. Two biocontrol fungi *Paecilomyces lilacinus* and *Paecilomyces variotii* isolated from Narayangonj and Sirajgonj with low egg parasitic rate (13.33 and 19.11%) and juvenile mortality rate (15.63 and 29.81%).

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ABBREBIATIONS AND ARONYMS

Cm	: Centimeter
et al.	: And others
etc	: Etcetera
g	: Gram
ml	: Milliliter
%	: Percent
^{o}C	: Degree Celsius
PDA	: Potato dextrose Agar
SAU	: Sher-e-Bangla Agricultural University.
$US	: USA dollar
NaOCl	: Sodium hypochlorite
(40/.65X10x X4x)	: Magnification (Objective X Eye piece X Camera)
SD	: Standard Deviation
Pf	: Percent free
Pi	: Percent infected
RKN	: Root knot nematodes

CHAPTER - I

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are a serious pathogen of many economic crops including oil plants, vegetables, fruit trees, tea, tobacco and medicinal plants and a major constraint to successful economic crop production all over the world causing significant yield losses, which cost about 78 billion us dollar worldwide annually (Baker, 1998). Annual crop losses caused by plant parasitic nematodes have been estimated to exceed $US 100 billion (Bird and Kaloshian, 2003), with more than half caused by *Meloidogyne* spp. Control of root knot nematodes has been primarily accomplished through chemical nematicides (Widmer and Abawi, 2000), which are costly and detrimental to the environment and human health (Nolling and Becker, 1994). In addition, their use is typically does not provide long-term nematode suppression. However, due to the significant drawbacks of the chemical control including threats to human health and the environment, biological control has become one of the promising alternatives (Stirling, 1991).

At present biocontrol seems to be most relevant and practically demanding approach for the control of root knot nematodes. Some of the opportunistic biocontrol agents like soil hyphomycetes have shown great promise. Some species of soil fungi colonizing phytonematode eggs, egg mass, females and cysts have been known for many years (Goffart, 1932) and their biocontrol potential has been studied (Stirling, 1991). Survey of fungal parasites of *Meloidogyne* spp. have been conducted worldwide and more than 30 genera and 80 Species, such as *Arthrobotrys* spp., *Monacrosporium* spp., *Paecilomyces lilacinus* and *Pochonia chlamydosporia* have been reported (Godoy *et al.*, 1983; Morgan-Jones *et al.*, 1984; He and Ge, 1987; Zhang *et al.*, 1993; Roccuzzo *et al.*, 1993; Li *et al.*, 1994; Chen *et al.*, 1996; Wang *et al.*,

2001). A number of potentially useful biological control agents of root knot nematodes (*Meloidogyne* spp.) have been isolated from nematode eggs (Morgan-Jones and Rodriguez-Kabana, 1988), but *Verticillium chlamydosporium* Goddard, *Dactylella oviparasitica* Stirling and Mankau and *Paecilomyces lilacinus* (Thom) Samson are the only species that have been studied in a detail. *V. chlamydosporium* has been found primarily in association with cyst nematodes but is known to parasitise root-knot nematodes (Morgan Jones *et al*., 1981 and 1984; Godoy *et al*., 1983). *D. oviparasitica* is associated with decline of root-knot nematodes in Californian peach orchards (Stirling *et al*., 1979) while *P. lilacinus* has been tested widely for its biocontrol capacity (Jatala, 1986; Hewlett *et al*., 1988; Cabanillas *et al***.,** 1989).

Trifonova *et al.* (2009) carried out mycological surveys of root-knot nematodes from the southern region of Bulgaria indicated that three species were associated with *Meloidogyne* viz. *Fusarium oxysporum*, *Verticillium chlamydosporium* and *Gliocladium roseum*. Hidal Go-Diaz *et al.* (2000) isolated a total of 83 isolates from *Meloidogne* infested soil and identified morphologically as *V. chlamydosporium* var. chlamydosporium, *V. chlamydosporium* var. *catenulatum*, *V. psalliotae*, *V. suchlasporium* and an isolate of *V. chlamydosporium* var. *chlamydosporium* with unusually large dictyochlamydospores. Verdejo-Lucas *et al.* (2002) isolated nine fungal species from single eggs of the nematode. The fungi included *Verticillium chlamydosporium*, *V. catenulatum*, *Fusarium oxysporum*, *F. solani*, *Fusarium* spp., *Acremonium strictum*, *Gliocladium roseum*, *Cylindrocarpon* spp., *Engiodontium album*, and *Dactylella oviparasitica*. A total of 455 fungal isolates belonging to 24 genera and 52 isolates of actinomycetes were obtained from 28 samples from greenhouses and fields in Hainan, Yunnan, Fujian, Hebei, Shandong, and Beijing (Sun *et al*., 2006).

Although biocontrol fungi of nematode are encountered frequently, little is known about their abundance, diversity, distribution, and role in regulating

nematode population in nature. Plant parasitic nematodes increase in numbers on susceptible hosts, particularly in mono cropping systems. When population of nematodes increase in soil, natural enemies, such as nematophagous fungi are also activated which parasitized and kill their hosts and consequently cause a decline in nematode abundance (Linford, 1937; Kerry, 1987; Jaffee *et al*., 1992).

Egg-parasitic fungi vary in their cultural characteristics and pathogenicity (Stirling and Mankau, 1978; Rodriguez-Kabana *et al*., 1984; Kerry *et al*., 1986; Irving and Kerry, 1986), but there have been few attempts to ensure that isolates chosen for biological control studies encompass this range of variability. Instead, isolates generally have been chosen at random from a limited range of material and surveys for egg parasites have not been followed by comprehensive screening tests to detect virulent isolates.

The objectives of the present research work were as bellows:

- To isolate and identify of the fungi associated with root knot nematodes (*Meloidogyne* spp*)* eggs and egg masses.
- To evaluate their biological control potentiality *in-vitro*.

CHAPTER - II

REVIEW OF LITERATURE

The nematodes *Meloidogyne* spp. is plant pest causing losses in a wide range of host crops, worldwide. They are managed by cultural practices, resistant cultivars or chemicals. Due to health concerns, the use of nematicides prompts for alternative control methods, including use of biocontrol agents or natural compound. Very few studies on the related to the biological control potentiality of fungi associated with root knot nematode (*Meloidogyne* spp.) have been carried out in many countries of the world. Nevertheless some of the important and informative works and research findings so far been done in abroad on this aspect have been reviewed in this chapter.

Gasperd *et al.* (1990) determined population densities of *Meloidogyne incognita* and the nematophagous fungi, *Paecilomyces lilacinus* and *Verticillium chlamydosporium*, in 20 northern California tomato fields over two growing seasons. *Paecilomyces lilacinus* was isolated from three fields, *V. chlamydosporium* was isolated from one field and both fungi were isolated from 12 fields. All *P. lilacinus and V. chlamydosporium* field isolates parasitized *M. incognita* eggs in vitro. In a greenhouse study, numbers of *V. chlamydosporium* and *P. lilacinus* increased more in soils with *M. incognita*-infected tomato plants than in soil with uninfected tomato plants. After 10 weeks, the Pf/ Pi of second-stage juveniles in soils infested with *P. lilacinus, V. chlamydosporium,* and *M. incognita* was 47.1 to 295.6.

Stirling and West (1991) carried out a survey of 46 Queensland soils for fungi capable parasitising eggs of root-knot nematodes, *Verticillium chlamydosporium* and *Paecilomyces lilacinus* were recovered. When the soils were potted, planted to tomatoes and inoculated with root-knot nematodes,

eggs parasitised by these fungi were observed more commonly in soils that had been naturally infested with the nematode than in soils where the nematode was absent. *P. lilacinus* was commonly isolated from chitin-amended soil but the addition of chitin did not increase egg parasitism. To identify isolates with the best biocontrol potential, 26 isolates of *P. lilacinus* and 13 isolates of *V. chlamydosporium* were screened for parasitic activity against eggs of *Meloidogyne javanica* using three different tests. Within each test, the number of eggs parasitised by different isolates of the same fungus varied considerably, suggesting that isolates differed in virulence. Two isolates of *V. chlamydosporium* and one isolate of *P. lilacinus* were highly parasitic in all three tests.

Frans *et al.* (1991) investigated the potentiality of three *Verticillium chlamydosporium* isolates as biological control agents against *Meloidogyne arenaria* on tomato plants under glasshouse conditions. All three isolates survived well in soil but showed marked differences in their ability to colonise uninfected roots, nematode galls and nematode eggs. Significant population reductions of >80% after the first nematode generation, were achieved with one isolate, which resulted in significant damage control, but not population control, in subsequent generations. Establishment of *V. chlamydosporium* in soil was significantly greater if the fungus was introduced without a food base, i.e. as hyphal fragments and chlamydospores rather than colonised sand-bran. The fungus did not invade the root cortex and there were no adverse effects of the fungus on plant growth.

Odour-Owino and Waudo (1996) observed *Paecilomyces lilacinus, Phoma herbarum* and three isolates of *Fusarium oxysporum* differed significantly in their ability to parasitize eggs and females of *Meloidogyne javanica. P. lilacinus* and *F. oxysporum*-1 significantly (P0.05) parasitized more than 70% of eggs and females while *F. oxysporum*-3 parasitized less than 20%. Also, *P. lilacinus* and *F. oxysporum*-1 had the greatest suppresive effect on hatching. In

general, control Petridishes and those treated with *F. oxysporum*-3 had the highest proportions of hatched eggs, but exhibited the least levels of egg parasitism. The fungus *P. lilacinus* significantly (P0.05) parasitized eggs of *M. javanica, M. incognita* and *M. arenaria* but no significant differences were detected in the levels of parasitism.

Hidal Go-Diaz *et al.* (2000) isolated a total of 83 fungal isolates and identified morphologically as *V. chlamydosporium* var. chlamydosporium, *V. chlamydosporium* var. *catenulatum, V. psalliotae, V. suchlasporium* and an isolate of *V. chlamydosporium* var. c*hlamydosporium*. From these, 24 that represented a range of origins were selected and screened for their ability to parasitize eggs of root-knot nematodes, colonize the rhizosphere of barley roots and produce chlamydospores. These were also screened in the glasshouse and *V. chlamydosporium var. catenulatum* caused the greatest reduction in nematode populations. One isolate of each subspecies of *V. chlamydosporium* was tested with the standard, Rothamsted isolate 10, on a range of host plants. The greatest reduction in numbers of nematodes occurred on tomato plants (cv. Pixie). The Rothamsted isolate 10 reduced numbers of nematodes to a greater extent than the other isolates, and therefore has the greatest potential as a biological control agent of root-knot nematodes.

Vianene and Abawi (2000) evaluated *Hirsutella rhossiliensis* and *Verticillium chlamydosporium* infected second-stage juveniles (J2) and eggs of *Meloidogyne hapla,* respectively, in petridishes and in organic soil in pots planted to lettuce in the greenhouse. In vitro, *H. rhossiliensis* produced 78 to 124 spores/infected J2 of *M. hapla.* The number of J2 in roots of lettuce seedlings decreased exponentially with increasing numbers of vegetative colonies of *H. rhossiliensis* in the soil.

