Egg Mass Mycoflora of *Meloidogyne incognita* in Assam, India

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A B S T R A C T

Survey was conducted during 2014-15 for the isolation of mycoflora from the egg masses of *Meloidogyne incognita* infecting crops like tomato, brinjal, pea and amaranthus from five different locations viz., Charigaon, Alengmora, Danichopari, Namdeori and Barbheta of Jorhat and Golaghat district of Assam. The egg masses were collected and surface sterilized in 0.4 per cent sodium hypochlorite (NaOCl) for two minutes. Further these egg masses were washed thoroughly with sterile distilled water until the traces of NaOCl was removed and placed in potato dextrose agar plate. The inoculated petriplates were incubated at 25±2°C in BOD incubator for 4 days. A pure culture of each isolate was made by using hyphal tip technique. A total of 29 fungal isolates comprising of 7 genera with 9 species viz. *Trichoderma harzianum*, *Paecilomyces niphetodes*, *Acremonium falciforme*, *Fusarium oxysporium*, *F. solani*, *Aspergillus niger*, *A. flavus*, *Vermispora leguminacea*, *Penicillium* spp. and an unidentified species were recovered. All the species showed varied relative frequency of occurrence, *F. oxysporum* being the most frequently occurred species with 31.03 per cent of total fungal isolates.

K e y w o r d s

Eggmass, *Meloidogyne incognita*, *T. harzianum*, *P. niphetodes*, *A. falciforme*, *F. oxysporium*, *F. solani*, *A. niger*, *A. flavus*, *V. leguminacea*, *Penicillium* Jorhat and Golaghat

Introduction

Mycoflora *i.e.* fungi are classified as pathogenic, non-pathogenic, saprophytic, predator and parasitic etc. Some are pathogenic to plants; some are antagonistic towards pathogen and some are beneficial which increases resistance in plant against pathogen. In soil, fungi control pathogen including nematodes like *Meloidogyne* spp. are known as nematophagous fungi and that comprise more than 200 taxonomically diverse species. *Meloidogyne* spp is sedentary plant parasitic nematode and laid their eggs in gelatinous matrix (called as eggmass) which are exposed on rhizoplane. However, such exposed egg masses are heavily colonized by micro flora and become an important factor in finding the nematode antagonists (Kok *et al.*, 2001). Now a day’s efforts has been put for
the finding the missing parasite links in food-web studies and that helps to show the length of food chain (Huxham et al., 1995; Hernandez and Sukhdeo, 2008; Amundsen et al., 2009) and that triggers the possibility of any new antagonistic agents which are present in that particular niche. However, further observations fascinate that how the parasites play a 'hidden' role in mediating ecosystem stability (Dobson et al., 2006; Wood, 2007; Lafferty et al., 2008).

Assam is the northeast state of India situated south of the eastern Himalayas along the Brahmaputra and Barak River valleys. Assam is one of the richest biodiversity sources in the world. It is estimated that there are millions of fungal species worldwide. It is estimated that around 27000 fungal species are characterized by the taxonomical, morphological and physiological basis (Manoharachary et al., 2005). In Assam, to date, very small portion of them are described regardless of nematophagous fungi. The detection of such fungal species on the basis of cultural and morphological characters is not only one of the most adopted methods but also considered as traditional methods and widely used tools in fungal taxonomy. Hence, our study is among the first empirical quantifications of which fungal species are associated with egg mass of *Meloidogyne* spp in Assam.

**Materials and Methods**

**Survey for collection of samples**

Survey was conducted for the isolation, characterization and identification of egg mass mycoflora of *Meloidogyne* infecting vegetable and legume crops from different locations of Jorhat and Golaghat districts of Assam. The location of identified species of the mycoflora and isolate code are presented in Table 1. The root samples showing the symptoms of galls were collected from different crops, transported to the P. G. laboratory, Department of Nematology, AAU, Jorhat-13 and stored at 5°C temperature. The samples were processed for isolation of mycoflora within four days of collection. The remaining roots were washed thoroughly in running tap water, cut into small pieces and preserved in 5 percent formaldehyde for studying the perineal patterns of the female root knot nematodes for identification (Taylor et al., 1955).

