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Effect of VA Mycorrhiza on Root Disease (*Pythium aphanidermatum* and *Meloidogyne incognita*) in Tobacco

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ABSTRACT: The interaction studies between five VAM fungi, i.e., *Glomus mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and damping-off root rot pathogen (*Pythium aphanidermatum*) on tobacco (*Nicotiana tabacum* L.) seedbeds revealed that all the VAM fungi colonized the roots efficiently within 30 days of inoculation. However, the per cent colonization was higher in the seedlings inoculated with *G. fasciculatum.* The per cent disease severity in 30 day old seedlings was significantly less in treatments with VAM fungi compared to non-mycorrhizal treatment. The impact of VA-mycorrhizal fungi i.e., *Glomus mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and root-knot nematodes on tobacco plants and the possibility of reducing the harmful effects of root-knot nematodes using VAM fungi, was carried out in pot experiments. The VAM fungus *G. fasciculatum* significantly increased VAM infection of tobacco plants infected or not infected with nematodes, whereas *Meloidogyne incognita* significantly reduced them, Mycorrhizal infection percentage and number or galls were affected by variation in mycorrhizal strain. The results indicated that the use of *G. fasciculatum* as a biocontrol agent is a promising technology for tobacco production.

Key words: *Glomus fasciculatum, Meloidogyne incognita,* root-knot nematodes and tobacco.

Damping-off is the most common and widespread nursery disease of tobacco *(Nicotiana tabaccum* L.) caused by soil-borne, hydrophilic fungus *Pythium aphanidermatum* (Edson) Fitzp. It is responsible for poor seedling stand. Conspicuous symptom of this disease is the sudden collapse of young seedlings in patches leading to uneven stand. Brown watery soft rot of young seedlings, girdling of hypocotyls and finally toppling and death of seedlings, leading to wet rot are the characteristic symptoms. The pathogen spreads quickly and affects the entire seed bed causing enormous loss of seedlings. The use of fungicides at present is unavoidable. However, chemical control of the disease is expensive and disturbs soil ecology (Alagarasamy & Jeyarajan, 1989). VAM fungi are known to impart resistance against soil borne plant pathogens, especially causing root rots and wilts (Moiler Kaare *et al.,* 2009). Apart from this, the beneficial effects of vesicular-arbuscular mycorrhizal (VAM) fungi on the growth of various crop plants have been explored and documented in literature (Hamel & Strullu, 2006). Hence, the present study was carried out to see the effect of VAM fungi on the severity of damping off disease in tobacco seed beds. Root- knot disease is one of the major problems effecting crop production throughout India. The disease is caused by root-knot nematodes, which belong to the genus *Meloidogyne.* Among the susceptible crops the most important are tobacco, tomato, egg plant, papaya and cotton. The losses due to nematode damage in tobacco, egg plant, cotton and coffee were 50% or more (Borah *et al.,* 2009). Indirect losses associated with root-knot disease are caused by secondary attack of other pathogens, inefficient utilization of fertilizers and water and high cost of chemical treatments. Vesicular-arbuscular mycorrhizal (VAM) fungi are beneficial soil fungi which form a symbiotic association with roots of many plants. Many research papers about the importance of VAM fungi in agriculture have been published (Shreenivasa *et al.*, 2007).

The subject of biological antagonism in the rhizosphere and root region is of long standing. The VAM fungi and

root-knot nematodes are members of the microbial population of the root region and they can compete with each other for the same site in the rhizosphere. Hence, the beneficial VAM fungi might be expected to reduce or even eliminate the harmful effects imposed by root-knot nematodes. In plants infected with both mycorrhizal fungi and nematodes, mycorrhizal inoculation substantially reduced adult nematode development (Kantharaju *et al.* , 2005). Therefore, the concept of using VAM fungi as a biocontrol agents is a promising perspective of these fungi. Objective of this work is to study the interaction of VAM fungi and root pathogens on tobacco.Since the data available on the potential of mycorrhiza in controlling soil borne diseases of tobacco is meagre, the present investigation to study the effects of VA mycorrhiza on host resistance is an attempt in this direction.

MATERIALS AND METHODS

Under the present interaction studies between VAM and *P. aphanidermatum* (damping -off causing pathogen), four VAM fungi namely *G. mosseae, G. fasciculatum, G. constrictum, Acaulospora laevis* and damping-off root rot pathogen *(P. aphanidermatum)* were used. The inoculum of these mycorrhizal endophytes was multiplied on *Zea mays* (maize) in pot culture under sterile conditions.

The experiment was conducted during 2007-8 at Central Tobacco Research Institute nursery site. The soil was analysed before starting the experiment for its chemical characteristics such as pH-7.6, electrical conductivity-1.76 , available N-o.25, available P-0.23 and available K-118.

Tobacco seeds were sown on m2 seed- beds along with the VA mycorrhizal inoculum. Around 50g soil including root bits containing 10-20 viable arbuscular mycorrhizal fungal propagules / g soil were used as inoculum and spread as a thin layer one cm below soil surface on each seed- bed. Three replications of each treatment were grown for a period of 60 days with every day watering up to field capacity.

