Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP2: 892-896

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Eco friendly management of onion basal rot disease using *Trichoderma viride* and AM fungi

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Abstract
Four fungal and two bacterial antagonists were screened for their antifungal activity against onion basal rot pathogen *Fusarium oxysporumf sp.cepae in vitro*. Among these, the fungal antagonist *T.viride* was highly effective in reducing the mycelial growth and conidial germination by 77.5 and 81.5 per cent respectively. The combined application of *T.viride* and AM fungi was more effective in suppressing the basal rot incidence than the application of *T.viride* or AM fungi alone in pot culture under Green house. The results of the four field trials revealed that the coinoculation of *T.viride* (bulb treatment @ 10g/ kg + soil application @2.5kg/ ha) and AM fungi (soil application @12.5kg/ha) was promising in combating the basal rot incidence and increasing the bulb yield of onion.

Keywords: *Trichoderma viride*, AM fungi, onion, basal rot, management

Introduction
Onion (*Allium cepa* var. *aggregatum*) is one of the important vegetable crops of the world. Onion holds a special place in the culinary world because of its versatility and intense flavour. It contains excellent antioxidants with components that work in the same way as vitamin C. The sulfur content of the onion creates an overwhelming aroma. Successful cultivation, production and marketing of onion is amenable to many biotic factors. Among these, basal rot disease caused by *Fusarium oxysporum* Schlechtend. Fr. f. sp. *cepae* (Hans.) Snyder and Hansen is the most destructive disease which cause severe yield loss. The disease occurs in all the onion growing areas of the world (Ozer & Koycii, 2004) [15]. The occurrence of the disease was first reported by Selby (1910) [22] in UAS. Yield loss up to 50 per cent has been recorded in susceptible cultivar (Evert *et al*., 1985) [9] and 90 per cent losses during storage (Davis & Reddy, 1983) and storage losses accounted to 30 per cent (Barocakine & Stoilova, 1986) [3]. The infected plant shows chlorosis and curling. The stem plates become brown in colour with white mycelial growth. The root is typically rot causing the plant to die. Someplants infected in the field may appear healthy and later develop rot during storage (Brayford, 2006).

Onion growers commonly use chemical fungicides for controlling the basal rot disease. But the large scale utilization of fungicides has an intensified worldwide concern about environmental pollution, human health hazards and development of fungicide resistant pathogens. It is very difficult to control fusarial pathogens with fungicide because it is soil borne and perpetuates in the soil for several years by means of chlamydospores. Hence, biocontrol is one of the best alternative to chemical which alleviates all the ill effects of chemical fungicides. A variety of microbes have been demonstrated to be effective against various soli borne pathogens including fusarial wilt pathogens (Singh *et al*., 2006) [24]. Among these, *Trichoderma* spp is recognized as a potential fungal antagonist against fusarial pathogens (Rajendran and Renganathan 1996, Devika *et al*., 2009) [9, 7]. Their mode of action has been multifaceted including parasitism, competition, antibiosis and induced resistance (Benitez *et al*., 2004) [4]. Several studies demonstrated that the application AM fungi can benefit crop protection and production (Gianinazzi-Pearson *et al*., 1996) [11]. It play a role as a bio fertilizer and bio control agent. AM fungi are compatible with bio control agents. Co-inoculation of beneficial organim generally increased plant growth and decresed plant disease than the inoculation of sole beneficial organism (Whipps *et al*., 2004; Dileep Kumar *et al*., 2001) [29, 8]. In the present study *in vitro* screening was done using four fungal and two bacterial antagonists for their efficacy against *F.oxysporum fsp.cepae*. Based on the results, the most promising antagonist *Trichodermaviride* was selected and tested along with AM fungi in pot culture and field against onion basal rot.

Materials and methods

Isolation and maintenance of the onion basal rot pathogen *Fusariumoxysporum* fsp. *cepae*

Basal rot infected onion plants along with bulb were collected from the field and the pathogen *F.oxysporum*fsp*cepae* was isolated by half plate method using Potato Dextrose Agar medium(PDA).The pathogen was purified by hyphal tip method and the pure culture was maintained on PDA slopes for further studies.

