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HISTOLOGICAL INTERACTIONS OF PAECILOMYCES LILACINUS AND MELOIDOGYNE INCOGNITA ON BITTER GOURD

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ABSTRACT: Momordica charantia roots were histologically examined for the interaction of Meloidogyne incognita and the fungus Paecilomyces lilacinus which was applied at different time intervals. Meloidogyne incognita induced large sized galls on the plants which were treated with P.lilacinus. Fully mature females were found associated with giant cells. All the mature females on the roots of untreated, Meloidogyne incoginta infected plants laid egg masses. The xylem and the phloem exhibited abnormalities in structure near the giant cells.Abnormal vessel elements were occupying larger area near giant cells. The fungus P.lilacinus. Soon after the application, entered the roots and spread through the lumen of the vessel elements. The plants that were treated with fungus either one weak before nematode inoculation or simultaneously, produced significantly (P= 0.01) small sized galls in comparison to untreated plants. The size of galls remained unchanged after completion of one life cycle by the nematode. In fungus treated plants the giant cells were small sized and the abnormality of vascular plants was less. Paecilomyces lilacinus entered the giant cells and also into the body of mature females. It destroyed the eggs and egg masses in and out side females. The fungus by destroying eggs checked the possibility of secondary infection that ultimately arrested increase of gall size.
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Key words: Abnormality, gall, giant cell, histology, vascular tissue.

1. INTRODUCTION

The fungus Paecilomyces lilacinus (Thom) Samson has been reported as a potential biological control agent for root –knot nematode and other plant parasitic nematodes (jatala, et al., 1979, 1982, 1986; Adiko,1984;Cardona and Leguizamon,1997 and Khan and Williams ,1998).P. lilacinus is common soil hypomycete closely related to Penicillium (Samson,1975).The sedentary stages of the root - knot and cyst nematode are most vulnerable to P. lilacinus. The fungus is capable of colonising nematode reproductive structures thus causing destruction of females, cysts and eggs. (Franco, et al,. 1981, Jatala, 1982, 1986; Gintis, et al., 1983 and Cardona and Leguizamon,1997).

Paecilomyces lilacinus increased the yield of tomato and okra and lowered the population of M. incognita juveniles, at the mid and at the beginning of the next season in treated pots than in untreated pots(Neo and Sasser 1984). M.incognita juveniles when exposed to fungus resulted in reduced gall formation and egg mass production.

The association of P.lilacinus with the eggs of M.incognita is well documented but the exact mode of its parasitism is unknown. Root galling and giant cell formation were absent in tomato roots inoculated with fungus infected eggs of M.incognita. P. lilacinus colonised surface of epidermal cells as well as the internal cells of cortex of tomato roots (Cabanillas et al.,1988).The effects of fungus on M.incognita parasitizing the roots of Momordica charantia has not yet been reported.The objectives of this study were : (i) to examine the effect of P.lilacinus on M.incognita infected plant tissues.(ii) to know the effect of P.lilacinus on nematode development, (iii) to determine the effect of P.lilacinus on eggs and egg masses,(iv) to examine the effect of P.lilacinus on the giant cells and (v) to evaluate the efficacy of P.lilacinus in controlling the disease development, on applying the fungus at varying time intervals, before or after nematode inoculation.

2. MATERIALS AND METHODS

Nematode cultures of Meloidogyne incognita was maintained from single egg mass on egg plant (Solanum melongena L.)grown in green house in 15 cm diameter earthen pots containing a mixture of steam sterilized soil and sand (3:1). M.incognita originally was isolated from vegetable crop fields of Aligarh. The root-knot nematode was identified on the basis of characteristic perineal pattern and North Carolina differential host test. Freshly hatched second stage juvenile inoculum was prepared by hatching egg masses picked from egg plant roots, maintained as pure culture in green house. Paecilomyces lilacinus, used in the experiment, was obtained from international potato centre, Lima,

Peru. The fungus was cultured on PDA for 15 days at then inoculated to Richards Medium (Riker and Riker, 1936) for en-masse propagation .The mycelia (100 gm) were blended in distilled water (100 ml) in warring blender to make mycelial suspension for soil application (10 ml of suspension containing 1gm mycelia).The fungus was applied into the rhizosphere zone by making three or four holes around the plant.

Plant Materials : Seeds of Momordica charantia L. variety NSC were surface sterilised with 1% sodium hypochlorite (NaOCl) for five minutes and rinsed three times with sterile distilled water. 100-150 axenised seeds were placed on a moist sterilised filter paper kept in sterilised petri dishes. Seeds were allowed to germinate. The germinated seeds were transferred to 15 cm diameter clay pots filled with autoclaved soil, sand and farmyard manure in the ratio of 7:3:1. Two week old seedlings were inoculated with a suspension of 1000 J2 pipetted into root zone via the holes in the rhizosphere zone around plant in each pot. To achieve the aim and objective the experiment was designed as per the following treatment schedule