Zareen *et al.* (2001) tested the effect of 10 strain of *Fusarium solani* on *Meloidogyne javanica in vitro* and in controlled conditions. Culture filtrates of the strains varied with respect to parasitism on eggs and females of *M. javanica* and nematicidal activity in terms of juvenile mortality. Mortality in boiled culture filtrates was slightly lower than that caused by un-boiled filtrates. Aqueous and ethyl acetate extracts of *F. solani* produced higher nematicidal activity than a hexane extract indicating that the active compound(s) were polar in nature. Conidial suspensions of *F. solani* strains Fs5, Fs9 and Fs10 used as soil drench that significantly reduced nematode populations in soil and root-knot disease severity, resulting in enhanced growth of tomato plants. There was no significant difference among *F. solani* strains on shoot fresh weight. Strain Fs5 was frequently reisolated from surface sterilized tomato roots. When evaluated in a field test, strain Fs5, reduced *M. javanica* reproductive potential and promote growth of tomato plants. However, root length and fresh root weights were slightly lower in Fs5-treated plants.

Verdejo-Lucas *et al*. (2002) isolated nine fungal species from single eggs of the nematode. The fungi included *Verticillium chlamydosporium, V. catenulatum, Fusarium oxysporum, F. solani, Fusarium* spp., *Acremonium strictum, Gliocladium roseum, Cylindrocarpon* spp., *Engiodontium album,* and *Dactylella oviparasitica*. Two sterile fungi and five unidentified fungi were also isolated from *Meloidogyne* spp. eggs.

Abd EL-Raheem *et al.* (2005) evaluated the nematophagous fungui *Pochonia chlamydosporia, Paecilomyces lilacinus* and *Arthrobotrys dactyloide*s as biological control agents for *Meloidogyne incognita* under greenhouse conditions. Experiments confined the effectiveness of these predatory and parasitic fungi that actively reduced the number of infective larvae of *M. incognita*. The killing effect of these fungi is similar to the synthetic chemical nematicide. The fungi under consideration have the potentiality to reduce population density of *M. incognita* along the growing season of faba bean plant

from 95.4 to 98.9%. These nematophagous fungi enhanced shoot and root growth of faba bean.

Sun *et al.* (2006) conducted a survey to determine the microflora on eggs and females of *Meloidogyne* spp. collected from plant roots and infested soil in China. A total of 455 fungal isolates belonging to 24 genera and 52 isolates of actinomycetes were obtained from 28 samples from greenhouses and fields in Hainan, Yunnan, Fujian, Hebei, Shandong, and Beijing. The predominant fungal species were *Paecilomyces lilacinus* (49.3% of the isolates collected), *Fusarium* spp. (7.9%), *Pochonia chlamydosporia* (6.9%), *Penicillium* spp. (5.7%), *Aspergillus* spp. (3.2%), and *Acremonium* spp. (2.8%). A total of 350 isolates of nematophagous fungi and actinomycetes were evaluated for their parasitism of eggs and effects on egg hatch and juvenile mortality in vitro. Pathogenicity varied among isolates, and 29.1% of isolates parasitized over 90% eggs 4 days after inoculation. Results also showed that seven isolates of fungi and actinomycetes reduced egg hatch rates to less than 10% contrasted to the control of 65.8%, and three isolates killed all hatched juveniles after 7 days. Seventeen fungal isolates and four actinomycete isolates with high pathogenicity in vitro were selected to test biocontrol efficacy in the greenhouse. They reduced tomato root gall index by 13.4–58.9% compared to the no treatment control.

Kiewnick and Sikora (2006) evaluated the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251, for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. In growth chamber experiments, a pre-planting soil treatment reduced root galling by 66%, number of egg masses by 74% and the final nematode population in the roots by 71% compared to the inoculated control.

Saxena (2008) were isolated a total of 54 nematophagous fungi from soil samples from 4 different types of habitats in Edinburgh, Scotland. Nematode-trapping fungi included 11 species of *Arthrobotrys,* 2 each of *Dactylellina* and *Drechslerella* and 3 of *Stylopage.* Fifteen species of endoparasites were isolated. The highest frequency of occurrence in terms of habitat was of *Arthrobotrys gephyropaga* and *Drechslerella brochopaga* among predators and that of *Harposporium anguillulae* among endoparasites.

Trifonova *et al.* (2009) carried out mycological surveys on fungal parasites of root-knot nematodes from the southern region of Bulgaria. They found three fungal species associated with *Meloidogyne*, namely *Fusarium oxysporum*, *Verticillium chlamydosporium*, and *Gliocladium roseum*. The fungi infected up to 8.7% of the eggs in the females of *Meloidogyne* spp. In the field the egg parasitism by fungi was also observed.

Ibrahim *et al.* (2009) determined fungal colonization for females and cysts of *Heterodera avenae* on wheat roots or rhizosphere soil, and also determined for eggs and juveniles of *Meloidogyne incognita* on tomato. The common fungi isolated from *H. avenae* were *Fusarium oxysporum, Paecilomyces lilacinus, Verticillium chlamydosporium* and *Rhizoctonia solani.* Also, the common fungi isolated from *M. incognita* were *Aspergillus* spp., *Alternaria alternata, F. oxysporum, P. lilacinus* and *V. chlamydosporium.* The effect of biocontrol fungi which isolated from *H. avenae* or *M. incognita* as well as the antagonistic bacterium *Bacillus thuringiensis* were examined against root-knot nematode infected tomato plants and the results indicated that the highest reduction in galls was observed with *P. lilacinus* (82.92%) followed by *V. chlamydosporium* (77.6%), *B. thuringiensis* (60.91%) and *F. oxysporum* (27.92%) as compared with plants infected with *M. incognita* alone.

Aminuzzaman (2009) isolated seventy fungal isolates belonging to seven genera with naturally infected eggs and females of root-knot nematodes (RKN), *Meloidogyne* spp. The predominant fungal species were *Fusarium* spp. (44.33% of the isolates collected), *Paecilomyces lilacinus* (11.44%), *Fusarium oxysporum* (11.44%), *Aspergillus* spp. (5.72%) and *Pochonia chlamydosporia* (4.29%). The isolates were evaluated for their potentiality to parasitize root knot nematode egg in 24 well tissue culture plate where 50 nematode eggs were added into each well of 24-well tissue culture plate having 1ml spore suspension of 10^5 spore per ml. The average egg parasitism, egg hatch and juvenile mortality ranged from 1.3 to 100%, 2.3 to 16.2% and 0.00 to 66.6%, respectively, for the all isolates tested. The fungal pellet contains spores of nematophagous fungus *Paecilomyces lilacinus* YES-2-14 was evaluated in green house to assess its bio control potentiality against root knot of tomato. Fungal pellet significantly reduced root gall index over untreated control at the end of the season. *Paecilomyces lilacinus* also significantly reduced the number of nematode populations in soil and plant roots and increased 20.75% tomato fruit yield over untreated control.

Moosavi *et al.* (2010) investigated Fars province of Iran for the presence of *Pochonia* spp., compared pathogenicity of different *Pochonia* species on eggs of RKN in vitro and selected the best isolates for further studies. They collected 128 soil samples of fields infested with cyst nematodes and 18 soil samples infested with root knot nematode were from Fars province of Iran. In vitro pathogenicity tests were carried out on 36 isolates of *Pochonia* spp. obtained from CBS and IRAN culture collections. The seven best isolates of this experiment were selected for greenhouse test and their ability in controlling RKN was examined in natural soil. In vitro pathogenicity of *Pochonia* on RKN eggs varied between 39 and 95% eggs infected. In greenhouse experiment, three isolates are promising for control of RKN and selected isolates are

subjected to more extensive testing to determine their effectiveness in a range of conditions before being developed as commercial biological control agents.

Anita and Selvaraj (2010) studied on the occurrence and impact of native antagonistic fungi on the root knot nematode, *Meloidogyne hapla* in Nilgiris revealed that the most common antagonistic fungi associated were *Trichoderma viride*, *Paecilomyces lilacinus* and *Verticillium lecanii*. The fungal culture filtrates were tested for their larvicidal and ovicidal properties in vitro. The antagonistic effect of these fungi on nematode was further confirmed *in vivo* under green house conditions. Both *in vitro* and *in vivo* studies revealed that *T. viride* and *P. lilacinus* were the most effective species against *M. hapla.* The egg parasitic fungus completely parasitized the eggs and depleted the egg content. The mortality of the juveniles increased with increase in the concentration and exposure to fungal culture filtrates. *In vivo* studies indicated that these fungi were highly effective in reducing the population of root knot nematodes in soil and can be used effectively.

Regaieg *et al.* (2010) evaluated filtrates of three isolates of the nematophagous fungus *Verticillium leptobactrum* for their nematicidal activity against the root-knot nematode *Meloidogyne incognita*. The filtrates inhibited egg hatching, with maximum toxicity observed for isolate HR21 at 50% (v: v) dilution, after 7 days exposure. Filtrates also inactivated second-stage juveniles (J2) at 10-50% dilutions. A scanning electron microscopy study of treated eggs showed severe alterations caused by the filtrate of isolate HR43 on *M. incognita* eggs, which appeared collapsed and not viable, suggesting the production of chitin-degrading enzymes or other active compounds.

Ruanpanum *et al*. (2010) isolated 83 actinomycetes isolates and 67 fungal isolates from 23 plant parasitic nematode infested soils from Thailand. The predominant fungal taxa were *Penicillium* (37.3%) and *Fusarium* (32.8%). All actinomycete and fungal isolates were subjected for primary screening in vitro for their effects on egg hatching and juvenile mortality of *Meloidogyne incognita*. From primary screening, 7 actinomycete and 10 fungal isolates reduced egg hatching and kill juveniles of *M. incognita* after 7 days incubation.

Carneiro *et al.* (2011) conducted glasshouse experiments in order to evaluate the effect of the fungi *Paecilomyces lilacinus* and *Pochonia chlamydosporia* on a population of *M. enterolobii* growing on guava plants. Guava seedlings of about 15-20 cm growing in plastic bags were inoculated with 10000 eggs of *M. enterolobii* plant^{-1}. Two months later, three isolates of *P. lilacinus* and one isolate of *P. chlamydosporia* were inoculated in the infested plants. The most effective result (61.5% of control) was obtained with the isolate CG1003 of *P. chlamydoporia*, followed by *P. lilacinus* (CG959 and CG1038) with about 40% of control. These fungi showed the ability to colonise healthy guava roots in glasshouse experiments. These results suggest that *P. chlamydosporia* can be selected as a potential biological control agent to be employed with other strategies in integrated management to control *M. enterolobii* on guava.

Kiewnick *et al.* (2011) evaluated the fungal biocontrol agent, *Paecilomyces lilacinus* strain 251, for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato at varying application rates and inoculum densities. They demonstrated that a preplanting soil treatment with the lowest dose of commercially formulated PL251 (2×10^5 CFU/g soil) was already sufficient to reduce root galling by 45% and number of egg masses by 69% when averaged over inoculum densities of 100 to 1,600 eggs and infective juveniles per 100 cm^3 of soil.

Aminuzzaman *et al.* (2011) evaluated the alginate pellet formulation of *Paecilomyces lilacinus* YES-2, which was isolated from root-knot nematode egg, and evaluated for bio-control against *Meloidogyne* spp. on brinjal and tomato in green house pot trail. The result showed that the pellets of *Paecilomyces lilacinus* enhanced plant growth and reduced galling index and nematode population in all treatments of different dosages applied. The biological control efficiency of the fungus against root-knot nematode was significantly higher along with the increase of dosages of bioagent applied for of the crops. Root galling index and final nematode population decreased up to 40.7 and 73.8%, respectively for tomato and 55.6and 66.9%, respectively for brinjal at the highest rate (1.6%) of application of the bio-control fungus.