**Preparation of perineal pattern for identification root knot nematodes**

Collected root samples were kept in 5 percent formaldeyde. For female, galled portions of root were selected and fixed in acid fuchsin (Eisenback and Triantophyllu, 1991). The stained roots were picked and mounted on the dissecting microscope. The adult females of *Meloidogyne* spp. were removed from the root tissue by teasing apart with the help of fine forceps and were collected in a cavity block having warm lactophenol. The intact of *Meloidogyne* females are placed in 45% lactic acid on a Perspex slide and the posterior end of the female having vulva and anus was cut with a scalpel. Body tissue is removed by lightly brushing the inner surface of the cuticle with slightly flexible bristle. When all tissue is removed, the cuticle is transferred to a drop of glycerine where it is carefully trimmed so as to be only slightly larger than the perineal pattern. The piece of cuticle with the perineal pattern is then transferred to a drop of glycerine on a slide. A coverslip is applied and sealed with glycerine and species were identified on the basis of characteristics given by Taylor et al., (1955).

**Collection of egg masses**

Egg masses were collected from the galled root of five plants from each sample. Root pieces with galls were mixed thoroughly,
washed in running tap water for 5 minute to get rid of soil and placed under a stereomicroscope. Egg masses were handpicked from the galled roots with help of a sterilized forcep. The egg masses thus collected were kept in sterilized cavity block containing 2 ml sterile distilled water.

**Surface sterilization of egg masses**

The collected egg masses were surface sterilized in 0.4 per cent sodium hypochlorite (NaOCl) for two minutes (Singh and Mathur, 2010). Egg masses were washed thoroughly with sterile distilled water until the traces of NaOCl was removed and placed in cavity block for further use.

**Preparation of media**

The ingredients used for preparation of potato dextrose agar (PDA) are peeled potato (200 gm), dextrose (20 gm), agar-agar (20 gm) and distilled water (1000 ml). Peeled potatoes were boiled in 500 ml water. Potato extract was separated by using double layer muslin cloth and measured amount of dextrose was added to the extract. In another flask, remaining 500 ml distilled water was taken, required amount of agar-agar was added and melted by boiling. The molten agar- agar was strained through double layer muslin cloth and mixed with potato dextrose extract solution. The volume was made upto 1000 ml by adding distilled water. pH was measured and maintained at 7.0 by NaOH. The medium was poured into culture tubes and conical flask plugged by non-absorbent cotton and then sterilized in autoclave at 121°C for 20 minutes.

**Isolation of fungal species from egg masses**

The sterilized ten egg masses were placed on petriplates containing PDA (1 petriplate/1sample) with the help of sterilized forcep. The PDA on the petriplates were amended with antibiotic, streptomycin sulphate @ 1 ml/L under sterilized condition and petriplates were sealed with the help of plastic wrapper. Inoculated petriplates were incubated at 25±2°C in BOD incubator for 4 days. The plates were observed daily. The fungal colonies that were grown from egg masses were transferred to another PDA plate. The fungi were sub cultured for purification by selecting desired colonies. A pure culture of each isolate was made by transferring them to respective slants and petriplate following the technique of hyphal tip culture. Isolated and purified cultures were maintained by periodical transferring in fresh PDA slants.

**Identification of mycoflora**

For identification of the fungal isolates, cultural characters (colours and texture of colonies) and microscopic features were studied. For microscopic studies, colour, shapes and size of conidia were examined. Mycelia from each isolate were taken from PDA plate and spread onto a clean glass slide mounted with lactophenol cotton blue, covered with cover slip and then observed under a light microscope at 400X magnification. The size of conidia was measured using an ocular micrometer. Twenty five (25) measurements were taken and average size of conidia was calculated. The cultural and microscopic features were compared with the available literature.

**Results and Discussion**

**Identification of Meloidogyne spp.**

Perineal patterns of the females of root knot nematode collected from different places viz., Alengmora, Charigoan Namdeori, Barbheta of Jorhat district and Danichapori, of Golaghat district were prepared. Microscopic observations reveal that the perineal patterns
of all the populations (Fig. 1) appeared roughly oval with high, squarish, dorsal arch, composed of closely spaced, smooth to wavy striae without forking. Lateral fields were absent. These morphological characters of perineal patterns were compared with the reported literature of Chitwood 1949, Taylor et al., 1955 and Eisenback et al., 1981 and were confirmed to be Meliodogyne incognita.