In vitro screening of antagonists against *Fusarium oxysporum* fsp. *cepa*

a) Mycelial growth

The efficacy of four antagonistic fungi *viz; T.viride, T.harzianum, T.reesi* and *Gliocladiumvirens* and two bacteria*viz*; *Pseudomonas fluorescens* and *Bacillus subtilis* against mycelial growth of was *F. oxysporum* fsp.*cepae* tested by dual culture technique (Dennis and Webster, 1971) [6]. These antagonistic organisms were obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. A nine mm actively growing culture disc of *F.oxysporum* fsp.*cepae* was placed onto sterilized PDA medium approximately at a distance of 1.5 cm away from the periphery of the Petri plate. Similarly nine mmdisc of respective antagonistis fungi (*T.viride, T.harzianum, T.reesi* and *Gliocladiumvirens*) was placed onto the medium at the opposite side of the plate. In the case of bacterial antagonists*viz., P. fluorescens* and *B. subtilis*, an actively growing 48 h. old culture of the respective bacterium were streaked on to the medium for 1.5 cm length instead of placing the culture disc. The medium inoculated with the pathogen alone in the same position served as the control. Carbendazim (0.1%) was used the chemical check. Three replications were maintained for each treatment. The plates were incubated at room temperature (28±2°C). The radial growth of the *F. oxysporum* fsp.*cepae* was measured 12 days after incubation. The results were expressed as per cent inhibition of mycelial growth pathogen.

b) Conidial germination

The culture filtrates of the above mentioned antagonistic organisms were used for assessing their efficacy activity against the conidial germination of *F. oxysporum* fsp.*cepae*. Fungal antagonists*viz*; *T.viride, T.harzianum, T.reesi*and *Gliocladiumvirens* were grown separately in PDA broth at 28±2°C for 10 days. The mycelial mat was removed by filtering through Whatman No.1 filter paper. The culture filtrates of *P.fluorescens* and *B.subtilis* were obtained by growing these organisms in King's B and nutrient broth respectively at 28±2°C for 48 h. The culture filtrates were centrifuged at 5000 rpm for 20 min at 4°C for clarification. The efficacy of culture filtrates of the antagonists against the conidial germination of *F. oxysporum* fsp.*cepae* tested by cavity slide technique. One drop of the culture filtrate of the antagonistic fungi was placed in a sterile cavity slide and allowed to air dry. One drop of the conidial suspension of the *F. oxysporum* fsp.*cepae* ($5x10^4$conidia/ml) was added and mixed thoroughly. Conidial suspension in sterile distilled water served as control. Then the slides were kept in moist growth chamber and incubated at 20°C. Observation on conidial germination of pathogen was recorded 12 hours after incubation by microscopic examination. Percent germination and per cent germination inhibition were worked out.

Preparation of talc based formulation of *Trichoderma viride*

Based on the *in vitro* efficacy of both fungal and bacterial anatagonitic organisms against *F. oxysporum* fsp. *cepae*, the most promising antagonistic organism *viz*; *T.viride* was selected and tested along with AM fungi in pot culture under green house condition. *T. viride* was used as the talc based formulation and it was prepared by using the method developed by Vidhyasekaran and Muthamilan (1995) [28].

AM fungi inoculum

The commercial inoculum of AM fungi containing mixture of *Glomus mossae* and *G. fasiculatum* was obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. The AM fungi was mass multuiplied in maize plants (*Zea maize).* The inoculum comprised of soil, hyphal fragments, viable AM fungal spores and root bits of maize. The number of viable spores in AM fungal inoculum was determined by Most Probable Number Method (Porter, 1979) [16].

Pot culture experiment

A pot culture experiment was conducted with 10 treatments and three replications in Completely Randomized Block Design under green house to evaluate effect of *T. viride* and AM fungi on basal rot incidence. Five pots were maintained for each replication. Potting mixture was prepared by mixing red soil, sand, Farmyard at the ratio of 3: 1: 1 and it was sterilized for two consecutive days at 15lb pressure for 30 min. The sterilized pot mixture was filled in 30 cm pot (5 kg in each pot) and sand maize inoculum of *F. oxysporum* fsp. *cepae* was prepared and incorporated @ 50g/ kg of soil in pot. Ten days after inoculation, the treatments were imposed as given in Table 2. Onion bulb variety CO 4 was planted in the pot @ 4bulbs/ pot. The observation on basal rot incidence was recorded 30 and 45 days after planting and the per cent incidence of disease was calculated.

Field experiment

Four field trials were conducted during Kharif (two) and Rabi (two) seasonsts evaluate the efficacy of *T.viride* and AM fungi on the incidence of basal rot disease in the field. Four treatments were given as shown in Table 3.The treatment plot consisted of 4x 3 M size plot and each treatment was replicated five times in Randomised Block Design. The onion cultivar Co4 was used for all the four experiments and bulb treatment with *T.viride* @ 10g/ kg was done before planting. The onion bulbs were planted in the ridges @ 264 bulbs/ plot ($22/m^2$) with a spacing of 45 cm between the ridges and 10 cm between the plants. Soil application of *T. viride* and AM fungi was done after planting onion bulbs. Plots which received the above treatment, 3 grams of talc based formulation of *T.viride* (@2.5kg/ ha) and 15 grams of AM fungi (@12.5kg/ ha) was mixed with 50 grams of soil and applied uniformly as a band along the ridges. The observations were recorded on the incidence of bulb rot on 30 and 60 days after planting and at the time of harvest based on the per centage of bulb infected. Onion bulb yield was also recorded. All the data were analysed statistically as per Gomez & Gomez (1984) [12].