Aminuzzaman and Liu (2011) collected galled roots of eggplant infected with *Meloidogyne* spp. from farmer's field of Mymensing, Bangladesh. They isolated biocontrol fungus *Paecilomyces lilacinus* from eggs of *Meloidogyne* spp. The fungus showed more than 80% egg parasitism and 52% juvenile mortality of *Meloidogyne* spp. by screening method in 24-well tissue culture plate. In green house study, it was found that the fungus increased shoot height, fresh shoot weight, root length and fresh root weight and also reduced root galling index up to 63% and number of egg mass per root system up to 40% when compared with control treatment. This was a new record of *Meloidogyne* egg parasitic fungus reported in Bangladesh and the evaluation of the bio-control potential *Paecilomyces lilacinus* against root-knot nematodes.

Aminuzzaman *et al.* (2013) isolated nematophagous fungi from *Meloidogyne* spp. eggs and females on 102 field collected root samples in China. Of the 235 fungi isolated (representing 18 genera and 26 species), the predominant fungi were *Fusarium s*pp. (42% of the isolates collected), *F. oxysporun* (13.2%), *Paecilomyces lilacinus* (12.8%) and *Pochonia chlamydosporia* (8.5%). The isolates were screened for their ability to parasitize *M. incognita* egg in 24-well tissue-culture plates in two different tests. The most promising fungi included

four *Paecilomyces* isolates, 11 *Fusarium* isolates, 10 *Pochania* isolates and one *Acremonium* isolate in test 1 or 2. *P. lilacinus* YES-2 and *P. chlamydosporia* HDZ-9 selected from the *in vitro* tests were formulated in alginate pellets, and evaluated for *M. incognita* control on tomato in green house by adding them into soil with a sand mixture at rate of 0.2, 0.4, 0.8 and 1.6% (w/w). *P. lilacinus* pellets at the higher rate (1.6%) reduced root galling by 50%. *P. chlamydosporia* pellets at the higher rate reduced the final nematode number 91%. The result showed that alginate pellets are suitable formulations for control of root-knot nematode in soil.

CHAPTER - III

MATERIALS AND METHODS

Isolation, identification and evaluation of biological control potentiality of fungi associated with root knot nematodes, *Meloidogyne* spp. were carried out in the Disease Diagnosis Laboratory of the Department of Plant Pathology, Sher-e- Bangla Agricultural University, Dhaka, Bangladesh during the period of January, 2011 to August, 2012. This chapter includes major information regarding materials and methods that were used to conduct the experiment. It consist of short description of locations of the experimental site, climate, materials used for the experiment, data collection procedure, statistical analysis etc. The details regarding materials and methods of the experiments are presented below under the following headings

3.1. Laboratory experiment

3.1.1. Experimental site

The experiment was conducted at the disease diagnosis laboratory of the Department of Plant Pathology, Sher-e- Bangla Agricultural University. Dhaka - 1207.

3.1.2. Experimental period

The experiment was conducted during the period of January 2011 to August 2012.

3.1.3. Collection of plant roots

Forty two plant root sample showing typical root knot symptoms were collected from different region of Bangladesh namely Dhaka, Gazipur, Narayongonj, Sirajgonj, Natore, Jessore and Jhenaidah (Plate 1). Plant roots of brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum),* Indian spinach (*Basella alba*), papaya (*Carica papaya*), potato (*Solanum tuberosum*),

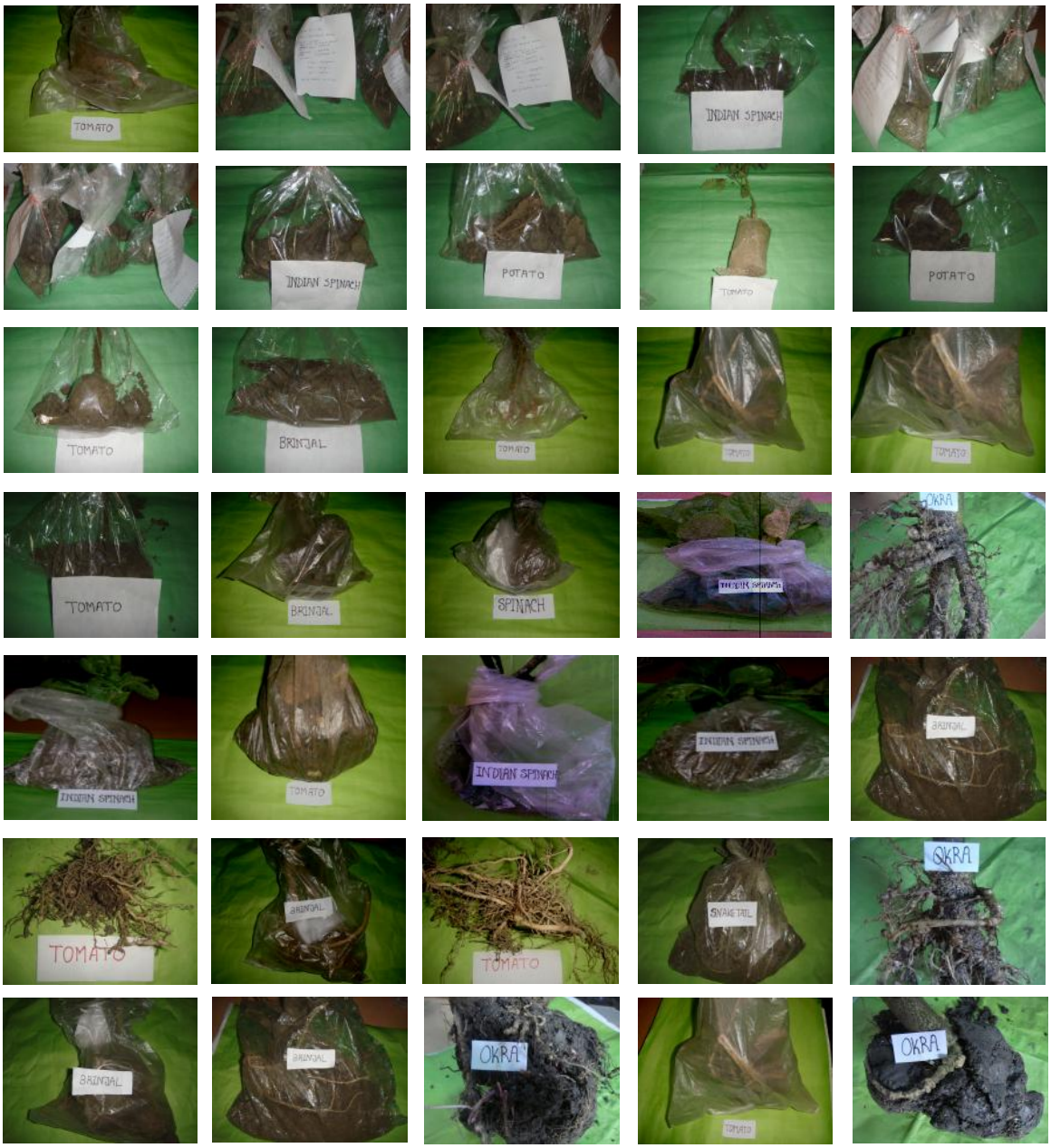


Plate 1. Root knot samples collected from different region of Bangladesh

Jute (*Corchorus capsularis*), spinach (*Spinacia oleracea*) and okra (*Abelmoschus esculentus*) with galls and adjacent soil were taken and placed in double sealed polyethene bags. All samples were stored in polyethylene bags at room temperature for quick isolation of fungi.

3.1.4. Microflora assay

Galled roots were washed in running tap water to get rid of soil. Gall were separated from roots, cut in small pieces and blended with water by blender. The suspension was passed through 200 meshes and 600 mesh sieve to separate eggs from root debris. Eggs suspension was then be treated by 1% sodium hypochloride (NaOCl) for 1 min to surface disinfest. The treated eggs were washed with sterile water for three times to remove residual NaOCl. 200µl egg suspension of approximately 50 eggs were smeared on each PDA plate and incubated at 23^0 C. Egg mass from roots were also picked and surface sterilized with 1% NaOCl and cultured on PDA plate as described above. Hyphae grown from eggs and eggs mass were transferred to PDA plate for purification and identification (Plate 2). The fungi were identified primarily according to ‘‘Compendium of Soil Fungi‘‘ (Domsch *et al*., 1980) and‘‘ Illustrated genera of imperfect fungi‘‘ (Barnett and Hunter, 1998). All isolates were maintained on PDA slant at 4^0 C and eppendorf tube at 0^0C for further use.

3.1.5. Pathogenicity test in vitro

The test was done following the method of Den Belder and Jansen (1994). The isolated fungal isolates were tested for their pathogenicity against *Meloidogyne incognita in-vitro*. The fungi were cultured on PDA plates at 25^0 C for 15 days. A plug of PDA was cut off from the margin of the colony and transferred to a 15ml sterile plastic centrifuge tube containing 5 ml sterile 0.05% Tween 80. The tube was agitated vigorously on a shaker for 1 min to dislodge and suspend the spores. The spore density was determined using a haemacytometer with the aid of microscope and adjusted to 10^5 spore/ml. For the isolates of sterile fungus hyphal fragments were used as inocula.

Meloidogyne incognita was cultured on tomato and brinjal plants and eggs were collected from the roots as described above. A 50 µl egg suspension, containing approximately 300 eggs, were pipetted into each well of a 24- well tissue culture plate containing 1 ml spore suspension of the tested fungus and incubated at 25-28 ^{0} C temperature (Plate 4). Instead of spore suspension, sterile water was added as control. After 4 days, the percentages of parasitized eggs were determined by randomly examining 100 eggs under compound microscope. The culture plates were incubated again at 25-28^{0} C for three more days. Egg hatch rate and juvenile mortality were determined by counting all eggs, juveniles, and dead juveniles under the compound microscope and calculated according to the following formulas: egg hatch rate=100×juveniles/(eggs + juveniles), and juvenile mortality = 100 × dead juveniles/total juveniles. The juvenile colonized by microbes, malformed or stiff were considered to be killed. The test was done with three replicates for each isolate. The means of microbial parasitism were calculated and the effects on eggs and juveniles were recorded by counting total number of *Meloidogyne incognita* in three wells (ca. 100 per well) to reduce error.

3.1.6: Data analysis

Mean values followed by ± standard deviation (±SD) were calculated for egg parasitism, egg hatch rate and juvenile mortality rate.