**Cultural, morphological and morphometric characterizations of fungal species**

*Trichoderma harzianum*

The colony textures of the isolates CHA-AMR-1, CHA-AMR-5, DA-P-3, DA-P-4, DA-P-5, DA-Br-1, DA-Br-2 and DA-Br-3 (Fig. 6, 7, 8, 10 and 11) were found to be compact, margins entirely regular and green colour with whitish sterile mycelium. On the reverse side, the colony was found to be colourless in isolates DA-Br-1, DA-Br-2, DA-Br-3, CHA-AMR-1 and CHA-AMR-5 and yellowish in isolates DA-P-3, DA-P-4 and DA-P-5. However in all isolates the colony formed 1-2 rings like of zonation. The conidial characters shows that all the isolates have smooth conidial wall, subglobose in shape with green coloured conidia and they varied in different size 1.66-3.32±0.83×1.66-3.32±0.68µm in CHA-AMR-1, 1.66-3.32 ± 1.66-3.32µm in CHA-AMR-5, 1.66-3.32±0.76×1.66-3.32±0.85µm in DA-Br-1, 1.66-3.32±0.81×1.66-3.32±0.76µm in DA-Br-2, 1.66-3.32±0.68×1.66-3.32±0.85µm in DA-Br-3, 1.66-3.32± 0.79 × 1.66-3.32±0.83µm in DA-P-3, 1.66-3.32±0.68×1.66-3.32±0.84µm in DA-P-4and 1.66-3.32± 0.72×1.66-3.32±0.81µm in DA-P-5, respectively. No chlamydospore was observed in any of the isolates. The cultural, morphological and morphometric characters of the all isolates were compared with the reported literature of Rifai (1969) and Gams and Bissett (2002) and confirmed as *Trichoderma harzianum* Rifai.

*Paecilomyces niphetodes*

The colony texture of the isolates ALLEN-To-1 and ALLEN-To-6 (Fig. 2 and 3) were found to be with arachnoid growth and white in colour. On the reverse side of petriplate, the colony was found to be colourless in all the isolates. However in all the isolates, the colonies appeared to be white powdery with basal felt. The conidial characters of these isolates have smooth wall, hyaline and ellipsoidal /triangular shaped conidia and varied from 3.32 × 1.66 µm in size. No chlamydospore was observed in isolates ALLEN-To-1 and ALLEN-To-6. The cultural, morphological and morphometric characters of both isolates were also compared with the reported literature of Samson (1971) and were confirmed as *Paecilomyces niphetodes* Samson.

*Fusarium oxysporum*

The colony texture of isolates DA-P-2, CHA-AMR-2, CHA-AMR-3, CHA-AMR-4, ALLEN-TO-3, ALLEN-TO-4, ALLEN-TO-5, ALLEN-TO-7 and ALLEN-TO-9 (Fig. 2, 3, 4, 7 and 10) were found to be flocose. The colony was found to be with smooth margin in isolates ALLEN-TO-3, ALLEN-TO-9, CHA-AMR-2, CHA-AMR-3 and CHA-AMR-4 and with lobes margin in isolates DA-P-2, ALLEN-TO-4, ALLEN-TO-5 and ALLEN-TO-9. The colour of mycelium varied from white (ALLEN-TO-3), light pink (DA-P-2), white (ALLEN-TO-9) with exudations (CHA-AMR-3 and CHA-AMR-4) and vinaceous (ALLEN-TO-4, ALLEN-TO-5, ALLEN-TO-7). The colony colour on the reverse side of petriplate was vinaceous in isolates ALLEN-TO-3, ALLEN-TO-4, ALLEN-TO-5, ALLEN-TO-7, CHA-AMR-2, CHA-AMR-3, CHA-AMR-4, DA-P-2 and colourless in isolates ALLEN-TO-9. The observation on the conidial characters showed abundance in microconida in the isolates DA-
P-2, CHA-AMR-2, CHA-AMR-3, CHA-AMR-4, ALLEN-TO-3, ALLEN-TO-4, ALLEN-TO-5, ALLEN-TO-7 and ALLEN-TO-9) in the present investigations were compared with the literature of Booth (1971), Hussain et al., (2012) and Xalxo et al., (2013) and confirmed to be *Fusarium oxysporum* Booth 1979.

