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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. niphetode*, *A. falcifrome*, *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. falcifrome*, *F. oxysporum*, and *F. solani* established as dormant spores inside or outside of *M. incognita* eggs.

Key words : *P. niphetodes, A. falcifrome, 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 fugal parasitism of *Meloidogyne incognita* eggs was conducted.

Material and Methods

In the current study, the fungi *viz*., *Fusarium oxysporium*, *Paecilomyces niphetodes*, *Acremonium*

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.

$$\text{Per cent egg parasitism} = \frac{\text{Total parsitised eggs}}{\text{Total number of eggs}} \times 100$$

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. oxysporium* (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 *Meloidogyn*e spp and recorded 79.30 ± 3.10 and 68.70 ± 14.20 per cent of parasitism of *Meloidogyn*e eggs, respectively. Siddiqui and Shaukat (2003) reported *F. solani* strain Fs5 to parasite the eggs of *M. javanica*.

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

Treatment	Percentage of egg parasitism
P. niphatodes	75.75a
F. oxysporum	66.00b
F. solani	64.50b
A. falciformi	55.50c
CD @ 5%	2.84

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

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 schahtii* 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-

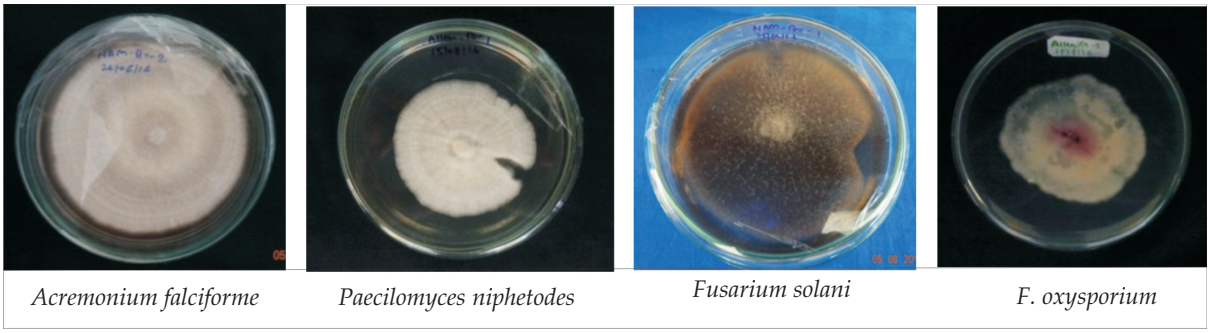

Fig. 1. Fungi used in the present investigation

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. oxysporm*, 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, proliferates, 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 J_2 that emerged from the eggs (Figure 2d and 3d). Critical observation also revealed that *Fusarium* species parasitized the eggs in the absence of J_1. 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 2e, 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 J_2 that were ready to hatch.

Additionally, microscopic examinations showed that *P. niphetodes* and *F. oxysporium* were formed conidia (Figures 2b and 3b), while *F. oxysporium* and *A. falciforme* were formed chalmydospore both in-

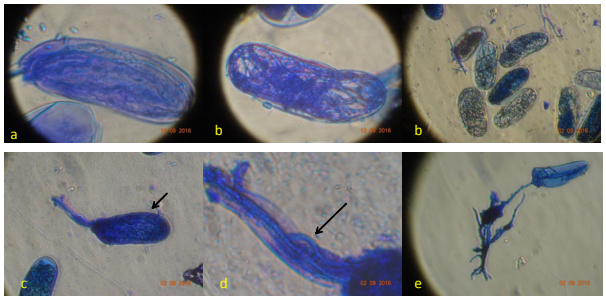

Fig. 2. *Meloidogyne incognitaeggs*/juvenile parasitized by *Paecilomycesniphetodes*
a Penetration of the egg shell and degradation of egg embryo, **b.** Ramification of hyphae inside the eggs **c**. Parasitized J_2 emerging from the egg (arrow pointing at J_2)**d.** Proliferation of hyphae within J_2 (arrow pointing at J_2) **e**. Degradation of egg shells