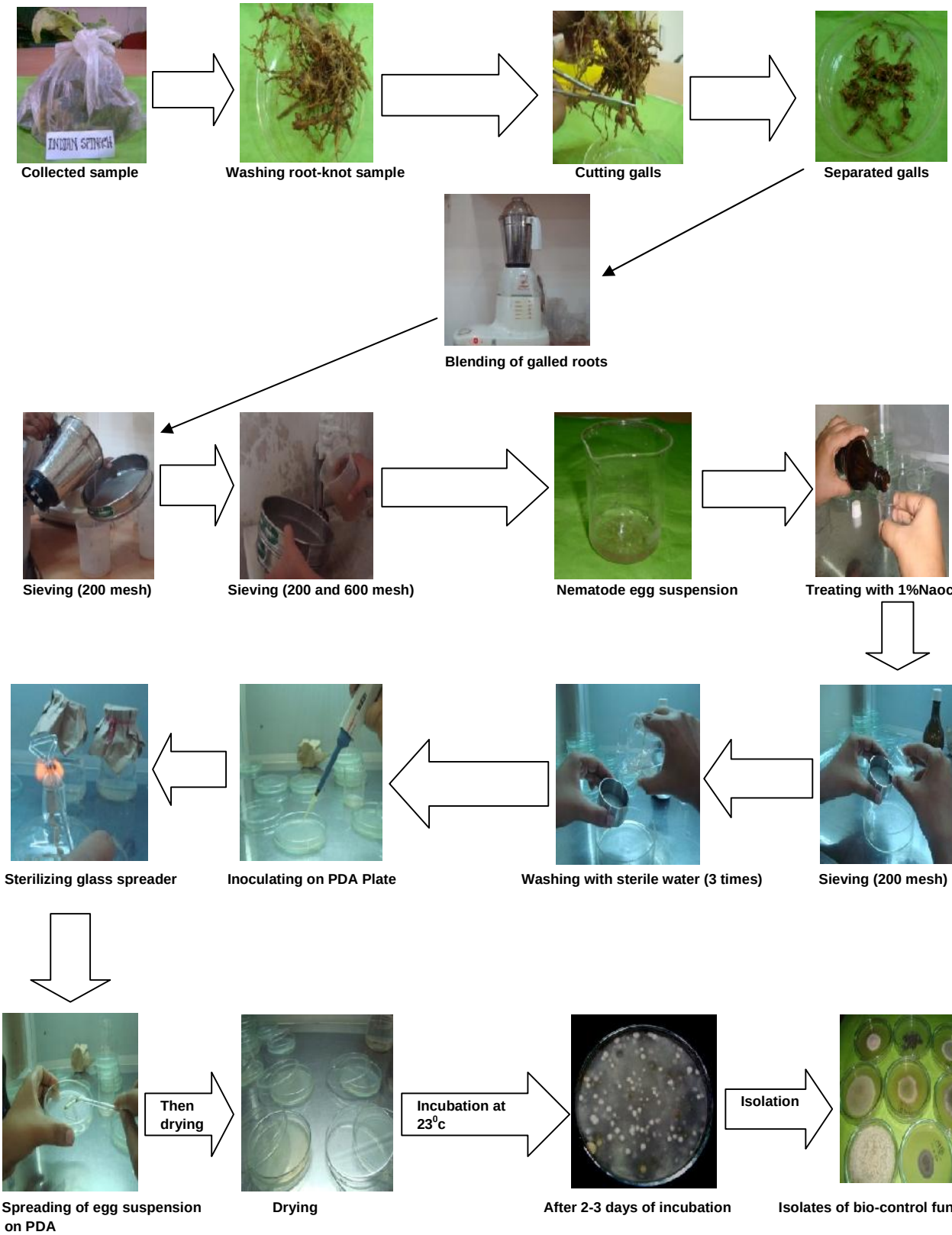
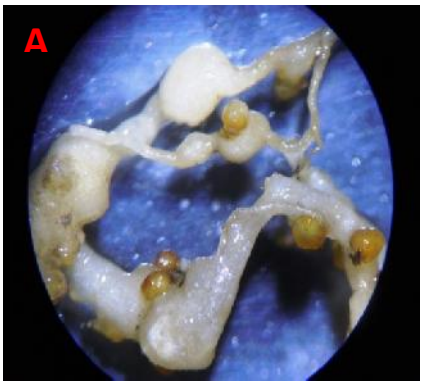


Plate 2. Steps involved in isolation of bio-control fungi from root-knot nematodes eggs



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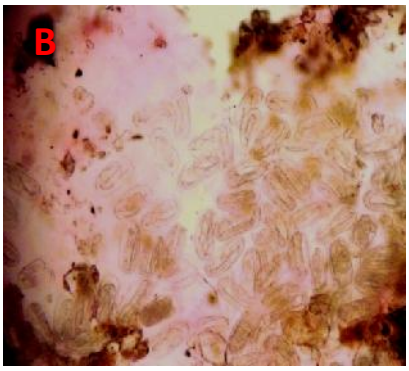

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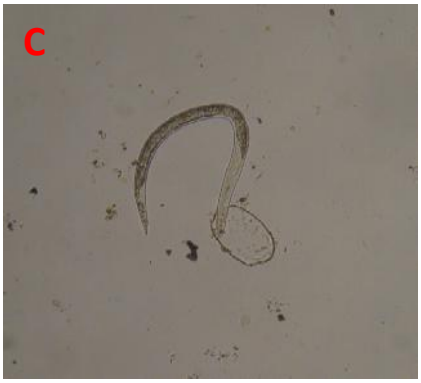

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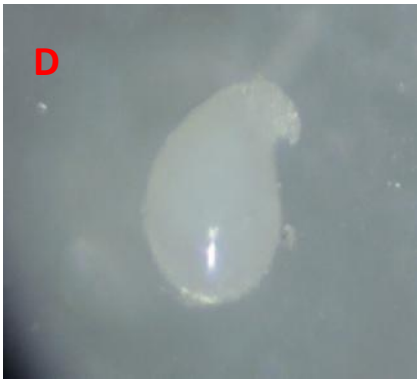

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Plate 3.Different stages of *Meloidogyne* spp. A. Egg mass B. Eggs in egg mass C. Juvenile and D. Female

Plate 4. Preservation of isolates in A. PDA slant B. Eppendorf tube and C. Pathogenicity test *in-vitro*

CHAPTER - IV

RESULTS

4.1. Microflora of *Meloidogyne* spp. eggs and egg masses

Microbes associated with *Meloidogyne* spp. were collected from 13 sites of 7 districts of Bangladesh (Table 1). A total of 26 samples were encountered with fungi from total 42 samples (Table 2). A total of 53 isolates fungi were obtained from *Meloidogyne* spp. eggs and 16 isolates from egg masses (Table 2). No parasites were isolated from egg and egg masses in sample Gazipur, and Natore (Table 2). The host plants are brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum)*, Indian spinach (*Basella alba*), papaya (*Carica papaya*), potato (*Solanum tuberosum*), jute (*Corchorus capsularis*), spinach (*Spinacia oleracea*) and okra (*Abelmoschus esculentus*) (Table 1).

The microbial communities associated with *Meloidogyne* eggs and egg masses were varied among sampling sites. Maximum 38 isolated were recovered from Dhaka, followed by 10 from Sirajgonj, 10 from jessore , 7 from Narayangonj, 4 from Jhenaidah (Table 2). A total of 69 fungal isolates were recovered represented 15 genera. Although a wide range of species were isolated, few of them occurred with high frequency. *Aspergillus* spp. was the most prominent species and accounted for 28.99% of the total collected fungal isolates (Table 3). Other fungal species frequently encountered were *Penicillium* spp., (15.94%), *Colletotrichum* spp. (10.14%), *Fusarium* spp. (5.80%), *Monocillium* spp (4.35%) and *Paecilomyces* spp. (2.90%), *Trichoderma* spp (2.90%) and *Thysanophora* spp. (2.90%) (Table 3). *Aspergillus* spp. and *Penicillium* spp. were mostly frequent in Dhaka where *Colletotrichum* spp. were prevalent in Narayangonj, *Fusarium* spp. were prevalent in Sirajgonj and *Paecilomyces* spp were prevalent in Nrayangonj and Sirajgonj. In the present study eight isolates were considered as sterile fungi as they had no sporulation ability.

CHAPTER - V

DISCUSSION

The experiment was conducted to isolate and identify the fungi associated with root knot nematodes (*Meloidogyne* spp.) eggs and egg masses and to evaluate their biological control potentiality *in-vitro*. In the present study forty two samples were examined of which only 26 samples were found to be associated with 69 fungal isolates representing 15 genera. In similar experiment Viaene and Abawi (1998) isolated a total of 24 and 16 isolates from egg masses and juveniles, respectively. Sun *et al.* (2006) isolated a total 455 fungal isolates belonging to 24 genera and 52 isolates of actinomycetes from *Meloidogyne* spp. eggs and females from 28 samples of green houses and fields of north and south China. In another study Aminuzzaman (2009) isolated seventy fungal isolates belonging to seven genera with naturally infected eggs and females of root-knot nematodes (RKN), *Meloidogyne* spp. Again, Aminuzzaman *et al.* (2012) isolated 235 (representing 18 genera and 26 species) nematophagous fungi from eggs and females of *Meloidogyne* spp from 102 fields root samples in China. Ruanpanun *et al*. (2010) isolated 83 actinomycetes isolates and 67 fungal isolates from 23 plant parasitic nematode infested soils from Thailand. Hidalgo-Diaz *et al.* (2000) isolated a total of 83 fungal isolates from plant parasitic nematode infested soils in Cuba. Saxena (2008) isolated 54 nematophagous fungi from soil samples of four different types of habitats in Edinburgh, Scotland. Verdejo-Lucas *et al*. (2002) isolated nine fungal species from single eggs of the nematode from vegetables in Almeria and Bercelona, Spain.

In the present study frequently encountered species were *Aspergillus* spp. (28.99 %) *Penicillium* spp. (15.94%), *Colletotrichum* spp. (10.14%), *Fusarium* spp. (5.80%), *Monocillium* sp. (4.35%) *Paecilomyces* spp. (2.90%), *Thysanophora* sp. and *Trichoderma* spp. (2.90%). Ruanpanun *et al*. (2010) isolated predominant fungal taxa *Penicillium* (37.3%) and *Fusarium* (32.8%).

Aminuzzaman (2009) reported the frequently encountered fungal species were *Fusanium* spp. (44.33% of the isolates collected), *Paecilomyces lilacinus* (11.44%), *Fusarium oxysporum* (11.44%), *Aspergillus* spp. (5.72%) and *Pochonia chlamydosporia* (4.29%). Aminuzzaman *et al.* (2012) isolated the predominant nematophagous fungi were *Fusarium s*pp. (42% of the isolates collected), *F. oxysporum* (13.2%), *Paecilomyces lilacinus* (12.8%) and *Pochonia chlamydosporia* (8.5%) in China. Sun *et al.* (2006) isolated frequently encountered fungal species were *Paecilomyces lilacinus* (49.3% of the isolates collected), *Fusarium* spp. (7.9%), *Pochonia chlamydosporia* (6.9%), *Penicillium* spp. (5.7%), *Aspergillus* spp. (3.2%), and *Acremonium* spp. (2.8%). Verdejo-Lucas *et al*. (2002) isolated nine fungal species from single eggs of the nematode. The fungi included *Verticillium chlamydosporium, V. catenulatum, Fusarium oxysporum, F. solani, Fusarium* spp., *Acremonium strictum, Gliocladium roseum, Cylindrocarpon* spp., *Engiodontium album*, and *Dactylella oviparasitica*. Trifonova *et al.* (2009) carried out mycological surveys of root-knot nematodes from the southern region of Bulgaria and reported three fungal species namely *Fusarium oxysporum, Verticillium chlamydosporium*, and *Gliocladium roseum* were associated with *Meloidogyne* spp. In this study eight non spore forming (Sterile) fungi has been isolated from eggs of root knot nematodes. Verdejo-Lucas *et al*. (2002) isolated two sterile fungi from single egg of the nematode eggs. Isolation of five sterile fungi from eggs of *Meloidogyne* spp. was reported from China (Sun *et al*, 2006 and Aminuzzaman , 2009).