**Fusarium solani**

The colony of isolate NAM-Br-1(Fig. 1) had brown in colour pigmentation on both sides of petriplate. The isolate NAM-Br-1 had abundant microconidia which were ellipsoidal to oval and straight in shape with zero septations. The microconidia ranged from 4.98-13.25±1.87×1.66-3.32µm±0.85m in size. These results were in agreement with Ciampi et al., (2009) also reported that microconidia of *F. solani* were varied from 8.00 - 16.00 x 2.00 - 4.00 µm in size with brown pigmentation. Mwaniki et al., (2011) reported that pigmentation of the aerial mycelium of the *F. solani* species complex (FSSC) isolates varied from white to cream while colony on reverse side varied from white to brown. Further they reported that microconidia of *Fusarium solani* species complex (FSSC) were one- or two-celled and oval in shape. Thus, the cultural, morphological and morphometric characters of the isolate NAM-Br-1 was compared with the literature of Mwaniki et al., (2011) and Ciampi et al., (2009) and confirmed to be confirmed as *Fusarium solani* Booth.

**Acremonium falciforme**

Colony texture of the isolate NAM-Br-2 (Fig. 2) was found to be velvety and margin entirely circular and colour white off. On the reverse side of petriplate, the colony was yellowish in colour and zonation was slightly raised in center with depressed a margin. The observation on the conidial characters showed that the conidia were hyaline, non-septate with slightly curved and crescentic in shape.
conidia. The conidia were ranges from 4.98-6.64±0.62 × 1.66-3.32±0.72 µm in size. The chlamydospores were terminal, elongate in shape brown in colour.

The chlamydospores were varied from 4.94-6.64 × 4.94-6.64µm in size. These results were found to similar with Jicinska (1974) observed that *A. falciforme* had crescentic conidia that are either non-septate or have a single septum. Williams (1987) who recorded that colony of *A. falciforme* became off-white to pale cream, velvety, with a slightly raised centre and a depressed margin on agar media. Further observed that aseptate coinidia and measured about 5.00-9.00 µm long, 2.00 to 3.00-5.00 to 5 µm wide and also produced terminal chlamydomspores. Chander and Sharm (1994) observed that *A. falciforme* produced light yellow colour pigmentation on the reverse side of plate after 4-5 days of incubation.

In the present investigation also, similar cultural, morphological and morphometrics characters as reported Jicinska, (1974), Williams, (1987) and Chander and Sharma, 1994 were observed. Thus, the cultural, morphological and morphometric characters of the isolate NAM-Br-1 in the present investigations was compared with the literature of literature of Jicinska, (1974), Williams, (1987) and Chander and Sharma, 1994 and confirmed to be *Acremonium falciforme* (Carrion) Gams, 1971.

**Aspergillus niger**

The colony characters of the isolate CHA-AMR-6 (Fig. 11) had velvety type colony texture with entirely circular margin and black in colour. On the reverse side of petriplate, the colony was pale yellow in colour. The conidia were rough, globose in shape and brown in colour. The conidia ranged from 1.66-4.98×1.66-3.32 µm in size. No chlamydospores were observed. The cultural, morphological and morphometric characters of the isolate was compared with the reported literature of Raper and Fennell (1965), Sharma and Pandey (2010) and Nithiyaa *et al*., (2012) and was confirmed as *Aspergillus niger*.

**Aspergillus flavus**

The colony characters of the isolates JOR-TO-1 and JOR-TO-2 (Fig. 9) were found to be velvety and circular with entirely circular margin and green in colour. On the reverse side of petriplate, the colonies were found to be yellow in colour. The observations on conidial characters revealed that conidia smooth, subspherical in shape and green in colour. The conidia were ranges from 1.66-3.32 × 1.66-3.32 µm in size. No chlamydospores were observed. The cultural, morphological and morphometric characters of all isolates were compared with the reported literature of Nithiyaa *et al*., 2012 and were confirmed as *Aspergillus flavus*.