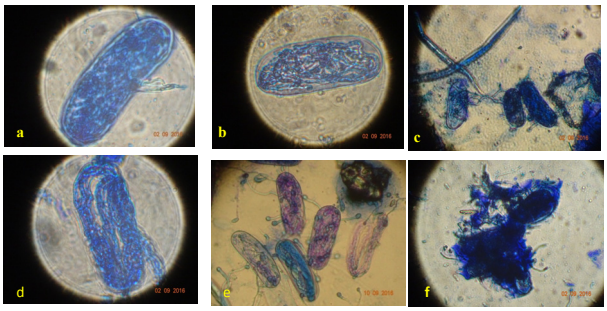

Fig. 3. *Meloidogyne incognita* eggs parasitisedby *Acromonium falciformi*
a. Penetration of the egg shell and degradation of egg embryo**b.**Ramification of hyphae inside the eggs, **c.** Ramifying several eggs. **d.** Parasitized J_2 inside the egg **e.** Terminal chlamydospore inside and outside of the eggs. **f**. Degradation of egg shells

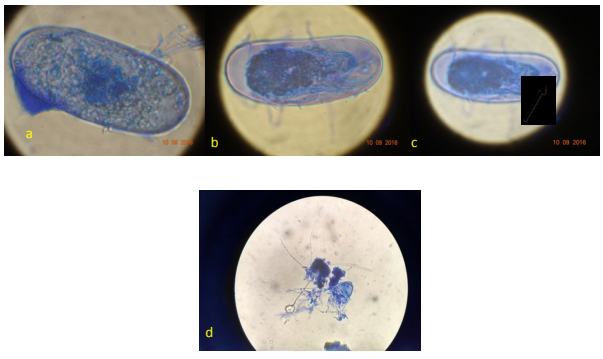

Fig. 4. *Meloidogyne incognita* eggs parasitised by *Fusarium oxysporum*
a. Ramification of hyphae inside the egg **b**. Degradation of the embryo **c**. Terminal chalmydospore within egg (arrow) **d**. Degradation of egg shell.

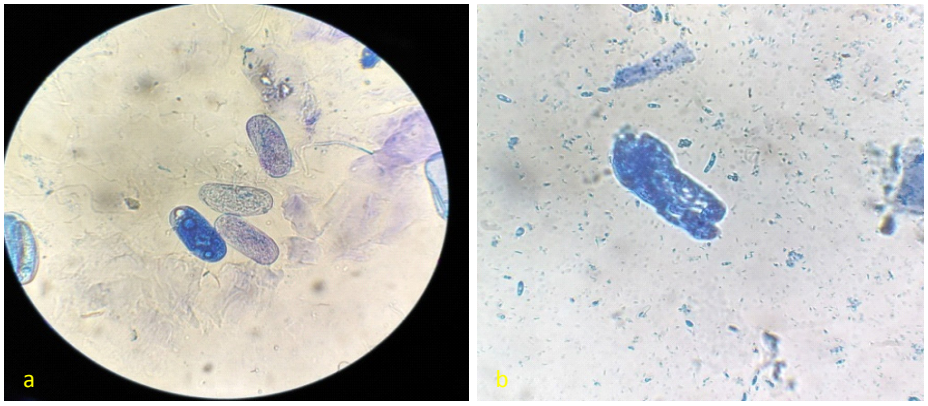

Fig. 5. *Meloidogyne incognita* eggs parasitised by *Fusarium solani*
a. Extensive network of hyphae inside the egg **b.** Degradation of egg shell and condensation of conidia around the egg

side and outside the parasitize eggs (Figure 3e and 4b). In addition, Nigh *et al*. (1980) exposed *Fusarium* spp. hyphae, chlamydospores, and micoconidia 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.

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