In the present study 24 well tissue culture plate technique was used to evaluate the biocontrol potential of nematophagous fungi *in-vitro*. The technique was found simple and effective to screen potential micro flora. Sun *et al.* (2006) and Aminuzzaman (2009) also used 24 well tissue culture plate technique to evaluate biocontrol potential of nematophagous fungi. Among the isolated fungi 50 were tested for pathogenicity in vitro. Most of the isolates parasitized eggs of *Meloidogyne* spp. after 4 days of incubation. Only 28% of the isolates

had failed to parasitize eggs. Sun *et al.* (2006) evaluated a total of 350 isolates of nematophagous fungi and actinomycetes for their potentiality to parasitize eggs, egg hatch rate and juvenile mortality in vitro. And observed 29.1% of isolates parasitized over 90% eggs and only 4.3% of the isolates had no parasitism on eggs. Aminuzzaman (2009) evaluated 70 fungal isolates *in-vitro* and found that average % egg parasitism ranged from 1.3 to 100%. Trifonova *et al.* (2009) carried out mycological surveys of root-knot nematodes from the southern region of Bulgaria. They found three fungal species associated with *Meloidogyne* spp. the fungi infected up to 8.7% of the eggs in the females of *Meloidogyne* spp.The highest Parasitism with 47.56% eggs was observed in *Aspergillus fumigatus*. In similar experiment Sun *et al.* (2006) were recorded highest Parasitism with 100% eggs parasitized by one isolates of *Aspergillus* sp. Aminuzzaman (2009) recorded only 1.3% eggs parasitized was observed in one isolates of *Aspergillus fumigatus*. In the present study different isolates of same fungal species Showed different rate of pathogenecity against *Meloidogyne* spp. eggs, it may be due to different virulent strain. Stirling and West*et al*. (1991) were screened 26 isolates of *P. lilacinus* and 13 isolates of *V. chlamydosporium* for parasitic activity against eggs of *Meloidogyne javanica* using three different tests. Within each test, the number of eggs parasitized by different isolates of the same fungus varied considerably, suggesting that isolates differed in virulence.

Fifty fungal isolates were used for pathogenicity tests in vitro and % egg hatched rate was observed after 7 days of incubation. *Paecilomyces variotii* perform better in arresting egg hatch rate. Sun *et al.* (2006) recorded the lowest egg hatched rate was in one isolate of *Paecilomyces* sp. (26.8) compared to egg hatch rate of 65.8% in sterile water. Aminuzzaman (2009) recorded the lowest egg hatched rate in one isolate of *Aspergillus flavus* (2.3) compared to egg hatch rate of 25.26% in sterile water. However, The egg hatch rate of *Meloidogyne* spp. was enhanced by some isolates of *Penicillium* spp., *Colletotrichum* spp., *Aspergillus* spp., *Aspergillus flavus* and *Monilia* sp. Sun *et*

al. (2006) also recorded the enhancement of egg hatched rate of *Meloidogyne hapla* in some isolates of *Cladosporium cladosporoides, Fusarium* sp, *Paecilomyces lilacinus and Penicillium sp.* (Were up to 100%). In the present study % juvenile mortality rate was observed after 7 days of incubation. More than half of fungal isolates showed their capability to kill *Meloidogyne* spp. juveniles compare to natural juvenile mortality rate. The highest average % juveniles mortality was observed in *Fusarium culmorum.* In similar experiment, Sun *et al.* (2006) evaluated a total of 350 isolates of nematophagous fungi and actinomycetes for their parasitism of eggs and effects on juvenile mortality in vitro after 7 days of incubation observed that more than half of the fungal and actinomycetes isolates were capable of killing *Meloidogyne hapla* Juveniles comparing to natural juvenile mortality in sterile water. One isolate of each *Aspergillus* sp., *Fusarium* sp and *Penicillium* sp. killed all hatch juvenile. In similar experiment, Aminuzzaman (2009) recorded compared to natural juvenile mortality in sterile water the highest average% juveniles mortality was observed in two isolate of each of *Pestalitopsis* spp. Findings of the present study indicate that parasitic fungi associated with naturally infected eggs and egg masses of root-knot nematodes have biocontrol potential. The findings of the present study may be used for further research to find out a potential biocontrol agent to control root knot nematodes (*Meloidogyne* spp.) in ecofriendly manner.

CHAPTER - VI

SUMMARY AND CONCLUSION

Isolation, identification and evaluation of biological control potentiality of fungi associated with root knot nematodes, *Meloidogyne* spp. were done in the disease Diagnosis Laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period of January, 2011 to August, 2012. Microbes associated with *Meloidogyne* spp. collected from 13 sites of 7 districts of Bangladesh that were used in this experiment.

The microbial communities associated with *Meloidogyne* spp. eggs and egg masses were varied among sampling sites. Maximum 38 isolated were recovered from Dhaka, followed by 10 from Sirajgonj, 10 from jessore, 7 from Narayangonj, 4 from Jhenaidah. A total of 69 fungal isolates were recovered the fungal isolates represented 15 genera.

The most prominent species was *Aspergillus* spp. (28.99% of the total fungal isolates). Other prominent fungal species were *Penicellium* spp., (15.94%), *Colletotrichum* spp. (10.14%), *Fusarium* spp. (5.80%), *Monocillium indicum* (4.35%) and *Paecilomyces lilacinus* (2.90*)* and *Paecilomyces variotii* (2.90%), *Trichoderma* spp (2.90%) and *Thysanophora* spp. (2.90%).

The average egg parasitism, egg hatch and juvenile mortality ranged from 0.22 to 47.56%, 14.28 to 79.64% and 2.01 to 45.73%, respectively for all isolate tested. Only 28% of the isolates had no ability to parasitize eggs. The highest parasitism with 47.56% eggs was observed in *Aspergillus fumigatus*. The lowest egg hatch rate 14.28% was observed in *Paecilomyces variotii.* And the highest average juveniles mortality 45.73 % was observed in two isolate of each of *Fusarium culmorum.*

Findings of the present study indicate that parasitic fungi associated with naturally infected eggs and egg masses of root-knot nematodes have biocontrol potential. Further investigation need to be carried out incorporating more and more sample to isolate potential bioagent from large geographic area of Bangladesh.

CHAPTER - VII

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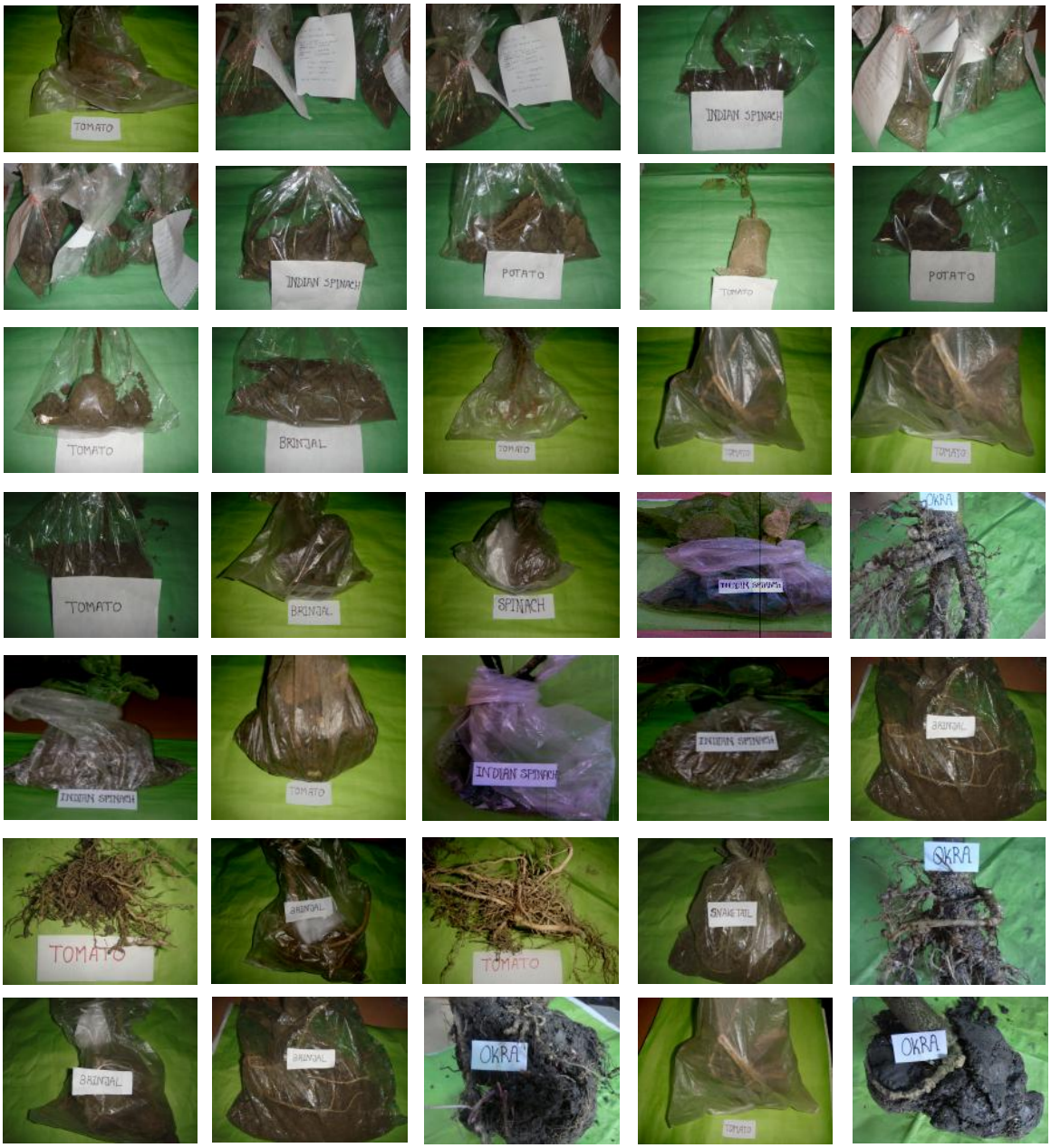

Plate 1. Root knot samples collected from different region of Bangladesh

Sample No	Name of crop	Location	Date of Collection	Egg/ Egg mass	Isolate name	Fungi Identification
1	Tomato	BARI campus, Gazipur	24.01.11	Egg	NILL	
2	Potato	Village: Potazia Thana:Shahjadpur Dist:sirajgonj	17.01.11	Egg mass	MEMPOS-1 MEMPOS-2 MEMPOS-2	*Monilia sp.* *Penicillium chrysogenum.* *Rhinocladiella* sp.
3	Brinjal	Village: Potazia Thana:Shahjadpur Dist:sirajgonj	17.01.11	Egg mass	MEMBRS-1 MEMBRS-2	*Aspergillus* sp. *Penicillium* sp.
4	Brinjal	Shere Bangla agricultural university campus, Dhaka	12.02.11	Egg mass	MEMBRD-1 MEMBRD-2 MEMBRD-3 MEMBRD-4	*Aspergillus* sp. *Penicillium* sp. *Catenophora* sp. *Penicillium* sp.
5	Papaya	Thana: Agargoan, (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMPAD-1 MEMPAD-2	*Penicillium* sp. *Aspergillus* sp.
6	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMPAD-3 MEMPAD-4 MEMPAD-5	*Fusarium culmorum* *Aspergillus niger* *Penicillium* sp.
7	Papaya	Thana: Agargoan (Nursery)	06.02.11	Egg	MEPAD-1 MEPAD-2	Sterile fungus *Pestalotia* sp.

		Dist: Dhaka			MEPAD-3	Sterile fungus
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg/ Egg mass**	**Isolate name**	**Fungi Identification**
					MEPAD-4	*Rhinotrichum* sp.
					MEPAD-5	Sterile fungus
					MEPAD-6	Unidentified

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

8	Tomato	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMTOD-1	Unidentified
9	Jute	WRC quarter, Dist: Dhaka	19.05.10	Egg mass	MEMJUD-1	*Penicillium* sp.
10	Tomato	SAU campus Dhaka	11.05.11	Egg	NILL	
11	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	NILL	
12	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	NILL	
13	Spinach	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	8.04.11	Egg	NILL	
14	Tomato	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	08.04.11	Egg	NILL	
15	Tomato	Village: Dumurtola Thana: Ovoinagor	08.06.11	Egg	METOJ-1	*Trichoderma harzianum*.