**Penicillium spp.**

The colony characters of the isolate JOR-TO-3 (Fig. 3) was found to be smooth texture, entirely circular margin and creamy white in colour on both sides of petriplate. The observation on conidial characters shows that both the isolates had smoothwall, globose in shape and green in colour conidia. The conidia were varied from 1.66-3.32±0.68×1.66-3.32±0.62µm in size. No chlamydospores were observed. The cultural, morphological and morphometric characters of all isolates were compared with the reported literature of Tiwari *et al*., (2011) and were confirmed as *Penicillium spp.*

**Vermispora leguminacea**

The colony characters of the isolates ALLEN-TO-2, ALLEN-TO-8 and ALLEN-10 (Fig. 2, 3 and 10) were found to be floccose, with
curled circular margin with white coloured aerial mycelium. On the reverse side of petriplate, the colony was white in colour. The colony zonations of all isolates were raised in center. The observation on the conidial characters showed abundance in microconidi in all above isolates and the microconida were hyaline, cylindrical to fusiform look like pod-shaped and slightly curved with 1-2 septations. The variation was also observed in size of microconida. In the isolate ALLEN-TO-2, it varied from 24.36-27.84±0.96×4.98µm, ALLEN-TO-8 from 23.20-25.52±0.94×4.98µm and ALLEN-10 from 23.20-27.84±1.68×4.98µm. No chlamydosporos were observed.

The result was confirmed with Chen et al., (2007) who observed that colonies of Vermispora leguminacea on PDA were white, finely powdery with aerial mycelium, conidia hyaline, cylindrical-fusiform, pod-shaped, slightly curved, 1-5 (mainly 3)- septate, 20.00 (17.5)-34.00x4-(4.5)-5.00µm in size. The cultural, morphological and morphometric characters of all isolates were compared with the reported literature of Chen, et al., 2007 and were confirmed as Vermispora leguminacea.

Unidentified species
The isolate DA-P-1 (Fig. 7) had with floccose texture, entirely circular margin and off white coloured aerial mycelium. On the reverse side of petriplate, the colony was pale yellow in colour. The observation on conidial characters showed that hyaline, smooth wall and spherical in shape conidia. The conidia were varied from 9.96±1.10×6.64±1.02µm in size. No chlamydosporos were observed. The isolate DA-P-1 is difficult to identified and regarded as unidentified species.

Occurrence of fungal species from M. incognita egg masses in Jorhat and Golaghat district of Assam
The fungal communities associated with M. incognita eggmasses were diverse and varied among sampling sites. A total of 29 fungal isolates comprising of 7 genera with 9 species (Table 2) (T. harzianum, P. niphedotes, A. falciforme, F. oxysporium, F. solani, A. niger, A. flavus, V. leguminacea, Penicillium sp) were recovered. All the species showed varied relative frequency of occurrence, F. oxysporum being the most frequently occurred species with 31.03 per cent of total fungal isolates.

**Table 1** Collection site of root-knot nematode (Meloidogyne spp.) infected samples from different place of Jorhat and Golaghat district of Assam

| Collection site | Sample                      | Isolate code |
|-----------------|-----------------------------|--------------|
| Charigaon       | Ameranthus (A. spinosus)    | CHA-AM       |
| Alengmora       | Tomato (L. esculentum)      | ALLEN-TO     |
| Danichapari     | Brinjal (S. melongena)      | DA-Br        |
|                 | Pea (P. sativum)            | DA-P         |
| Namdeori        | Brinjal (S. melongena)      | NAM-Br       |
| Barbheta        | Tomato (L. esculentum)      | JOR-TO       |
Table.2 Mycoflora recorded from *Meloidogyne incognita* egg masses in Jorhat and Golaghat district of Assam