1) T_1- un-inoculated control
2) T_2- inoculated with 1,000 J2 only
3) T_3- inoculated with 1,000 J2 and treated with fungus one week before inoculation
4) T_4- inoculated with 1,000 J2 and treated with fungus simultaneously
5) T_5- inoculated with 1,000 J2 and treated with fungus two week after inoculation

Each treatment was replicated five times, arranged in randomised block design. Each set of plants was uprooted carefully after 45days . The roots were cut into small pieces and processed for histopathological studies. Root pieces were fixed in formalin- aceto - alcohol (F.A.A) and then dehydrated through tertiary-butyl-alcohol (T.B.A.) schedule as given by Johansen (1940). Infiltration and embedding of root pieces in paraffin wax was followed and sections of 12µm thickness were taken with the help of rotary microtome in the form of ribbon. The ribbons were cut into small pieces and mounted on clean slides with the help of Haupt's adhesive and 3% formalin and stained with safranin and fast green (Sass1951). The slides were taken out from xylene. The mounting medium was applied on the surface of slide before evaporation of xylene and cover slip was lowered gradually. Finished slides were left at room temperature at least for 24 hours and then kept in an incubator at 40^0C.The slides were examined under light microscope and necessary photographs were taken.

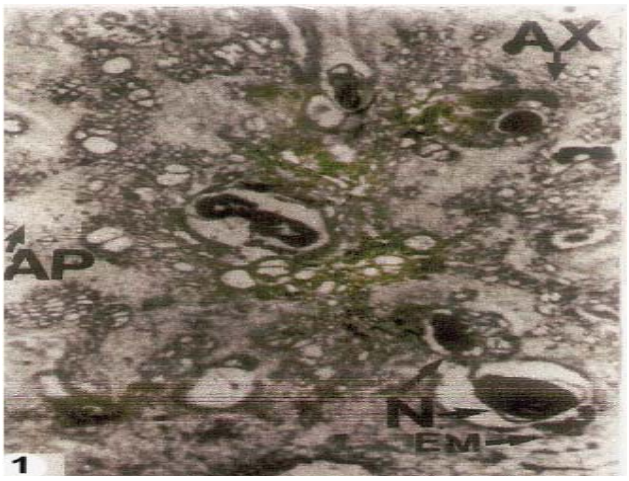

Fig 1: Showing heavy infestation of Meloidogyne incognita (N). The mature females have egg masses (EM). Abnormal xylem (AX) and abnormal phloem (AP) are in abundance.(25X)

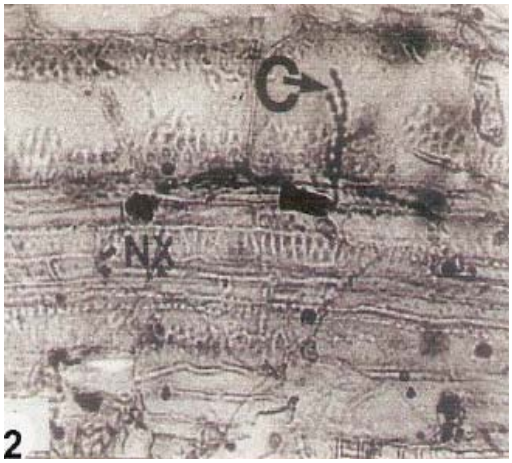

Fig 2: Showing normal xylem (NX) strands with conidiophores and conidial chain C in the lumen of vessel elements.

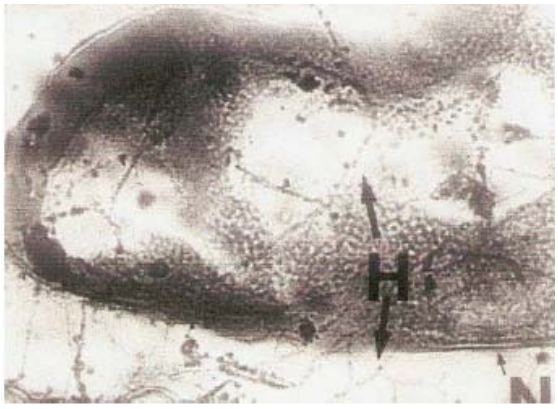

Fig 3: Showing abundant growth of Peacilomyces lilacinus hyphae (H) in and around the females of M. incognita (N).(32 X).

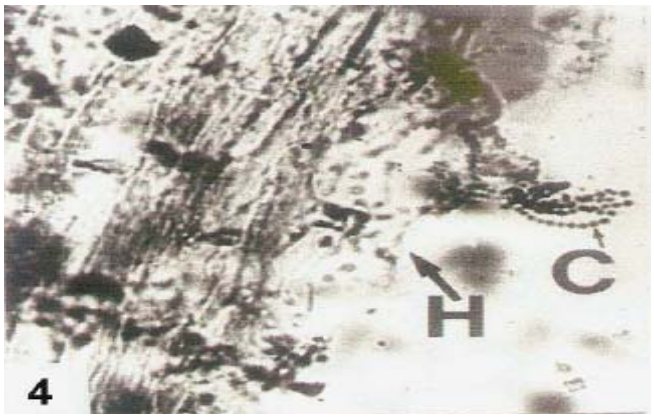

Fig 4: Showing hyphae (H) and conidial chains (C) at the root surface. (12.5 X)