		Dist:Jessore				

Sample No	Name of crop	Location	Date of Collection	Egg /Eggmass	Isolate name	Fungi Identification

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

16.	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist: Jessore	08.06.11	Egg	MEBRJ-1	*Penicillium* sp.
17	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist: Jessore	08.06.11	Egg	MEBRJ-2 MEBRJ-3	*Fusarium culmorum.* *Aspergillus flavus*
18	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	08.06.11	Egg mass	MEBRJ-4 MEBRJ-5	*Penicillium* sp. *Scopulariopsis* sp.
19	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-1 MEISD-2 MEISD-3 MEISD-4	*Aspergillus terreus* *Aspergillus niger* *Aspergillus sp.* *Trichoderma sp.*
20	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-5 MEISD-6 MEISD-7 MEISD-8	*Thysanophora* sp. *Aspergillus terreus* *Aspergillus niger* *Monocillium indicum*
21	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-9 MEISD-10 MEISD-11 MEISD-12	*Aspergillus niger* *Aspergillus terreus* *Thysanophora* sp. *Aspergillus* sp

22.	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-13	Sterile fungus
					MEISD-14	*Aspergillus* sp.
					MEISD-15	*Colletotrichum* sp.
					MEISD-16	*Sphaeropsis pyriputrescens*
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg /Eggmass**	**Isolate name**	**Fungi Identification**

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

23	Indian Spinach	SAU campus, Horticulture farm,Dhaka	23.06.11	Egg	MEISD-17 MEISD-18 MEISD-19	*Penicillium digitatum* *Aspergillus terreus* *Colletotrichum* sp.
24	Indian Spinach	SAU campus Horticulture farm,Dhaka	23.06.11	Egg	MEISD-20 MEISD-21	Sterile fungus *Aspergillus flavus*
25	Okra	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	20.07.11	Egg	NILL	
26	Indian spinach	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	20.07.11	Egg	MEISJ-1	*Aspergillus terreus*
27	Indian spinach	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	22.07.11	Egg	NILL	
28	Brinjal	Village: Kotchandpur Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	MEBRJH-1 MEBRJH-2	*Aspergillus flavus* *Aspergillus terreus*

29	Indian Spinach	Village: Dhopadi	20.07.11	Egg	MEISJ-2	*Penicillium* sp.
Sample No	**Name of crop**	**Location** Thana: Ovoinagor Dist: Jessore	**Date of Collection**	**Egg /Eggmass**	**Isolate name**	**Fungi Identification**

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

30	Okra	Village: Fulbari Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	MEOKJH-1 MEOKJH-2	*Aspergillus terreus* *Monocillium* sp.
31	Brinjal	SAU campus. Dhaka	29.07.11	Egg	NILL	
32	Okra	Village: Kotchandpur Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	NILL	
33	Okra	Village Sundoli Thana: Ovoinagor Dist: Jessore	28.08.11	Egg	MEOKJ-1 MEOKJ-2	*Monocillium* sp. Unidentified
34	Okra	Village: Sundoli Thana: Ovoinagor Dist: Jessore	28.08.11	Egg	NILL	
35	Tomato	SAU campus, Dhaka	20.09.11	Egg	NILL	
36	Tomato	Village: Araihazar Dist:Narayon	24.12.11	Egg	METONA-1 METONA-2	Sterile fungus *Colletotrichum* sp.

		gonj			METONA-3	*Colletotrichum* sp.
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg /Egg mass**	**Isolate name**	**Fungi Identification**

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

Sample No	Name of crop	Location	Date of Collection	Egg/ Egg mass	Isolate name	Fungi Identification
37	Tomato	Village: Araihazar Dist:Narayon gonj	24.12.11	Egg	METONA-4	Sterile fungus
					METONA-5	Sterile fungus
					METONA-6	*Colletotrichum* sp.
					METONA-7	*Paecilomyces lilacinus.*
38	Tomato	Village: Araihazar Dist:Narayon gonj	24.12.11	Egg	NILL	
39	Tomato	BARI campus,Gazi pur	03.01.12	Egg	NILL	
40	Brinjal	Village: Maligasa Thana: Bagatipara Dist: Natore	03.01.11	Egg	NILL	
41	Tomato	BARI campus,Gazi pur	03.01.11	Egg	NILL	
42	Brinjal	Village: Jarila Thana+Dist: Sirajgonj	01.01.11	Egg	MEBRSH-3	*Colletotrichum* sp.
					MEBRSH-4	*Colletotrichum* sp.
					MEBRSH-5	*Fusarium solani*
					MEBRSH-6	*Paecilomyces variotii*
					MEBRSH-7	*Fusarium solani*

Sample No	Name of crop	Location	Date of Collection	Egg/ Egg mass	Isolate name	Fungi identification
1	Tomato	BARI campus, Gazipur	24.01.11	Egg	NILL	
2	Potato	Village: Potazia Thana:Shahj adpur Dist:sirajgonj	17.01.11	Egg mass	MEMPOS-1 MEMPOS-2 MEMPOS-2	*Monilia* sp. *Penicillium chrysogenum.* *Rhinocladiella* sp.
3	Brinjal	Village: Potazia Thana:Shahj adpur Dist:sirajgonj	17.01.11	Egg mass	MEMBRS-1 MEMBRS-2	*Aspergillus* sp. *Penicillium* sp.
4	Brinjal	Shere Bangla agricultural university campus, Dhaka	12.02.11	Egg mass	MEMBRD-1 MEMBRD-2 MEMBRD-3 MEMBRD-4	*Aspergillus* sp. *Penicillium* sp. *Catenophora* sp. *Penicillium* sp.
5	Papaya	Thana: Agargoan, (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMPAD-1 MEMPAD-2	*Penicillium* sp. *Aspergillus* sp.
6	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMPAD-3 MEMPAD-4 MEMPAD-5	*Fusarium culmorum* *Aspergillus niger* *Penicillium* sp.
7	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg	MEPAD-1 MEPAD-2 MEPAD-3 MEPAD-4 MEPAD-5 MEPAD-6	Sterile fungus *Pestalotia* sp. Sterile fungus *Rhinotrichum* sp. Sterile fungus Unidentified

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

8	Tomato	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	MEMTOD-1	Unidentified
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg /Eggmass**	**Isolate name**	**Fungi Identification**
9	Jute	WRC quarter, Dist: Dhaka	19.05.10	Egg mass	MEMJUD-1	*Penicillium* sp.
10	Tomato	SAU campus Dhaka	11.05.11	Egg	NILL	
11	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	NILL	
12	Papaya	Thana: Agargoan (Nursery) Dist: Dhaka	06.02.11	Egg mass	NILL	
13	Spinach	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	8.04.11	Egg	NILL	
14	Tomato	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	08.04.11	Egg	NILL	
15	Tomato	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	08.06.11	Egg	METOJ-1	*Trichoderma harzianum*.

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

16.	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist: Jessore	08.06.11	Egg	MEBRJ-1	*Penicillium* sp.
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg /Egg mass**	**Isolate name**	**Fungi Identification**
17	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist: Jessore	08.06.11	Egg	MEBRJ-2 MEBRJ-3	*Fusarium culmorum.* *Aspergillus flavus*
18	Brinjal	Village: Dumurtola Thana: Ovoinagor Dist:Jessore	08.06.11	Egg mass	MEBRJ-4 MEBRJ-5	*Penicillium* sp. *Scopulariopsis* sp.
19	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-1 MEISD-2 MEISD-3 MEISD-4	*Aspergillus terreus* *Aspergillus niger* *Aspergillus sp.* *Trichoderma sp.*
20	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-5 MEISD-6 MEISD-7 MEISD-8	*Thysanophora* sp. *Aspergillus terreus* *Aspergillus niger* *Monocillium indicum*
21	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-9 MEISD-10 MEISD-11 MEISD-12	*Aspergillus niger* *Aspergillus terreus* *Thysanophora* sp. *Aspergillus* sp
22.	Indian Spinach	SAU campus Horticulture farm,Dhaka	22.06.11	Egg	MEISD-13 MEISD-14 MEISD-15 MEISD-16	Sterile fungus *Aspergillus* sp. *Colletotrichum* sp. *Sphaeropsis pyriputrescens*

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

Sample No	Name of crop	Location	Date of Collection	Egg /Egg mass	Isolate name	Fungi Identification
23	Indian Spinach	SAU campus, Horticulture farm,Dhaka	23.06.11	Egg	MEISD-17 MEISD-18 MEISD-19	*Penicillium digitatum* *Aspergillus terreus* *Penicillium* sp.
24	Indian Spinach	SAU campus Horticulture farm,Dhaka	23.06.11	Egg	MEISD-20 MEISD-21	Sterile fungus *Aspergillus flavus*
25	Okra	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	20.07.11	Egg	NILL	
26	Indian spinach	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	20.07.11	Egg	MEISJ-1	*Aspergillus terreus*
27	Indian spinach	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	22.07.11	Egg	NILL	
28	Brinjal	Village: Kotchandpur Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	MEBRJH-1 MEBRJH-2	*Aspergillus flavus* *Aspergillus terreus*
29	Indian Spinach	Village: Dhopadi Thana: Ovoinagor Dist: Jessore	20.07.11	Egg	MEISJ-2	*Penicillium* sp.

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

30	Okra	Village: Fulbari Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	MEOKJH-1 MEOKJH-2	*Aspergillus terreus* *Monocillium* sp.
Sample No	**Name of crop**	**Location**	**Date of Collection**	**Egg /Eggmass**	**Isolate name**	**Fungi Identification**
31	Brinjal	SAU campus. Dhaka	29.07.11	Egg	NILL	
32	Okra	Village: Kotchandpur Thana: Kotchandpur Dist: Jhenaidah	22.07.11	Egg	NILL	
33	Okra	Village Sundoli Thana: Ovoinagor Dist: Jessore	28.08.11	Egg	MEOKJ-1 MEOKJ-2	*Monocillium* sp. Unidentified
34	Okra	Village: Sundoli Thana: Ovoinagor Dist: Jessore	28.08.11	Egg	NILL	
35	Tomato	SAU campus, Dhaka	20.09.11	Egg	NILL	
36	Tomato	Village: Araihazar Dist:Narayongonj	24.12.11	Egg	METONA-1 METONA-2 METONA-3	Sterile fungus *Colletotrichum* sp. *Colletotrichum* sp.

Table 1: Sampling site and source of isolates of *Meloidogyne* spp. associated fungi (Contd.)

37	Tomato	Village: Araihazar Dist:Narayon gonj	24.12.11	Egg	METONA-4 METONA-5 METONA-6 METONA-7	Sterile fungus Sterile fungus *Colletotrichum* sp. *Paecilomyces lilacinus.*
38	Tomato	Village: Araihazar Dist:Narayon gonj	24.12.11	Egg	NILL	
39	Tomato	BARI campus,Gazi pur	03.01.12	Egg	NILL	
40	Brinjal	Village: Maligasa Thana: Bagatipara Dist: Natore	03.01.11	Egg	NILL	
41	Tomato	BARI campus,Gazi pur	03.01.11	Egg	NILL	
42	Brinjal	Village: Jarila Thana+Dist: Sirajgonj	01.01.11	Egg	MEBRSH-3 MEBRSH-4 MEBRSH-5 MEBRSH-6 MEBRSH-7	*Colletotrichum* sp. *Colletotrichum* sp. *Fusarium solani* *Paecilomyces variotii* *Fusarium solani*

Table 2. Sampling locations and number of fungal isolates isolated from root-

Fungus	Number of Isolates	R

knot Nematodes (*Meloidogyne* spp.)