| Place       | *T. harzianum* | *P. niphatodes* | *F. oxysporum* | *F. solani* | *A. falciforme* | *A. niger* | *A. flavus* | *V. leguminacea* | *Penicillium sp.* | Unidentified species | Total | Relative frequency (%) |
|-------------|----------------|-----------------|----------------|-------------|----------------|------------|-------------|-------------------|-----------------|----------------------|-------|------------------------|
| **Jorhat district** |                |                 |                |             |                |            |             |                   |                 |                      |       |                        |
| Charigaon   | 2              | -               | 3              | -           | 1              | -          | -           | -                 | -               | -                    | 6     | 20.69                  |
| Alengmora   | -              | 2               | 5              | -           | -              | -          | -           | 3                 | -               | -                    | 10    | 34.48                  |
| Namdeori    | -              | -               | -              | 1           | 1              | -          | -           | -                 | -               |                      | 2     | 06.90                  |
| Barbheta    | -              | -               | -              | --          | -              | 2          | -           | 1                 | -               |                      | 3     | 10.34                  |
| **Golaghat district** |                |                 |                |             |                |            |             |                   |                 |                      |       |                        |
| Danichapori | 6              | -               | 1              | -           | -              | -          | -           | -                 | -               | 1                    | 8     | 27.59                  |
| **Total**   | 8              | 2               | 9              | 1           | 1              | 1          | 1           | 2                 | 3               | 1                    | 29    | 100                    |
| **Relative frequency (%)** | 27.59          | 6.90            | 31.03          | 3.45        | 3.45          | 3.45       | 3.45        | 6.90              | 10.34           | 3.45                 |       |                        |

"-", no fungi were recovered

*Relative frequency of occurrence of different species of fungi at different locations*= Number of isolates per species ×100/Total number of isolates

**Relative frequency of occurrence of different species of fungi** = Number of isolates per species ×100/Total number of isolates
Fig. 1 Perineal pattern of *Meloidogyne incognita*

a- Charigoan, b- Alengmora, c- Barbheta, d- Namdeori, e- Danichopari
**Fig. 2** Cultural and morphological characteristic of isolates of Alengmora

| Front side view | Reverse side view | Microscopic structure |
|-----------------|-------------------|-----------------------|
| ![Image of ALLEN-TO-1](image1.png) | ![Image of ALLEN-TO-1](image2.png) | ![Image of ALLEN-TO-1](image3.png) |
| ![Image of ALLEN-TO-2](image4.png) | ![Image of ALLEN-TO-2](image5.png) | ![Image of ALLEN-TO-2](image6.png) |
| ![Image of ALLEN-TO-3](image7.png) | ![Image of ALLEN-TO-3](image8.png) | ![Image of ALLEN-TO-3](image9.png) |
| ![Image of ALLEN-TO-4](image10.png) | ![Image of ALLEN-TO-4](image11.png) | ![Image of ALLEN-TO-4](image12.png) |
**Fig. 3** Cultural and morphological characteristic of isolates of Alengmora

| Front side view | Back side view | Microscopic structure |
|-----------------|----------------|-----------------------|
| ![Front side view](image1) | ![Back side view](image2) | ![Microscopic structure](image3) |
| **ALLEN-TO-5** | ![Front side view](image4) | ![Back side view](image5) | ![Microscopic structure](image6) |
| **ALLEN-TO-6** | ![Front side view](image7) | ![Back side view](image8) | ![Microscopic structure](image9) |
| **ALLEN-TO-7** | ![Front side view](image10) | ![Back side view](image11) | ![Microscopic structure](image12) |
| **ALLEN-TO-8** | ![Front side view](image13) | ![Back side view](image14) | ![Microscopic structure](image15) |
Fig. 4 Cultural and morphological characteristic of isolates of Alengmora

| Front side view | Back side view | Microscopic structure |
|----------------|----------------|-----------------------|
| ![Front side view](image1) | ![Back side view](image2) | ![Microscopic structure](image3) |
| ALLEN-TO-9 |

| Front side view | Back side view | Microscopic structure |
|----------------|----------------|-----------------------|
| ![Front side view](image4) | ![Back side view](image5) | ![Microscopic structure](image6) |
| ALLEN-TO-10 |

