Parasitism of *Meloidogyne incognita* eggs by Native Fungi of Assam

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**ABSTRACT**

In the current investigation, four fungi *viz.*, *Paecilomyces niphetodes*, *Acremonium falciforme*, *Fusarium oxysporium*, and *F. solani* were capable of parasitizing *Meloidogyne incognita* eggs. The high percentage of egg parasitism was recorded by *P. niphetodes*. Microscopic examinations showed that the hyphae of *P. niphetodes*, *A. falciforme*, *F. oxysporum* and *F. solani* were closely attached to the egg surface of the parasitized eggs, made perforations, penetrated, ramified, and completely fed upon the internal content of the eggs. The parasitization of the second stage juvenile that emerged from the eggs by *P. niphetodes* and *A. falciforme* was seen. Whereas, *A. falciforme*, *F. oxysporum*, and *F. solani* established as dormant spores inside or outside of *M. incognita* eggs.

**Key words**: *P. niphetodes*, *A. falciforme*, *F. oxysporum* and *F. solani*, Egg parasitism, *M. incognita*.

**Introduction**

More than 2,000 plant species have been attacked by root-knot nematodes, which contributed to 5% of the world’s crop losses (Hussey and Jansma, 1988). In Assam, *M. incognita* reduced tomato, okra, and brinjal yield by 13.20, 15.80-17.80, and 27.30 percent, respectively (Anonymous, 2011 and Bhatti, 1994). The application of chemical nematicides will be severely prohibited due to environmental damage, animal welfare, and the development of resistance among the target pathogens. The use of bioagents has been demonstrated to provide an effective, safe, long-lasting, and natural robust protection against nematode pests (Anita and Samiyappan, 2012). *Meloidogyne* spp. in soil is openly attacked by natural enemies (Kok et al., 2001) and such enemies can be exploited as bioagents for successful control of *Meloidogyne* spp. (Karssen et al., 2006). Among the natural enemies, fungi are more wide-ranging and saprophytic in nature. These fungi could be opportunistic and capable of showing antagonistic behavior, such as predation, parasitism, and antibiosis against plant pathogenic nematodes (Cayrol, 1983; Zaki, 1994; Kalita et al., 2012 and Kurulkar, 2017).

These fungi have the ability to produce antibiotics, metabolites, protease enzymes, and other compounds in the environment, which has a negative impact on nematode viability (Blaxster and Robertson, 1998 and Sharon, 2001). The potency of bioagents was reported to vary from species to species by Irving and Kerry (1986). Utilizing native biocontrol agents is one method of enhancing the potential of bioagents (Singh et al., 2013). In order to employ effective native biocontrol agent(s), the possible advantages and fit fall must be studied. Therefore, research on the fungal parasitism of *Meloidogyne incognita* eggs was conducted.

**Material and Methods**

In the current study, the fungi *viz.*, *Fusarium oxysporium*, *Paecilomyces niphetodes*, *Acremonium oxysporium*,
*Falciforme*, and *F. solani* (Figure 1) were used. These were isolated and identified by the previous author Kurulkar (2017) from the egg mass of *M. incognita* in Assam. The pure culture of fungi was maintained on the potato dextrose agar. The pure culture of *M. incognita* egg masses were obtained from Experimental plot, Department of Nematology, AAU, Jorhat-13. Collection and surface sterilization of egg masses of *M. incognita* were done according to protocol described by Kurulkar (2017). Egg parasitism of test fungi was done by growing the fungi on PDA plate. After three days of fungal growth, the sterilized two egg masses were kept at the periphery of fungal growth and incubate for 7 days at 25±2 °C temperature in BOD incubator. After incubation, the percent egg parasitism was calculated by counting the parasitized and non-parasitized eggs under a microscope at 40X magnifications by using cotton blue staining. The percentage of egg parasitism was calculated by using the formula given below.

\[
\text{Per cent egg parasitism} = \frac{\text{Total parasitised eggs}}{\text{Total number of eggs}} \times 100
\]

Statistical analysis was done by using WASP version 1.0. The difference between treatments was estimated by using Duncan’s Multiple Range Test (DMRT) at 5% probability level.

### Results and Discussion

In the present study, the maximum egg parasitism was recorded in the treatment with *P. niphetodes* (75.75%) followed by *F. oxysporum* (66.00%), *F. solani* (64.50%) and *A. falciforme* (55.50%)(Table 1). Fungus, *P. niphetodes* emerged as statistically most superior over other treatments. Pau (2012) demonstrated that the isolates of *P. lilacinus* like, PLA, PLM and PLB caused 78.50, 73.40 and 66.00 per cent of egg parasitism of *M. incognita*. Aminuzzaman et al. (2013) evaluated egg parasitism potential of *F. oxysporum* (WC06-12F-1) and *Fusarium* sp. (FZ07-5F-8) against *Meloidogyne* spp and recorded 79.30 ± 3.10 and 68.70 ± 14.20 per cent of parasitism of *Meloidogyne* eggs, respectively. Siddiqui and Shaukat (2003) reported *F. solani* strain Fs5 to parasitize the eggs of *M. javanica*.

