Journal of Biological Control, 24 (1): 70–74, 2010

Research Article

Interaction between arbuscular mycorrhizal fungi and *Pythium aphanidermatum* in tobacco seedbeds

D. V. SUBHASHINI[*] and K. PADMAJA
Division of Crop Chemistry and Soil Science, Central Tobacco Research Institute, Rajahmundry 533105, Andhra Pradesh, India.
[*]*Corresponding author E-mail*: dv_subhashini@rediffmail.com

ABSTRACT: The interaction studies between five VAM fungi, *i.e.*, *Glomus mosseae, G. fasciculatum, G. constrictum, G. intraradices, Glomus* spp. (local isolate*), Acaulospora laevis* and damping-off root rot pathogen (*Pythium aphanidermatum*) along with fungicide (mancozeb + metalaxyl) check 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 local isolate *Glomus* spp. The per cent disease severity in 24 day old seedlings was significantly less in treatments with VAM fungi compared to non-mycorrhizal treatment. The disease severity in mycorrhizal seedlings ranged from 31.33 to 63.33, and it was 26.6 per cent in fungicide treatment and 100 per cent in non-mycorrhizal pathogen inoculated control seedlings. The minimum disease severity was observed in the fungicide treatment and in the seedlings inoculated with *Glomus* spp. The increase in plant growth characteristics such as seedling height, leaf area, fresh and dry weights of the seedling was significantly higher in pathogen inoculated mycorrhizal seedlings as compared to non-mycorrhizal pathogen inoculated seedlings. Total leaf cholorophyll content and nutrients N, P, K, Zn, Cu, Fe and Mn were higher in mycorrhizal seedlings compared to the non-mycorrhizal seedlings.

KEY WORDS: *Pythium aphanidermatum,* seed beds, tobacco, VA-mycorrhiza

(Article chronicle – Received: 12.11.2009; Sent for revision: 26.12.2009; Accepted: 29.01.2010)

INTRODUCTION

Damping-off is the most common and widespread nursery disease of tobacco (*Nicotiana tabacum* L.) caused by soil-borne, hydrophilic fungus *P. 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 and Jeyarajan, 1989). VAM fungi are known to impart resistance against soil borne plant pathogens, especially those causing root rots and wilts (Whipps, 2001; Moller 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 (Durgannavar *et al.,* 2004; Hamel and 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.

MATERIALS AND METHODS

Under the present interaction studies between VAM and *P. aphanidermatum* (damping - off causing pathogen), six VAM fungi namely *G. mosseae, G. fasciculatum, G. constrictum, G. intraradices, Glomus* spp. (local tobacco isolate*), Acaulospora laevis* and damping-off root rot pathogen (*P. aphanidermatum*) along with fungicide (mancozeb + metalaxyl) check were used. The inoculum of these mycorrhizal endophytes was multiplied on *Zea mays* (maize) in pot culture under sterile conditions.

Experimental soil

The experiment was conducted during 2007 and 2008 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 (1.77 dSM^{-1}), available %N (0.25), available

%P (0.23) and available %K (118). Tobacco seeds were sown on m^2 seed- beds along with the VA mycorrhizal inoculum. Around 50g soil including root bits containing 10-20 viable arbuscular mycorrhizal fungal propagules g^{-1} 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.

Inoculation by plant pathogen

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, spore population, plant growth characterisitics, chlorophyll content, nitrogen, phosphorus, potassium and micro-nutrients such as Zn, Cu, Fe and Mn contents were recorded after 60 days of pathogen inoculation. The severity of damping-off disease was recorded regularly up to 30 days after sowing, on the basis of per cent root area affected by the pathogen after washing and subjecting the root samples based on infection to 0-5 scale, where 0 = apparently free from infection, 1 = 1-10, 2 = 10-20, 3 = 21-50, 4 = 51-75 and 5 = 76-100 (Bharat and Bhardwaj, 2001).

The presence of mycorrhizae in the tertiary roots was ascertained by differential staining technique of Philips and Hayman (1970) and the per cent mycorrhizal colonization in roots was determined by the slide method (Hayman, 1970). The population of VAM spores in soil was estimated by wet sieving and decanting method (Gerdemann and Nicolson, 1963). The height of the seedlings was measured. The leaf area was measured by using leaf area meter (LICOR - 3100). Fresh and dry weight of the seedlings was determined after drying them in an oven at 65°C for 72 hours.

