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Morphopathological Changes Induced by Root Knot Nematodes in Luffa Plant *(Luffa cylindrica L.)* and Their Alleviation By Vesicular Arbuscular Mycorrhizae Fungi

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Abstract: Root-knot nematode penetration and morphopathlogy of the infection sites were studied in roots, stem and hypocotyls of *Luffa cylindrica*, L. and effect of combined inoculation of Vesicular Arbuscular Mycorrhizae (VAM) fungi included *Glomus mossae, Glomus intraradices, G. fasciculatum, Acaulospora* sp. and *Meloidogyne incognita* (Root-knot Nematode) were investigated. The maximum number and size of galls were noticed in nematode inoculated plants. The anatomical observations of roots, stems and hypocotyl were done with the aid of a light microscope which demonstrated that *Meloidogyne incognita* caused rupturing of epidermis of the root of *Luffa cylindrica,* L. while penetrating into the inner tissues. They caused the formation of giant cells in the form of clusters. The giant cell clusters modify the internal morphology of the affected tissues. Abnormal xylem and abnormal phloem occupy a major portion near the giant cells and endodermal and pericycle cells showed hypertrophy and granular appearance of cytoplasm. Vascular discoloration (Blackness) was greater in the presence of *Meloidogyne incognita* compared with that by VAM alone. Histological studies showed that plants grown in VAM-infested soils contained spores in cortical region of roots and hypocotyls. In plants inoculated with both fungus and *Meloidogyne incognita,* the giant cells were small sized and the abnormality of vascular bundles was less. The results of this study also confirmed VAM (Arbuscular mycorrhizae fungi) as a potential bio-control agent for *Meloidogyne incognita* on Luffa plant.

Key words: Root-knot nematodes · VAM · Histopathology · Bio-control agent

INTRODUCTION

Root-Knot Nematodes: Root-knot nematodes are plant-parasitic nematodes from the genus *Meloidogyne*. They exist in soil in areas with hot climates or short winters. About 2000 plants are susceptible to infection by root-knot nematodes and they cause approximately 5% of global crop loss. They induce the formation of giant cells and root galls that impair water and nutrient uptake to the shoots[7] reducing the yield and fruit size and causing the mineral deficiency that decreases plant longevity and a delay in the crop production.

Histopathology of Plants Infected by Nematodes: Root-knot nematodes are known to cause galls on roots of more than 2000 plant species [8]. Gall formation is a visible sign of root-knot nematode infection resulting from the hypertrophy and hyperplasia indicative of feeding site formation within the roots [9].The giant cells which are ten times the normal size interfere with the development of the root.Moreover, an increase of dehydrogenase and diaphorase activity has been detected around the head of *M. incognita* infecting soybean. Auxin activity and auxin inhibitors in extracts of *Meloidogyne* also have been reported [10]. Kaplan *et al*., [11] observed cellular browning in the soybean cultivar, 'Centennial', which is resistant to *M. incognita*. When giant cells were present they were small and deteriorated before the nematodes could mature these structures were first described by Treub in 2002 [12]. Bird observed giant-cell formation by the 4th day on susceptible tomato plants at 24°C and by the 9th and 13th day the giant-cells had reached their average maximum size. The first sign of giant-cell induction is the formation of several binucleate cells [13]19].

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According to Dundon [5] the number of nuclei of a coenocyte would correspond to the number of cells that are formed by the fusion of several cells.The multinucleate condition of the giant cell is produced by the accumulation of nuclei from several cells and from divisions without cytokinesis [14]. Swamy & Krishnamurthy [15] observed frequent occurrence of dumbbell—shaped and dissimilar nuclei in the coenocyte of *Enterolobium*and they feel that amitotic budding of nuclei could be a method of increase in nuclear number. In the present study minute wall ingrowths of some giant cells in the rhizome of ginger are observed. According to Gravato[3] the transfer cells have functions of absorption and/or secretion. The giant cells function as a storage house for the feeding purpose of the female nematode as well as for its young larvae [16].Once the female nematode selects a feeding cite it is stationary.

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 [17]. It has also been reported that the fungus can destroy neither the juveniles nor the adult females [18]. The eggs however seem to be the most preferred target for obtaining the nourishment by the fungus.. Khan And Williams [19] found *P.lilacinus* entering into the body of the females through natural opening.

Vesicular Arbuscular Mycorrhizal (VAM) Fungi and Root-knot Nematode Interaction: In 2003 Fassuliotis studied the soil microbial-plant relationships, introduced the Greek term 'mycorrhiza', which literally means 'fungus roots. Mycorrhizal fungi form symbiotic relationships with plant roots in a fashion similar to that of root nodule bacteria within legumes. Of the seven types of mycorrhizae described (arbuscular, ecto, ectendo-, arbutoid, monotropoid, ericoid and orchidaceous mycorrhizae), arbuscular mycorrhizae and ectomycorrhizae are the most abundant and widespread. [1][2][20] Arbuscular mycorrhizal (AM) fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants [21]. AM fungi are obligate mutualists belonging to the phylum Glomeromycota and have a ubiquitous distribution in global ecosystems [22].