In the present investigation, the fungi *P. niphetodes*, *A. falciforme*, *F. oxysporum*, and *F. solani* were found to be attached to the gelatinous matrix (gm) surrounding the egg masses of *M. incognita* and ramifying multiple eggs within eggmasses, however all other fungi were unable to grow on other nearby eggs except *A. falciforme*. According to Pau et al. (2015), a large network of *P. lilacinus* hyphae was seen ramifying numerous *M. incognita* eggs, but not developing on other nearby eggs. According to Yao et al. (2015), *A. implicatum* conidia and hyphae were attached to the surface of the *M. incognita* egg. *A. strictum* and *F. oxysporum* hyphae were found surrounding the *Heterodera schachtii* eggs, according to Nigh et al. (2018). When describing the chemical composition of the gelatinous matrix of nematode egg masses, Sharon and Spiegel (1993) noted that it contained amino-acid, amino-sugar, and some glycoproteins. It is crucial for the patho-

*Table 1. Efficacy of native fungal bioagents on the parasitism of *M. incognita* eggs*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of egg parasitism</th>
</tr>
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<tbody>
<tr>
<td><em>P. niphetodes</em></td>
<td>75.75a</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>66.00b</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td>64.50b</td>
</tr>
<tr>
<td><em>A. falciforme</em></td>
<td>55.50c</td>
</tr>
<tr>
<td>CD @ 5%</td>
<td>2.84</td>
</tr>
</tbody>
</table>

Mean with different letters in the column are significantly different from each other based on DMRT (P≤0.0005)

![Fig. 1. Fungi used in the present investigation](image-url)
genesis that fungal parasites adhere to host substrates. A fungus and egg mass of nematodes are bound together via an adhesion mechanism mediated by glycoproteins and which act as a “glue” (Epstein and Nicholson, 1997). This could be the explanation why in the present investigation, fungi like *P. niphetodes*, *A. falciforme*, *F. oxysporum*, and *F. solani* exhibit such type of traits to display parasitic capacity towards *M. incognita* eggs. Nematode egg shells have three layers: an inner lipid layer, a middle chitinous layer, and an outer vitelline layer (Mansfield et al., 1992; Wharton, 1980 and Burgwyn, 2003). In the present investigation, it was observed that the hyphae of *P. niphetodes*, *A. falciforme*, *F. oxysporum*, and *F. solani* were closely connected to the egg surface of the parasitized eggs, made perforations, penetrated, ramified, and extensively fed upon the inner content of the eggs while exploring the egg parasitism in the present study (Figure 2a, 3a, 4a and 5a). Recent research has demonstrated that when fungus comes into contact with egg shells, they start producing lytic enzymes such chitinase, protease, and collagenase. As a result, the lipid layer, vitelline layer, and chitin of eggs were all degraded (Tikhonov et al., 2002 and Khan et al., 2004). Researcher like Mortan et al. (2004) discovered that *P. lilacinus* produced the chitinase enzyme when it came into touch with nematode eggs, allowing the fungi to enter the eggs, feed on the inside, and multiply there. In a similar manner, *A. implicatum* secretes chitinase (Lin et al., 2013), *Fusarium* spp. generates acetic acid and moniliformin (Ciancio et al., 1984) against plant parasitic nematodes, and this aids fungi in dissolving eggshells and enabling them to penetrate, proliferate, and feed on the embryo of eggs. Through microscopic examinations, it was discovered that some eggs and eggs containing juveniles were appeared to be abnormal, malformed, and shrunken (Figure 2a, 2c, 2d and 3d). It was interesting to see that *P. niphetodes* and *A. falciforme* parasitize the J₁ that emerged from the eggs (Figure 2d and 3d). Critical observation also revealed that *Fusarium* species parasitized the eggs in the absence of J₁. It could be because these fungi prefer to parasitize eggs where J₁ has not yet formed. Additionally, some parasitized eggs showed full egg shell degeneration (Figure 2c, 3f, 4d and 5d). According to Pau et al. (2012) *M. incognita* eggs of an early age were more vulnerable to *P. lilacinus* infection than eggs containing J₁ that were ready to hatch. Additionally, microscopic examinations showed that *P. niphetodes* and *F. oxysporum* were formed conidia (Figures 2b and 3b), while *F. oxysporum* and *A. falciforme* were formed chalmydospore both in-
side and outside the parasitize eggs (Figure 3e and 4b). In addition, Ngh et al. (1980) exposed Fusarium spp. hyphae, chlamydospores, and microconidia inside Heteroderas chachtii eggs. The same thing was noted by Westphal and Becker (2001), who discovered that Fusarium spp. hyphae and chlamydospores were usually present in H. schachtii eggs. However, the types of mechanisms favoured by various researchers and mentioned above may be involved in the egg parasitism of M. incognita by P. niphetodes, A. falciforme, F. oxysporum, and F. solani in the current investigation.

References


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