The chlorophyll content in the leaves was estimated by employing dimethyl sulfoxide method (Hiscox and Israelstam 1979). Nitrogen (N), phosphorus (P), potassium (K) and micronutrients such as Zn, Cu, Fe and Mn concentrations in tobacco seedlings were determined according to the methods developed by Jackson (1973). Seedlings were dried at 70°C and made into fine powder. This was used for analyzing total nitrogen by Microjeldhal method, phosphorus by vanado- molybdo- phosphoric yellow color method and potassium by flame photometry.

$$\text{Diseases severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total no. of seedlings examined} \times \text{Maximum disease grade}} \times 100$$

Copper, iron, manganese and zinc concentrations in the plant samples were determined by employing Atomic Absorption Spectrophotometer (Perkin Elmer).

Statistical analysis

Since there was no seasonal variation 2007 and 2008, pooled analysis is given after analyzing the data statistically to know the levels of significance and at P = 0.05.

RESULTS AND DISCUSSION

Disease severity and mycorrhizal colonization

The perusal of data presented in Table 1 indicates that all the VA-mycorrhizal fungi colonized the root tissue and sporulated 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, VAM colonization and number of spores per 100 g soil varied with VAM species. Amongst the various mycorrhizal endophytes tested, *Glomus* spp. (local VAM isolate of tobacco) showed highest root colonization (68%) and number of spores (345/100g soil) followed by *G. intraradices* and *Acaulospora laevis, G. constrictum* and *G. fasciculatum* while minimum colonization and spore population was registered in *G. mosseae*. Higher colonization with *Glomus* spp. (the local VAM isolate) may be attributed to the adaptation of VAM fungi in native soil.

Table 1. Effect of VAM Fungi on severity of damping-off (*Pythium aphanidermatum*) of tobacco

VAM	Disease severity	(%) VAM root colonization (%)	No. of spores / 100 g soil
G. mosseae	63.33	36.00	145
G. fasciculatum	57.33	42.00	162
G. constrictum	51.66	46.00	165
G. intraradices	38.33	61.00	306
Glomus spp. (local isolate)	31.33	68.67	345
Acaulospora laevis	45.00	52.27	209
Mancozeb + metalaxyl (Ridomil – 0.2%) Check	26.66	0.00	00
Control	100	0.00	00
SEM±CD	0.89	1.85	5.54
(P = 0.05)	2.71	5.61	16.81
CV %	2.99	8.37	5.75

The native soil VAM isolates (*Glomus* spp.) showed highest reduction in the damping-off severity compared to other VAM fungi. Tobacco seedlings inoculated with this VAM endophyte registered 31.33% disease severity compared to 100.00% in non-mycorrhizal seedlings (control and the treatment with fungicide) recorded 26.66%. *Glomus* spp. inoculated seedlings had healthy feeder roots except some rotting of the main root, where as in control plants, complete rotting of the feeder roots and coverage of the main root with the mycelium of the pathogen was observed. The VAM fungus *G. intraradices* was the next best in disease reduction followed by *Acaulospora laevis*, *G. constrictum* and *G. fasciculatum*, while *G. mosseae* proved least effective against *P. aphanidermatum.* Similar observation on the reduction of root infection due to VAM inoculation in chilli seedlings has been reported by Alejo-Iturvide (2008).

Plant growth characteristics

The data in Table 2 reveal significantly higher increase in height, fresh and dry weight of seedlings inoculated with VAM fungi as compared to non-mycorrhizal seedlings (control). Similarly, the leaf area was also more in mycorrhizal seedlings than non-mycorrhizal seedlings. Seedlings inoculated with local *Glomus* spp. in general, had better growth than the seedlings inoculated with other VAM fungi. Several workers have also reported increased growth of seedlings upon VAM inoculation (Binet *et al.,* 2007 and Wang *et al.,* 2007). Germination count in VAM treated seed beds ranged from 81.53 to 54.73. The performance of native isolate was almost equal to that of fungicide treatment 82.53, whereas the germination in control was only 35%. Regarding healthy transplantable seedlings again *Glomus* spp was found to be superior by yielding 1396 seedlings / m^2 bed. *G. mosseae* treatment resulted in 726 seedlings where as fungicide treated bed resulted in 1503 and control 312 seedlings / m^2 bed.

Chlorophyll content and Leaf area

The total chlorophyll content (Table 3) in mycorrhizal seedlings ranged between 1.31 to 0.95 mg per g leaf fresh weight, whereas in non-mycorrhizal seedlings, the chlorophyll content was 0.72 mg/g and 1.42 in case of fungicide treatment. Baqual *et al.* (2005) observed increased chlorophyll content in mulberry upon VAM inoculation and reported that this increase may be due to the increased uptake of nutrients compared to non-mycorrhizal ones. Leaf area in mycorrhizal seedlings ranged between 590 to 387 cm^2 in fungicide treatment, it was 606 in control it was 153 cm^2.