Results

In dual culture technique, among the six anatagonists tested the fungal antagonist *T.viride* was significantly superior in inhibiting the mycelia growth of *F. oxysporum* fsp. *cepae*. *T.viride* recorded the lowest mycelial growth of 2.0 cm which accounted for the growth inhibition of 77.5 per cent. *T.harzianum* ranked next by reducing the growth by 69.7 per cent. The bacterial antagonist *B. subtilis* was least effective in inhibiting the growth *F. oxysporum* fsp. *cepae*. The fungicide Carbendazim (0.1%) used for comparisiontoatally inhibited the growth of *F. oxysporum* fsp. *cepae* (Table 1).

T.viride also showed promising results in conidial germination assay. The culture filtrate of *T.viride* inhibited the conidial germination of *F. oxysporum* fsp. *cepae* by 81.5 per cent. The culture filtrate of *T.harziaum* and *P.fluorescens* suppressed the conidial germination by 75 and 70 per cent respectively. The Carbendazim (0.1%) completely arrested the conidial germination of *F. oxysporum* fsp. *cepae* (Table 1).

In pot culture experiment, the combined application of *T.viride* (bulb treatment @ 10g/ kg and soil application @ 10g/pot) and AM fungi (soil application @ 20 g/ pot) was the most effective treatment in suppressing the basal rot incidence. This treatment recorded the disease incidence of 23per cent as against 85 per cent in the control which accounted for the disease reduction of 73 per cent. The disease reduction was comparatively less in sole application of either *T.viride* or AM fungi. The application of AM fungi (soil application @ 20g/pot) alone suppressed the disease only to the extent of 49.9% (Table.2).

The results of the two field trials conducted during Kharif season revealed that co inoculation of *T.viride* (bulb treatment @10g/kg + soil application @2.5 kg/ ha) and AM fungi (as soil application @ 12.5kg/ha) reduced the bulb rot incidence by 66.5 per cent. The combined application of *T.viride* and AM fungi also registered the highest yield of 9.3t/ ha as against 3.4 t/ ha in control. Application of *T.viride* (bulb treatment @10g/kg + soil application @ 2.5 kg/ha) alone suppressed the disease incidence only by 61.6%. The Carbendazim (bulb treatment @2g/kg +soil drenching @0.1%) treatment used for comparision recorded the lowest disease incidence (19.9%) with the highest (71.3%) disease reduction. Similar results were also recorded during Rabi season (Table 3).

Table 1: *In vitro* efficacy of antagonist against mycelial growth and conidial germination of *Fusarium oxysporum* fsp.*cepae*

S. No	Antagonists	Mycelial growth(cm)*	Growth inhibition (%)	Conidial germination*	Germination Inhibition (%)
1.	*Trichoderma viride*	2.0[b]	77.5	17.0(24.4)[b]	81.5
2.	*T.harzianum*	2.7[c]	69.7	23.0(28.6)[c]	75.0
3.	*T.reesi*	4.5[e]	49.4	38.1(38.1)[e]	58.6
4.	*Gliocladiumvirens*	4.7[ef]	47.2	40.0(41.4)[f]	55.0
5.	*Pseudomonas fluorescens*	4.0[d]	55.1	33.5(30.4)[d]	70.0
6.	*Bacillus subtilis*	4.8[f]	46.1	41.2(43.2)[f]	53.0
7.	Carbendazim (0.1%)	0[a]	100.0	0(0.6)[a]	100
8.	Control	8.9[g]	-	92.0(73.9)[g]	-

*****Mean of three replications
Value in parentheses represents are sine transformed values
In a column means followed by same letters are not significantly different at 5 % levelby DMRT

Table 2: Basal rot incidence of onion treated with *T.viride* and AM fungi under green hosuse