Sampling locations	Number of sample	Number of sample encountered with fungi	Number of isolates		Total
			From eggs	From egg masses	
Dhaka, 3sites	17	12	27	11	38
Narayongonj,1 site	03	02	07	_	07
Sirajgonj, 2 Sites	03	03	05	05	10
Jessore, 3 sites	12	07	10	_	10
Jhenaidah, 2 sites	03	02	04	_	04
Gazipur, 1 site	03	00	00	_	00
Natore, 1 site	01	00	00	_	00
Total no of isolates	42	26	53	16	69

	Dhaka	Gazipur	Narayangonj	Shirajgonj	Natore	Jessore	Jhenaidah	Total
Penicillium spp.	6	-	-	2	-	3	-	11
Aspergillus spp.	14	-	-	1	-	2	3	20
Fusarium spp.	1	-	-	2	-	1	-	4
Colletotrichum spp.	2	-	3	2	-	-	-	7
Paecilomyces spp.	-	-	1	1	-	-	-	2
Monilia sp.	-	-	-	1	-	-	-	1
Rhinocladiella spp.	-	-	-	1	-	-	-	1
Catenophora sp.	1	-	-	-	-	-	-	1
Pestalotia sp.	1	-	-	-	-	-	-	1
Rhinotrichum sp.	1	-	-	-	-	-	-	1
Trichoderma spp.	1	-	-	-	-	1	-	2
Scopulariopsis sp.	-	-	-	-	-	1	-	1
Thysanophora sp.	2	-	-	-	-	-	-	2
Monocillium spp.	1	-	-	-	-	1	1	3
Sphaeropsis sp.	1	-	-	-	-	-	-	1
Sterile Fungi	5	-	3	-	-	-	-	8
Not Identified	2	-	-	-	-	1	-	3
Total	38	0	7	10	0	10	4	69

Table 3. Fungal species isolated from *Meloidogyne* spp. eggs and egg masses

"-", no isolate was recovered

Table 2. Sampling locations and number of fungal isolates isolated from root-

Fungus Species	Number of Isolates	R F

knot Nematodes (*Meloidogyne* spp.)

Sampling locations	Number of sample	Number of sample encountered with fungi	Number of isolates		Total
			From eggs	From egg masses	
Dhaka, 3sites	17	12	27	11	38
Narayongonj,1 site	03	02	07	_	07
Sirajgonj, 2 Sites	03	03	05	05	10
Jessore, 3 sites	12	07	10	_	10
Jhenaidah, 2 sites	03	02	04	_	04
Gazipur, 1 site	03	00	00	_	00
Natore, 1 site	01	00	00	_	00
Total no of isolates	42	26	53	16	69

	Dhaka	Gazipur	Narayangonj	Shirajgonj	Natore	Jessore	Jhenaidah	Total
Penicillium spp.	6	-	-	2	-	3	-	11
Aspergillus spp.	14	-	-	1	-	2	3	20
Fusarium spp.	1	-	-	2	-	1	-	4
Colletotrichum spp.	2	-	3	2	-	-	-	7
Paecilomyces spp.	-	-	1	1	-	-	-	2
Monilia sp.	-	-	-	1	-	-	-	1
Rhinocladiella spp.	-	-	-	1	-	-	-	1
Catenophora sp.	1	-	-	-	-	-	-	1
Pestalotia sp.	1	-	-	-	-	-	-	1
Rhinotrichum sp.	1	-	-	-	-	-	-	1
Trichoderma spp.	1	-	-	-	-	1	-	2
Scopulariopsis sp.	-	-	-	-	-	1	-	1
Thysanophora sp.	2	-	-	-	-	-	-	2
Monocillium spp.	1	-	-	-	-	1	1	3
Sphaeropsis sp.	1	-	-	-	-	-	-	1
Sterile Fungi	5	-	3	-	-	-	-	8
Not Identified	2	-	-	-	-	1	-	3
Total	38	0	7	10	0	10	4	69

Table 3. Fungal species isolated from *Meloidogyne* spp. eggs and egg masses

"-", no isolate was recovered

Table 4. Pathogenicity of fungal isolates to *Meloidogyne* spp. eggs and juveniles *in-vitro*

Fungal Species	No. of isolates	Percentage of eggs parasitized (after 4 days)			Egg hatch rate (%)(after 7 days)		
		Maximum	Minimum	Average*	Maximum	Minimum	Average*
Penicillium spp.	8	30	0	7.46±1.34	91.82	8.82	51.90±3.
Penicillium chrysogenum	1	1.33	1.33	1.33	57.94	57.94	57.
Aspergillus terreus	5	1.33	0.44	0.98 ± 0.42	53.95	13.96	22.10±1.
Aspergillus flavus	5	2.22	0	0.71± 0.35	84.79	11.59	43.67±1.
Aspergillus fumigatus	1	47.56	47.56	47.56	29.66	29.66	29.
Aspergillus spp.	7	0.44	0	0.25 ± 0.41	83.88	62.69	78.24±1.
Monillia sp.	1	3.34	3.34	3.34	79.64	79.64	79.
Rhinocladiella sp.	1	19.67	19.67	19.67	65.83	65.83	65.
Fusarium Culmorum	2	16.44	14.22	15.33±0.00	16.49	15.57	16.03±2.
Fusarium solani	2	3.11	2.67	2.89± 0.40	56.81	54.02	55.41±0.
Colletotrichum spp.	5	23.11	11.56	15.91±1.29	84.12	8.33	37.04±2.
Paecilomyces lilacinus	1	13.33	13.33	13.33	48.71	48.71	48.
Paecilomyces variotii	1	19.11	19.11	19.11	14.28	14.28	14.
Trichoderma spp.	2	2.22	1.33	2.00±1.63	47.59	21.86	34.72±1.
Monocillium spp.	2	0	0	0	40.62	35.25	37.94±2.
Thysanophora sp.	2	0.44	0	0.22±0.54	19.61	17.99	18.80±0.
Scopulariopsis sp.	1	0	0	0	20.00	20.00	20.
Sterile fungus	4	5.33	0	2.22±1.09	49.96	26.21	37.62±3.
Control		0	0	0	50.97	50.97	50.

*Mean followed by ± SD (Standard deviation)

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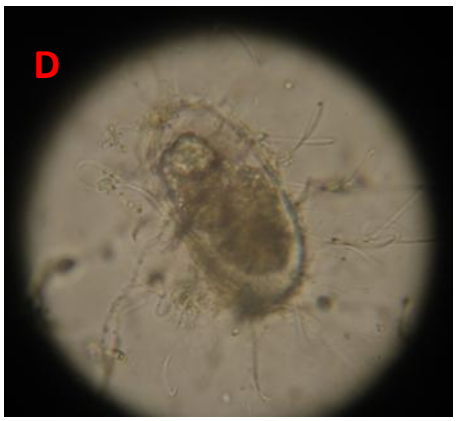
(40/.65X10x X4x)

(10/.25X10x X4x)



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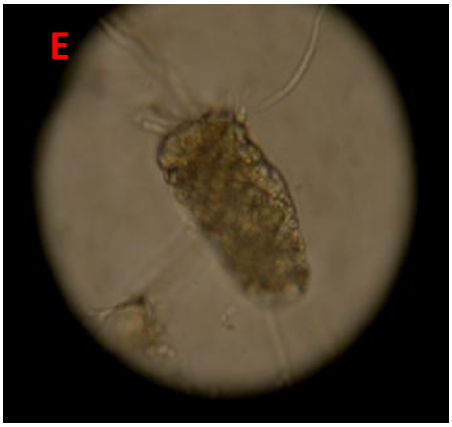

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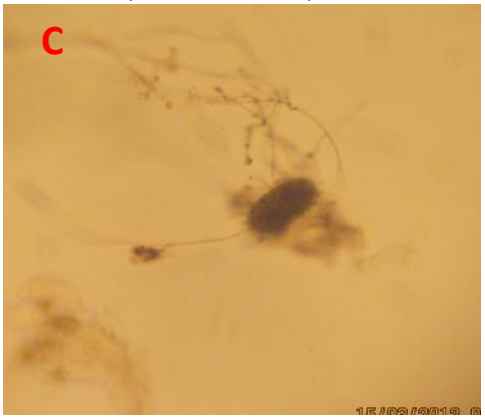
(40/.65X10x X4x)

Plate 18. *Meloidogyne* spp. eggs parasitized by A-B. *Fusarium culmorum.*

C-D. *Paecilomyces variotii* E-F. *Fusarium solani*

(40/.65X10x X4x)



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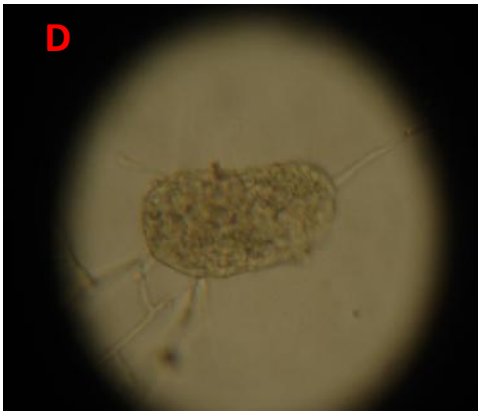

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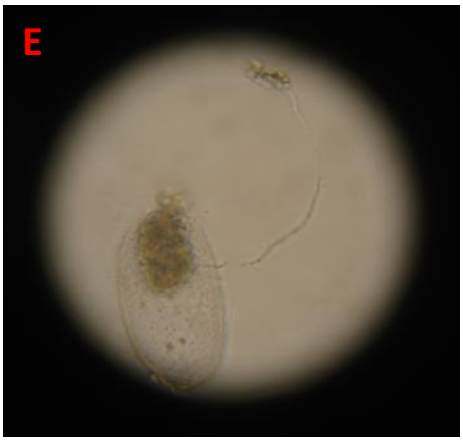
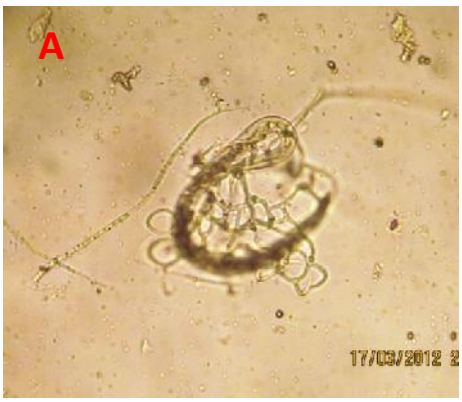


(40/.65X10x X4x)

Plate 19. *Meloidogyne* spp. eggs parasitized by A-B *Colletotrichum spp.* C. A*spergillus fumigatus* D. *Penicillium* sp. E. by *Paecilomyces lilacinus*