Table 2. Effect of VAM X *P. aphanidermatum* interaction on growth characteristics of tobacco seedlings

VAM	Seedling height (cm)	Germination count (%)	Seedling wt (g)		Healthy transplantable seedlings/m^2 bed
			fresh	dry	
G. mosseae	9.63	54.73	31.66	3.66	726
G. fasciculatum	10.30	63.63	31.00	4.20	983
G. constrictum	10.53	70.26	34.00	4.96	985
G. intraradices	10.90	77.10	42.00	5.78	1218
Glomus spp. (local isolate)	10.93	81.53	45.00	6.72	1396
Acaulospora laevis	10.77	74.96	38.66	5.40	1092
Mancozeb+metalaxyl (Ridomil – 0.2%) Check	11.30	82.53	48.00	6.90	1503
Control	8.80	35.00	15.00	2.20	312
SEM±	0.19	1.48	1.33	0.21	23.62
CD (P = 0.05)	0.57	4.48	4.03	0.64	71.6
CV %	3.18	3.80	6.45	7.30	63.98

Table 3. Effect of VAM x *P. aphanidermatum* interaction on leaf area and chlorophyll content in tobacco leaves

Treatment	Leaf area (cm^2)	Chlorophyll content in leaves (mg/g leaf fresh wt.)
G. mosseae	387	0.95
G. fasciculatum	450	1.15
G. constrictum	489	1.14
G. intraradices	572	1.26
Glomus spp. (local isolate)	590	1.31
Acaulospora laevis	530	1.24
Mancozeb + metalaxyl (Ridomil – 0.2%) Check	606	1.42
Control	153	0.72
SEM±	3.49	0.03
CD (P = 0.05)	10.60	0.11
CV %	1.28	5.26

Jalali *et al.* (1990) also reported increased concentration of nutrients in mycorrhizal plants co-inoculated with root rot fungi compared to non-mycorrhizal plants. The native strain of *Glomus* also resulted in better uptake of micronutrients, 75.73, 273, 1126, 51.4ppm of Zn, Cu, Fe and Mn, respectively compared to the fungicide treatment. Cavagnaro (2008) reported increase in zinc uptake due to VAM inoculation.

From the above observations, it can be concluded that the local VAM isolate from tobacco rhizosphere, *i.e.*, *Glomus* spp. has the potential of being used as one of the biological tools in the management of damping-off disease in tobacco nurseries caused by *P. aphanidermatum.* Artificial inoculation of this VAM endophyte not only lowered the disease severity but also increased the number of healthy transplantable tobacco seedlings even in the presence of damping-off causing pathogen.

Table 4. Effect of VAM x *P. aphanidermatum* interaction on nutrient concentration in tobacco seedlings

Treatment	N (%)	P (%)	K(%)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)
G. mosseae	3.40	0.27	3.04	62.43	197	685	37.43
G. fasciculatum	3.53	0.31	3.16	64.17	222	815	39.96
G. constrictum	3.53	0.35	3.29	68.14	240	868	42.00
G. intraradices	3.56	0.39	3.71	74.35	257	1025	48.90
Glomus spp. (local isolate)	3.86	0.41	4.04	75.73	273	1126	51.40
Acaulospora laevis	3.57	0.37	3.61	67.79	240	929	45.23
Mancozeb + metalaxyl (Ridomil – 0.2%) Check	3.53	0.31	3.24	69.60	251	924	43.73
Control	3.03	0.22	2.64	57.70	163	574	27.06
SEm ±	0.17	0.01	0.05	1.08	4.10	5.09	0.95
CD (P = 0.05)	NS	0.04	0.15	3.29	12.44	15.45	2.88
CV(%)	8.39	7.23	2.50	2.82	3.08	1.02	3.93

Nutrient concentration

The data on N, P, K and micronutrients Zn, Cu, Fe and Mn is presented in Table 4. It indicated significantly higher P and K concentration in the roots of VAM inoculated tobacco seedlings when compared to non-mycorrhizal seedlings, *Glomus* spp. and *G. intraradices* inoculated seedlings had higher P concentration 0.41 and 0.39% and K concentration 4.04 and 3.71%, respectively, compared to seedlings inoculated with other mycorrhizal fungi.

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