Treatment No	Treatments	Disease incidence (%)*	Disease reduction (%)
T_1	*T.viride* (Bt @ 10 g/kg)	44.2(41.7)[e]	48.0
T_2	*T.viride* (SA @ 10 g/Pot)	36.50(37.2) [d]	57.1
T_3	$T_1 + T_2$	34.0(35.7)[c]	60.00
T_4	AM Fungi(SA@ 20 g / pot)	43.4(41.2)[e]	49.9
T_5	$T_3 + T_4$	23.0(28.7)[b]	73.0
T_6	Carbendazim(Bt @ 2 g/kg)	38.0(38.1)[d]	55.3
T_7	Carbendazim (SD @ 0.1 %)	33.4(35.3)[c]	60.7
T_8	$T_6 + T_7$	13.6(21.6)[a]	84.0
T_9	Uninoculated control (Healthy**)**	-	-
T_{10}	Pathogen inoculated control	85.00(67.2)[f]	-

*Mean of three replications. Bt – Bulb treatment, SA – Soil Application, SD-Soil Drenching.
Values in parentheses represents are sine transformed values.
In a column means followed by same letters are not significantly different at 5 % by DMRT

Table 3: Efficacy of *T.viride* and AM fungi against incidence of onion basal rot in the field

Treatment No	Treatments	Kharif*			Rabi*		
		Basalrot incidence (%) **	Disease reduction (%)	Bulb yield (t/ha)**	Basal rot incidence (%) **	Disease reduction (%)	Bulb yield (t/ha)**
T_1	*T.viride* (bulb treatment) @ 10 g/kg + soil application @ 2.5 kg/ha	26.7 (31.16)[c]	61.6	6.0[b]	24.3 (29.4)[c]	59.6	6.3[b]
T_2	T_1 + AM Fungi soil application @ 12.5 kg/ha	23.4 (28.9)[b]	66.5	9.3[a]	21.6 (22.7)[b]	64.1	7.1[a]
T_3	Carbendazim (bulb treatment) @ 2 g/kg + soil drenching (0.1%)	19.9 (26.5)[a]	71.3	8.4[a]	19.2 (25.9)[a]	68.1	7.8[a]
T_4	Control	69.6 (56.6)[d]	-	3.4[d]	60.1 (51.9)[d]	–	4.2[c]

*Mean of two trials (Kharif and Rabi)
** Mean of five replications
Values in parentheses represents are sine transformed values.
In a column means followed by same letters are not significantly different at 5 % level by DMRT

Discussion

The present study revealed that the fungal anatagonist *T.viride* was highly effective in reducing the mycelial growth and conidial germination of *F.oxysporum fsp.cepae in vitro*. The antagonistic potential of *T. viride* against fusarial pathogens was previously demonstrated in onion (Floriand Roberti, 1993), cotton (Mohan, 1999) and chilli (Devika Rani *et al*., 2009) [7]. *T. viride* was also foundto be effective in suppressing the disease in the pot culture and field condition. This observation was in accordance with the findings of Floriand Roberti (1993) in onion and Shashi and Ashish (2010) [23] in pigeonpea. *T.viride* was reported to be the best antagonist against damping off (Srivastava and Tiwari, 2003) [25] and white rot (McLean *et al*., 2005) [14] in onion.

In the present investigation, co inoculation of *T. viride* and AM fungi was more effective than sole inoculation of single microbes. The combination of AM fungi with biocontrol agents increased chitinase activity in plant which indirectly reduced disease incidence. Transient activation of chitinase has been reported in several AM fungi symbiosis (Pozo *et al*., 1996, 1998) [17, 18]. The mixture of taxonomically different biocontrol organisms require different optimum temperature, pH and moisture conditionto colonize the root more aggressively, improve plant growth and efficacy of biocontorl agents (Sayeed Akhtar and Siddiqui, 2008) [21]. The efficacy of AM Fungi *Glomus intraracices*against Fusarial wilt of tomato wasearlier reported by Akkopru *et al*., (2005) [1]. The potential mode of action of AM fungi includes direct pathogen growth alteration, competiton for food or space or improved nutritional status of the plant, modified root morphology of the host, development of environment conducive for the antagonistic microbes in the rhizosphere and induced resistance (Avis *et al*., 2008) [2].

In the present study thecombined inoculation of *T. viride* and AM fungialso registered the highest bulb yield in onion. Torres *et al.*, (1996) [26] observed decresed white rot incidence and increased yield in onion inoculated with *Glomus* spp. *Trichoderma viride* produced plant growth promoting substancs*viz*; cytokinin like molecules (Tsavkelova *et al.*, 2006) [27] which may also provide added advantage for increased yield in addition to suppression of basal rot disease. It is concluded that the combined application of *T. viride* and AM fungi is the best ecofriendly method for managing the basal rot disease and making the onion cultivation more sustainable.

Acknowledgement

Authors are thankful to the Department of Plant Pathology, TNAU, Coimbatore for providing bacterial and fungal antagonists.

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