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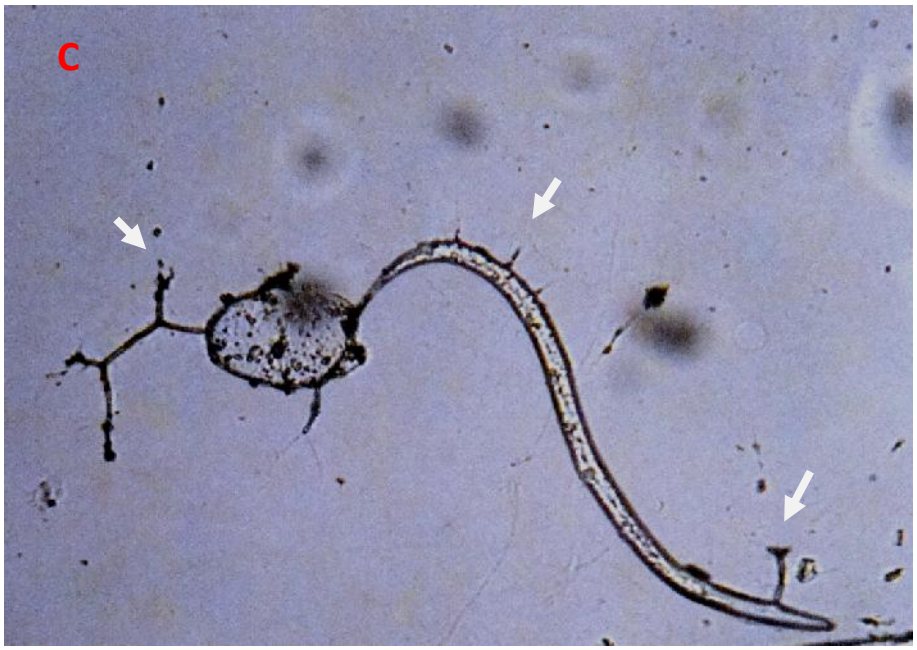
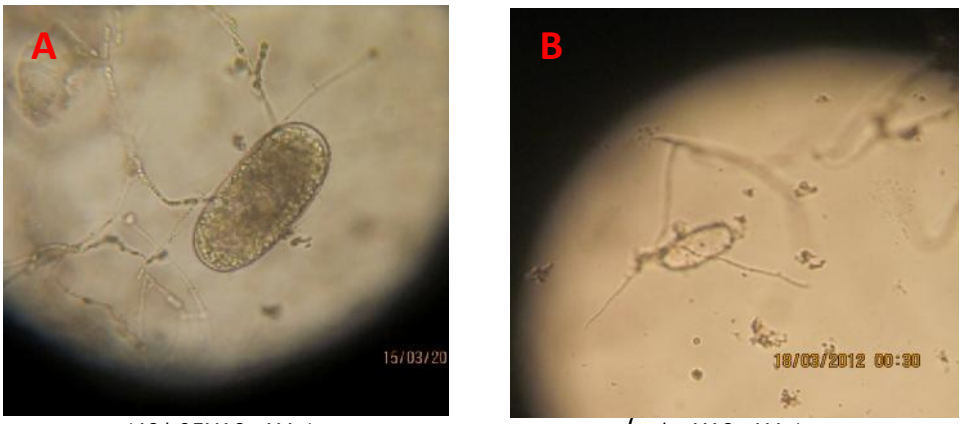


(40/.65X10x X2x)

(40/.65X10x X2x)

Plate 20. *Meloidogyne* spp. juvenile infected by A-B. *Rhinocladiella* sp. C. *Penicillium* sp. D. *Fusarium culmorum*.



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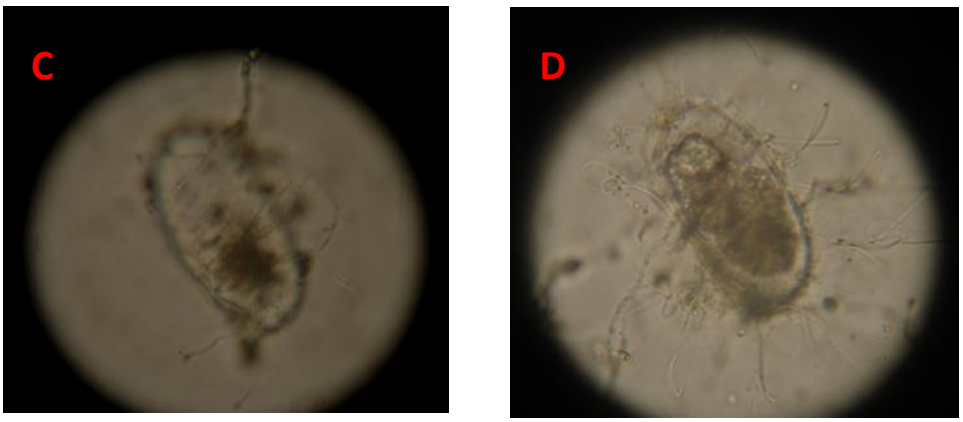

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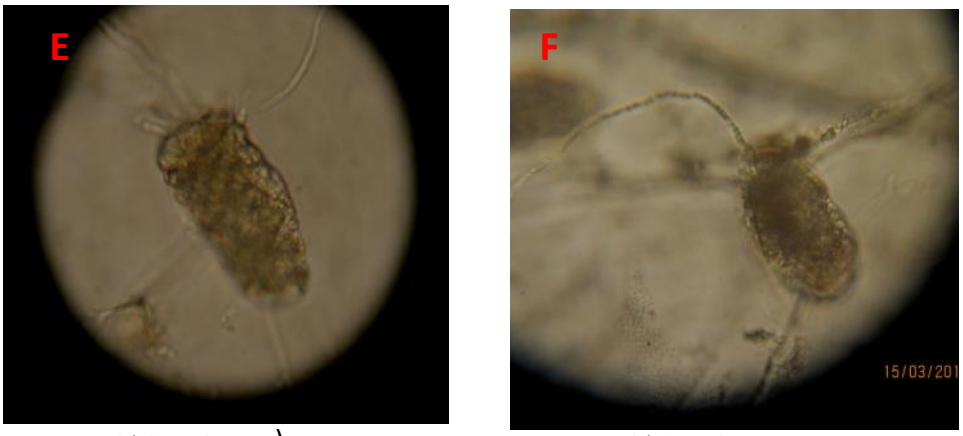
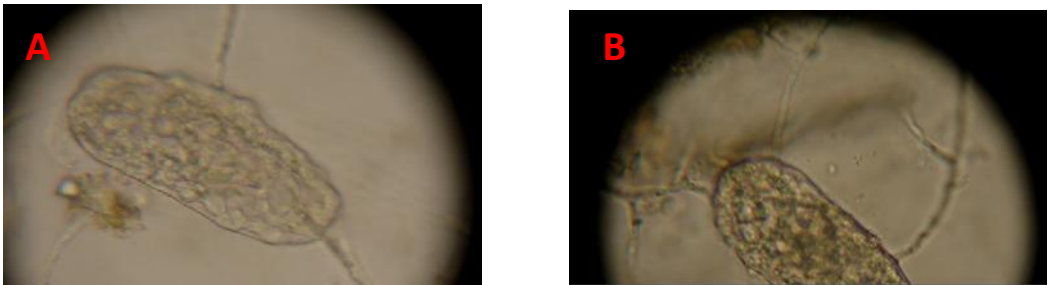


40/.65X10x X4x) (40/.65X10x X4x)

Plate 18. *Meloidogyne* spp. eggs parasitized by A-B. *Fusarium culmorum.*

C-D. *Paecilomyces variotii* E-F. *Fusarium solani*



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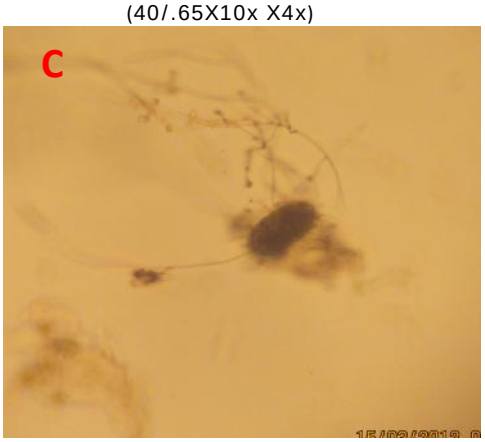
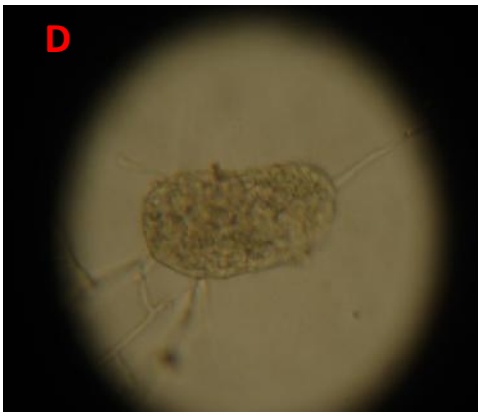


(40/.65X10x X4x)

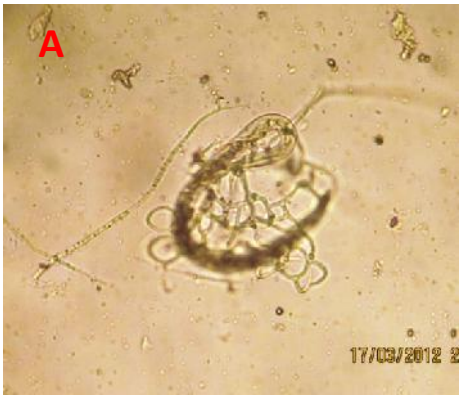


(10/.25X10x X4x)

(40/.65X10x X4x)

(40/.65X10x X4x)

Plate 19. *Meloidogyne* spp. eggs parasitized by A-B *Colletotrichum spp.* C. A*spergillus fumigatus* D. *Penicillium* sp. E. by *Paecilomyces lilacinus*



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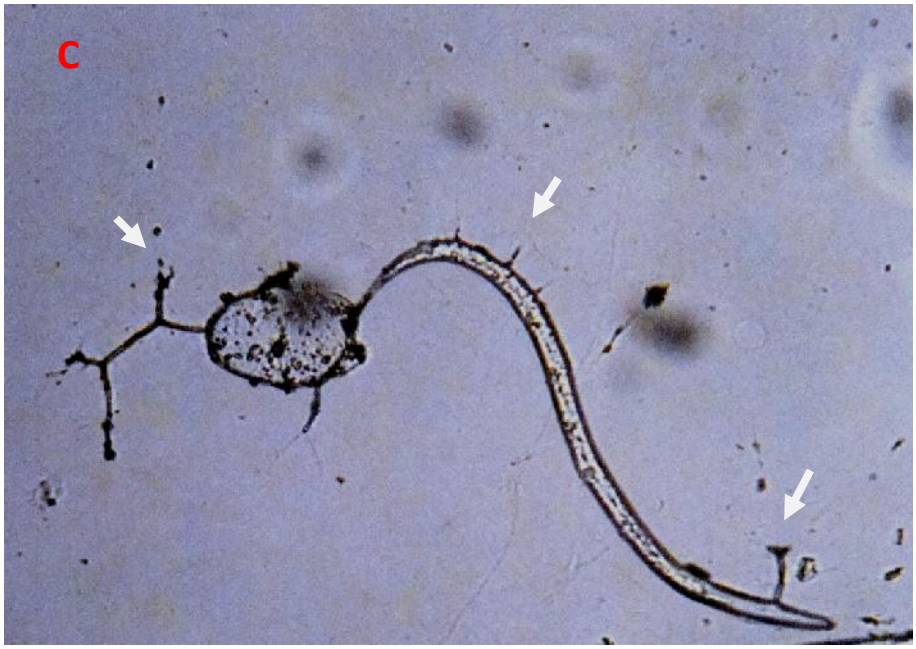

(40/.65X10x X2x)

(40/.65X10x X2x)

Plate 20. *Meloidogyne* spp. juvenile infected by A-B. *Rhinocladiella* sp. C. *Penicillium* sp. D. *Fusarium culmorum*.