All the treatments were inoculated with propagules (mycelium) of P. *aphanidermatum* raised on wheat grain medium @ 50g/m^2 nursery bed. The observations on disease severity, mycorrhizal root colonization were recorded after 30 days of pathogen inoculation.Four weeks old mycorrhizal tobacco seedlings were transplanted at the rate of one seedling per pot Mycorrhizal tobacco plants received *Meloidogyne incognita* inoculum at the rate of 2000 eggs/plant. The treatments were Nematode, Mycorrhiza, Nematode + Mycorrhiza and control.Each treatment was replicated three times. Clay pots (30 cm in diam.) were filled with 50 kg silty soil and sterilized in oven at 140°C for 2 h. Each plant received about 500 ml of tap water every two days. Pots were arranged in a randomized complete block design. Roots were thoroughly washed from surrounding soil and the following parameters were determined. The infection percentage was determined by root technique (Giovanetti & Mosse, 1980), (c) for assaying nematode infection the number of galls per plant were counted. Also, gall rating index (R.I.) from 0-5, where 0 = 0, 1= 1-10; 2 = 11-30, 3 = 31-100, 4 = 100-200 and 5 = > 200 galls per plant was determined .

RESULTS AND DISCUSSION

The perusal of data presented in Table 1 indicates that all the VA- mycorrhizal fungi colonized the root tissue efficiently in the rhizosphere soil of tobacco seedlings, and significantly reduced the severity of damping-off disease casued by P. *aphanidermatum.* However, percentage of disease severity and VAM colonization varied with VAM species. Amongst the various mycorrhizal endophytes tested, G. *fasciculatum* showed highest root colonization (69%) followed by *G. mosseae* and *Acaulospora laevis. G. constrictum* showed minimum colonization Higher colonization with, *G. fasciculatum* may be attributed to the adaptation of VAM fungi in native soil.

VAM isolate (*G. fasciculatum)* showed highest reduction in the damping- off severity compared to other VAM fungi. Tobacco seedlings inoculated with this VAM endophyte registered less disease severity compared to 100% in non- mycorrhizal seedlings (control).G. *fasciculatum* inoculated seedlings had healthy feeder roots except some rotting of the main root, where as in control plants, complete rotting of the feeder

Table 1: Mycorrhizal Root Infection and number of *Pythium* infected seedlings before and after inoculation of *Pythium* in the soil treated with 4 mycorrhizal cultures.

Treatment	Mycorrhizal root infection%	Damped off seedlings	
		B.I.P	A.I.P
G. fasciculatum	69	1.60 (1.5)	6.22 (37.6)
G. constrictum	36	3.19 (9.1)	9.52 (89.6)
G. mosseae	54	2.91 (7.4)	13.19 (172.9)
Aculospora	47	4.19 (16.5)	9.73 (93.6)
Control	0	7.96 (62.3)	15.79 (248.3)
CD (P=0.05)		0.45	0.34

Figures in parentheses are retransformed means

roots and coverage of the main root with the mycelium of the pathogen was observed. The VAM fungus *G. mosseae* was the next best in disease reduction followed by *Acaulospora laevis* and *G. constrictum.* Similar observation on the reduction of root infection due to VAM inoculation in chilli seedlings has been reported by Alejo-lturvide (2008).

The *G. fasciculatum* successfully infected tobacco roots forming typical VAM structures. Amongst the various mycorrhizal endophytes tested, *G. fasciculatum* showed highest root colonization (63%) followed by *G. mosseae*(50%) and *Acaulospora laevis* (49%). *G. constrictum* (38%)showed minimum colonization.(Table-2) Higher colonization with, *G. fasciculatum* may be attributed to the adaptation of VAM fungi in native soil.

Root diseases caused by nematodes are influenced by the mycorrhiza, less nematode galls were found in most of the mycorrrhizal plants tested (Table-2). The penetration of the larvae into the roots was not inhibited but their development within the mycorrhizal roots was restricted (Subhashini & Ramakrishnan, 2011). *M. incognita* formed conspicuous galls on roots of tobacco which were significantly greater in nonmycorrhizal than in mycorrhizal plants.(Table-3) The reduction in galls number caused by mycorrhizal inoculation was also observed by Jalaluddin *et al.,* (2008). The tolerance of mycorrhizal plants to nematode infection reported here agreed with that documented in many crops such as tomato (Shreenivasa *et al.,* 2007) and green gram (Sorah

Table 2: Root-Knot index of tobacco plants as influenced by inoculation with 4 different strains of mycorrhiza

Treatment	Mycorrhizal root infection (%)	Root-knot index
G. fasciculatum	63	3.75
G. constrictum	38	4.87
G. mosseae	50	2.50
Aculospora	49	3.75
Control	0	5.00
CD (P=0.05)		0.52

Table 3: Root-knot index of tobacco plants as influenced by mycorrhizal inoculation and root-knot nematode (Pot culture)

Treatment	Mycorrhizal root infection (%)	Root-knot index
Nematode	0	5.00
Mycorrhiza	60	2.12
Nematode + Mycorrhiza	49	2.37
Control	0	4.12
CD (P=0.05)		0.37

et al., 2009). The tolerance of mycorrhizal plants to nematode damage was attributed to changes in root physiology which in turn reduced nematode penetration and/or retarded adult nematode development (Grandison

& Cooper, 1986). These physiological changes were described by Heald *et al.,* (1989) as increased concentration of lignin, sugar, amino acids, phenol synthesis, and ethylene production.

To conclude with the present investigation clearly indicates the beneficial role of VA mycorrhiza in improving the resistance of host plant both interms of damping off, root-knot nematode and substantiates the view of Jalaluddin *et al.,* (2008) that these biological systems could be profitably exploited in the control of soil borne diseases.

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