Fig. 5 Cultural and morphological characteristics of isolates of Namdeori

| Front side view | Back side view | Microscopic structure |
|----------------|----------------|-----------------------|
| ![Front side view](image7) | ![Back side view](image8) | ![Microscopic structure](image9) |
| NAM-Br-1 |

| Front side view | Back side view | Microscopic structure |
|----------------|----------------|-----------------------|
| ![Front side view](image10) | ![Back side view](image11) | ![Microscopic structure](image12) |
| NAM-Br-2 |
**Fig.6** Cultural and morphological characteristics of fungal isolates of Danichapori

| Front side view | Back side view | Microscopic structure |
|-----------------|----------------|-----------------------|
| ![DA-Br-1 Front side view](image1) | ![DA-Br-1 Back side view](image2) | ![DA-Br-1 Microscopic structure](image3) |
| ![DA-Br-2 Front side view](image4) | ![DA-Br-2 Back side view](image5) | ![DA-Br-2 Microscopic structure](image6) |
| ![DA-Br-3 Front side view](image7) | ![DA-Br-3 Back side view](image8) | ![DA-Br-3 Microscopic structure](image9) |
**Fig. 7** Cultural and morphological characteristics of fungal isolates of Danichaporid
**Fig. 8** Cultural and morphological characteristics of fungal isolates of Danichaporı

| Front side view | Back side view | Microscopic structure |
|-----------------|----------------|-----------------------|
| ![Front side view](image) | ![Back side view](image) | ![Microscopic structure](image) |

DA-P-5

**Fig. 9** Cultural and morphological characteristics of isolates of Barbheta

| Front side view | Back side view | Microscopic view |
|-----------------|----------------|------------------|
| ![Front side view](image) | ![Back side view](image) | ![Microscopic view](image) |

JOR-TO-1

| Front side view | Back side view | Microscopic view |
|-----------------|----------------|------------------|
| ![Front side view](image) | ![Back side view](image) | ![Microscopic view](image) |

JOR-TO-2

| Front side view | Back side view | Microscopic view |
|-----------------|----------------|------------------|
| ![Front side view](image) | ![Back side view](image) | ![Microscopic view](image) |

JOR-TO-3
Fig. 10 Cultural and morphological characteristics of isolates of Charigaon

| Front side view | Back side view | Microscopic view |
|-----------------|----------------|------------------|
| ![CHA-AMR-1](image1) | ![CHA-AMR-1](image2) | ![CHA-AMR-1](image3) |
| ![CHA-AMR-2](image4) | ![CHA-AMR-2](image5) | ![CHA-AMR-2](image6) |
| ![CHA-AMR-3](image7) | ![CHA-AMR-3](image8) | ![CHA-AMR-3](image9) |
| ![CHA-AMR-4](image10) | ![CHA-AMR-4](image11) | ![CHA-AMR-4](image12) |
The result was confirmed with Gine et al., (2013) isolated twenty fungal species belonging to 15 genera viz., Fusarium sp., F. oxysporum F. solani., Paecilomyces lilacinus, Plectosphaerella cucumerina, Pochonia chlamydosporia, and Thielavia sp., Cladosporium tenuissimum, Colletotrichum coccodes and F. equiseti, Chaetomium sp. Cladosporium sp., sphaerospermum, Cylindrocarpon oolidum, Dactylella oviparasitica, F. verticillioides, Monacrosporium thaumasi, Myrothecium verrucaria, Penicillium citrinum, P.olsonii and Verticillium sp. from Meloidogyne spp in Spain. Further, they reported that P. chlamydosporia, Fusarium spp. and P. cucumerina were most frequently isolated from eggs of root knot nematode.

Aminuzzaman et al., (2013) isolated fungi like Acremonium spp., Alternaria spp., Aspergillus spp., Aspergillus flavus, A. fumigates, A. nidulans, Botryotrichum sp., Chaetomium sp., Cladosporium sp., Cephalosporium sp., Cylindrocarpon sp., Cylindrocladium sp., Fusarium spp., F. chlamydosporium, F. moniliforme, F. oxysporum, F. solani, Mortierella spp, Paecilomyces lilacinus, Penicillium spp., P. janthinellum, Pestalotia sp., Pestalotiopsis spp., Pochonia chlamydosporia, Scopulariopsis brumptii, Trichoderma sp. and sterile fungi from eggs and females.

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