The interaction between AMF and nematodes results in improvement, reduction or has no effect on disease severity. The effect of the interaction VAM × plant nematodes depends on various factors such as nematode, fungus and plant species, environmental conditions, time of mycorrhization and period of exposure to the nematode.[23]

MATERIALS AND METHODS

Materials were used for preparation of Hoagland solution. All solutions were prepared in distilled water.The solutions used in the preparation of Hoagland are categorized as.

- Macronutrients.
- Micronutrients.
- $[Fe(EDTA)]^2$.

Macronutrients: The solutions of following composition were prepared as macronutrients.

1. $Ca(NO_3)_2$. 23.615gm/100ml.
2. KCl. 7.46gm/100ml.
3. KH_2PO_4. 13.61gm/100ml.
4. $MgSO_4$. 24.64gm/100ml

Micronutrients: These nutrients were dissolved together in 1Liter of distilled water.

1. $H_3BO_3$2.5gm.
2. $MnCl_2/MnSO_4.H_2O$
3. 1.5/1.281gm.
4. $ZnSO_4.4H_2O$ 0.1gm.
5. $H_2Mo_3/NaMoO_4.2H_2O$ 0.05/0.083gm.
6. $CuSO_4$0.8gm.
   $[Fe(EDTA)]^2$:

The last solution that was prepared separately is potassium ethlendiamine tetra -acetate ferrate (II) and it was prepared by dissolving 26.2 gm of EDTA in 250ml of 2M KOH. In a one-liter flask 24.9mg $FeSO_4.7H_2O$ was dissolved in 500 ml distilled water then EDTA solution was added and volume was made up

Preparation of Permanent Slides: Thin cross sections of freshly harvested roots, stem and hypocotyls from each treatment were cut under the water. Choose the best sections and they were passed through the ascending percentage series of alcohol for dehydration of tissues using safranin and light green stains. They were fixed in Canada balsam for histological studies.

RESULTS AND DISCUSSIONS

Effect of Nematode on Anatomy of Roots: Histologically the roots of *Luffa cylindrica*L. consists of uniseriate epidermis, with a single layer of compactly arranged thin-walled living cells. Epidermal cells produce root hairs, which are useful for absorption of water. The cortex is multiseriate, relatively homogenous and consist of general cortex and endodermis. Endodermis is single layered, with compactly arranged barrel shaped cells. Endodermal cells are characterized by the presence of Casparian strips. Stele forms the central part of the root. The stele consists of pericycle,vascular strands and conjunctive tissue. Pericycle is the outermost part of the stele and is uniseriate and parenchymatous. Vascular strands are radial; xylem is exarch.

Effect of Nematode on Anatomy of Stem: Histologically the stem of *Luffa cylindrical* L. have ridges and grooves.Primary stem consists of epidermis, cortex and stele. The epidermis is the outermost region surrounding the stem. It is uniseriate, with a single layer of compactly arranged barrel shaped living cells. The outer walls of the epidermal cells are cutinized. Cuticle reduces the transpiration. Few stomata are present in the epidermis for exchange of gases and transpiration. All the epidermal cells are colorless except the guard cells. Multicellular hairs are present on the epidermis.Below the ridges from three to four layers of angular collenchymas is present. Collenchyma is living mechanical tissue. The hypodermis gives considerable strength, flexibility and elasticity to young stem and, having chloroplasts, it may carry out photosynthesis. The middle cortex is also multilayered and parenchymatic. The cells may be round or oval with prominent intercellular spaces