3. RESULTS

Histologically the primary root of Momordica charantia consists of uniseriate epidermis, multilayered parenchymatous cortex and stele. The stele in primary root may be diarch, triarch but generally tetrarch, displaying a typical dicotyledonous pattern. Xylem and phloem are radially arranged alternating with one another. There is small pith consisting of parenchyma cells at the centre of four xylem arches. Meloidogyne incognita inoculated M.charantia plants receiving no disease controlling treatment produced large sized galls. The mature females feeding on giant cells, abnormal xylem and abnormal phloem was observed (Fig 1).All the mature females were found associated with egg masses. Histological examination of the galled roots which were given Paecilomyces lilacinus treatment, one week before the nematode inoculation, revealed that the fungus entered the root tissue and grew successfully. The hyphae and conidiophores, bearing chains of conidia were seen in normal vessel elements of the xylem (Fig 2).The giant cells, though smaller, resembled with those of T2 plants .In the vicinity of the giant cells abnormal xylem and phloem were present in Paecilomyces lilacinus treated plants. The abnormality in vascular elements was less. The fungal hyphae destroyed eggs and egg masses and also entered into the body of the females. Around the nematode body fungal growth was abundant (Fig 3).The root surface also exhibited profused growth of fungus (Fig 4). The simultaneous application of root knot nematode and Paecilomyces lilacinus not only destroyed eggs and egg masses, but also entered into the internal tissues of root, either intercellularly or intracellularly as is evident from the transverse sections of vessel elements (Fig 5).The egg masses were destroyed by the fungus and the growth of fungus was profound inside egg masses. There was no change in the size of giant cells and amount of vascular elements as compared to untreated plants. In plants treated with Paecilomyces lilacinus, one week after nematode inoculation, the fungal hyphae was observed inside the giant cells (Fig 6).The fungal growth was profuse around the body of developing nematodes. In the normal tissue the fungus spreads both inter and intracellularly as is evident from figure (Fig.7)

Fig 5: Showing vessel elements (VE) in transverse sections enclosing conidial chains (C).(40 X)

Fig 6: Showing hyphae (H) in giant cell (GC) and nematode (N) adjacent to giant cell.(25 X)

Fig 7: Showing normal xylem (NX) strands with hyphae(H) of Paecilomyces lilacinus traversing through the lumen of vessel elements. (32 X)

DISCUSSION

The fungus Paecilomyces lilacinus shows diverse modes of habits. Basically it is a saprophyte (Domsch et al., 1980) and can easily be grown on artificial culture media. It behaves as an epiphyte and grows on the surface of plant roots (Cabanillas et.al.,1988). It also grows inside the root tissue and behaves as an endophyte and does not cause any damage to the plant. Still at other times it parasitizes eggs and egg masses of Meloidogyne species and destroy them. Because of the lastly stated behaviour, P.lilacinus has been used by several workers, as biocontrol agent against root-knot nematode and other nematodes (Jatala, et al., 1979).

Paecilomyces lilacinus was encountered frequently in and around normal and abnormal xylem. In our opinion, vessels and vessel elements provide sufficient space for its development and also provide an uninterupted passage to grow inside the plant tissues. We consider that P.lilacinus develops inside the root tissues inter and intracellularly. Whether the fungus is beneficial or not to plant but, it is not harmful. In all the sections studied, the fungus was not found damaging the plant tissues even when it was in abundance. Further it did not affect the giant cells in which its occurrence was noted. In all the treatments it was regularly observed that P.lilacinus damage the eggs and egg masses. Various workers have reported egg destroying activity of this fungus (Jatala, et al,. 1979, Jatala, 1985,1986.) It has also been reported that the fungus can destroy neither the juveniles nor the adult females (Jatala ,1986). The eggs however seem to be the most preferred target for obtaining the nourishment by the fungus. Contrary to this (Cardona and Leguizamon,1997) reported 94% infection in Meloidogyne spp. by P. lilacinus strain-9207. KHAN and WILLIAMS (1998) found P.lilacinus entering into the body of the females through natural opening. They did not mention whether the fungus damaged the females or not, although it damaged the eggs inside the egg masses. Small sized giant cells and small amount of abnormal xylem and phloem indicated that the nematode activity and development was influenced by the presence of P.lilacinus. Large giant cells and more quantity of abnormal tissues showed that the nematodes which entered earlier were not affected by the fungus. On the basis of these observations we concluded that the fungus can not check primary infection of nematode when the plants are attacked by the juveniles. However, it can check secondary infection because it destroys eggs as and when these are deposited. As far as time of application of P.lilacinus is concerned, from our studies it can be suggested that incorporation of fungus P.lilacinus one week before and at the time of nematode inoculation is more effective in controlling the root-knot disease, as compared to later intervals of fungus applications.

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