Comparison of Anatomical Changes in Root, Hypocotyl and Stem by Root-Knot Nematode and its Alleviation by VAM Fungi: Rootknot nematodes infect the roots of different fruit, vegetable and cereals and induce gall formation at the terminal or sub-terminal portions of the affected roots and other underground plant parts. Second stage juvenile cause giant cell formation, hyperplasia of protophloem and abnormal xylem proliferation. These changes result in swelling at sites in stele and also surrounding cortex forming distant galls. Some histopathological changes brought about by *Meloidogyne*spp. have been reported [4]. The root knot nematodes, *Meloidogyne*species, are probably the major obstacle to crop production in the tropics. In the present study an attempt was made to examine histopathology of infected Mango roots. The first noticeable change is hypertrophy of the cortical cells, which can be very pronounced at 24 hours after juvenile penetration, especially when several J2 penetrate simultaneously. *Meloidogyne*species typically induce extensive pericyclic hyperplasia and cortical hypertrophy, which results in galls. The pericycle and endodermal cells near the path of J2 migration also can exhibit hypertrophy. Rootgrowth usually continues normally, but sometimes it can be retarded or stopped completely. [10][13]. Huang [6] described the developing giant-cell and associated wall modifications in *Viciafaba*and *Cucumissativus*infected with *M. javanica*. At 48 hours after nematode penetration, walls of giant-cells were not distinguishable from those of normal parenchyma cells. At 7 days after penetration, the primary walls started to show roughening of the inner wall surface and, in some areas, invaginations of the plasmalemma towards the protoplast. Rod-shaped structures later appeared in the invaginations of the plasmalemma Scientists studied the ultrastructure of the infection process of alfalfa cultivars (Ranger [susceptible] and Lahontan [tolerant]) to the stem nematode *D. dipsaci*. Electron micrographs of infected tissues showed that both cultivars undergo similar sorts of damage. However the infection rate and severity of damage were different between the two cultivars. Moreover, 24 hours after inoculation, more lipid bodies were present in the susceptible cultivar than in the tolerant one. The author concluded this might indicate that permeability of the membrane is altered, perhaps due to enzymatic reactions.Furthermore, chloroplasts in infected cells of the susceptible cultivar were swollen and the outer chloroplast membrane was ruptured 72 hours after initial inoculation. Other observed changes included swelling of tylacoid units, swollen nuclei and rupture of the nuclear membrane. When compared to controls, the cytoplasm of susceptible plants was denser with more ribosomes and numerous cisternae of smooth and rough endoplasmic reticulum.Arbuscularmycorrhizal fungi effectively reduce root disease by a number of soilborne Pathogen. The co- evolution of plants with arbuscularmycorrhizal fungi has resulted in strategies whereby the expression of defence processes in roots is maintained low during symbiosis development. Arbuscularmycorrhizal fungi do not elicit structural defense barriers but they can activate genes involved in the phenylpropanoid pathway and in PR- protein synthesis. However, defense responses are

Fig. 1: T_1=VAM

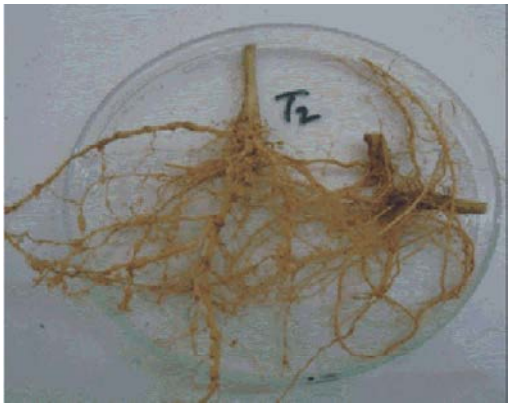

Fig. 2: T_2=NEMATODE

Fig. 3: T_3=VAM+Nematode simultaneouslynematodes)

Fig. 4: T_4=VAM+Nematode (VAM one week before nematodes)

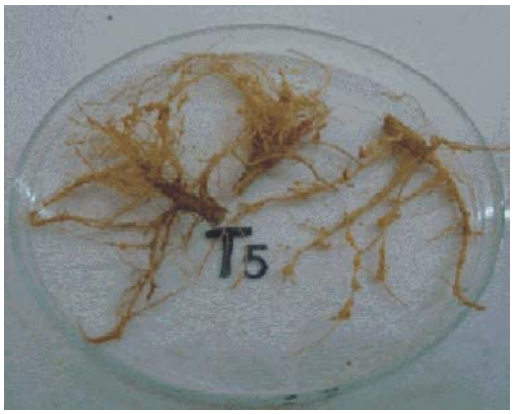

Fig. 5: T_5=Nematode+ VAM (VAM one week after nematodes)

Treatments	InfectionClass	IndexValue	DescriptionOfIndexValue
C=Control.	0	0	Free fromgalls.
T1=VAMonly.	0	0	Free fromgalls.
T2=Nematodes only.	6	75	Heavy,gallsverynumerous,mostlycoalesced,rootgrowthslightlyretarded.
T3=VAM+nematodessimultaneously.	2	5	Veryslight,traceto25galls.
T4=VAM+nematodes,VAMone week beforenematodes.	1	1	Trace, less than 5galls.
T5=Nematodes +VAM, VAMoneweekafternematodes.	5	50	Moderatelyheavy,gallsnumerous,manycoalesced.

triggered in host tissues at a low level, in an uncoordinated way or transiently, suggesting that they are repressed as plant fungal interactions progress. The eliciting factor is not known but it may be linked to active H + -ATPase, suggested to be part of a signalling pathway inducing defense related genes in plant-pathogen interactions at the symbiotic fungal- plant interface [24].

CONCLUSION

These studies show that the endotrophicmycorrhizal fungus *Glomus fasciculatus* causes an increase in *Luffa cylindrica,* L. plant resistance to nematode infection and development. Inoculation of plants with fungus before nematode infestation seemed necessary to allow the fungal symbiont to become established and colonize extensively the cortical cells of *Luffa* transplant and seedling.This could cause changes in root exudates pattern and in cell wall composition which could adversely affect nematode attraction and penetration.

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