Management of basal rot of onion caused by *Fusarium oxysporum*f. sp. *cepae* using bio-regulators

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ABSTRACT

Basal rot of onion caused by *Fusarium oxysporum*f. sp. *cepa* is one of the most serious soil borne diseases causing significant yield loss of up to 50 per cent. Efficacy of various biocontrol agents was evaluated for the potential to manage the basal rot of onion *in vitro*. Among the tested isolates of *Trichoderma viride* (Tv5) gave the greatest (82.86%) inhibition and *Pseudomonas fluorescens* (Pf 2) exerted significantly the greatest (80.82%) reduction of mycelial growth of *F. oxysporum*f. sp. *cepae*. Based on the laboratory analysis, effective biocontrol agents were evaluated in glass house conditions. Among the nine treatments tested in the field by RBD, the combination of bacterial and fungal biocontrol agents and *Glomusmosseae* (Tv5 +Pf2+*G.mosseae*) gave significantly the greatest (89.49%) disease reduction. These biocontrol agents were useful as an alternative to chemical control of the onion basal rot and to enhance growth and yield of onion.

Keywords: Onion, basal rot, *Trichoderma viride*, *Pseudomonas fluorescens*, *Glomusmosseae*

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INTRODUCTION

Onion (*Allium cepa*var. *aggregatum*) a bulbous, biennial herb, is one of the most important vegetable crops grown in India (Ilhe *et al*., 2013). India ranks first in area and second in onion production in the world (Kumar *et al.,* 2015). India has about 1.2 million hectare area under onion cultivation constituting about 10% of total acreage under vegetable with an annual production of 19.40 MT in 2015 (Singh *et al.,* 2017). The total area under production of onion in India during 2016-2017 was 1306 thousand ha with 22427 thousand MT production. However, in Tamil Nadu the total area was 239.286 thousand ha with a total production of 6559.8 thousand MT/ha (NHB 2016). Basal rot of onion caused by *Fusarium oxysporum* f.sp. *cepae* Schlecht. Emend. Snyder and Hansen (Jagatap and suryawnashi, 2015) is one of the most devastating diseases of onion and causes huge yield losses in all growing areas of the world. It causes severe losses in the productivity both in the field and storage condition (Lager, 2011). Management of this disease through chemicals and the use of resistant varieties are possible to some extent. Bio control is an important component of integrated disease management (IDM) that provides disease control while being relatively harmless to humans, non-polluting and bio-degradable, selective in mode of action, difficult for pathogens to develop resistance, unlikely to harm other beneficial microorganisms and generally improves soil health and sustainability of agriculture and enhances the growth parameters in a number of vegetable crops (Sheo Raj *et al.,* 2004; Jogaiah *et al*., 2013). Among the biocontrol agents the most commonly used in agriculture are the species of *Trichoderma* which are known to stimulate plant growth by increasing water and mineral uptake, notably that of phosphate, as well as suppressing plant diseases (Shoresh, 2010). The use of *Pseudomonas fluorescens* could provide significant levels of disease

suppression and substantially enhance plant growth and yield. In addition to these antagonists, obligate biotrophic endosymbionts and arbuscular mycorrhizae, are known as plant growth promoting fungi through increasing uptake of mineral nutrients, especially phosphorus (Gill *et al*., 2002). It is also reported that inoculation of mycorrhizae enhances the growth parameters of onion (Brown *et al*., 2008).Eco friendly approach will always be better. It ensures maximum suppression of the disease without any adverse effect on the ecosystem. An integrated approach would always ensure greater disease suppression and enhanced crop yield

MATERIALS AND METHODS

Isolation of *F.oxysporum*f.sp.*cepae*

The pathogen was isolated from the infected bulbs of onion by tissue segment method (Rangaswami, 1958). The infected portions of bulb were cut into small pieces using sterilized scalpel and these were surface sterilized with 1.0 per cent sodium hypo chloride for one minute and washed in three changes of sterile distilled water and then placed on Petri dish containing Potato Dextrose Agar (PDA) medium. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of fungus. The hyphal tips of fungi grown from the plates were transferred aseptically to PDA slants for maintenance of the culture. The pathogens were identified based on their cultural and morphological characters**.**

Isolation of native antagonists from rhizosphere soil *Trichoderma* spp.

Rhizosphere soil samples collected from ten different locations were used for the isolation of *Trichoderma* isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These *Trichoderma* cultures were purified by single hyphal tip method and were used for studies. *Trichoderma* spp., thus isolated was subjected to identification based on the key suggested by Domsch *et al*. (1980). On the whole ten isolates of *Trichoderma* spp. were isolated. These isolates were designated as Tv_1-Tv_{10}.

Isolation of native antagonistic bacteria

Antagonistic bacteria were isolated from the rhizosphere soil collected from different onion growing areas of Tamilnadu by serial dilution method on King's B medium, incubating at room temperature for 24 h. Colonies with characteristics of *Pseudomonas* sp., were isolated individually and purified by streaking them on King's B medium. For the identification of *Pseudomonas* sp. isolates, certain biochemical tests were conducted according to Bergey's Manual for Determinative Bacteriology (Breed *et al*., 1989). The identified isolates were designated as Pf_1-Pf_{10}.

Screening of the fungal and bacterial biocontrol agents against *F.oxysporum* f.sp. *cepae in vitro*

The antagonistic activity of biocontrol agents against *F.oxysporum*f.sp. *cepae* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium a 9 mm mycelial disc obtained from five day old culture of *Trichoderma* spp. was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a 9 mm culture disc of *F.oxysporum*f.sp.*cepae* was placed and incubated. Control was maintained by inoculating *F.oxysporum*f.sp.*cepae* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for three days. In case of *P. fluorescens* one cm long streak was gently made onto the medium using two- day-old culture. The radial growth (in mm) of the pathogen and the test antagonists and the extent of the inhibition zones (in mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927)

Per cent inhibition (I) = C-T/C × 100

Where, C- mycelial growth of pathogen in control

T- Mycelial growth of pathogen in dual plate

I- inhibition per cent

acid and tetramethyl hexadecenol constituted among total identified volatiles.

Efficacy of biocontrol agents against basal rot of onion in pot culture

To study the biocontrol potential of *T. viride*, *P.fluorescens*and *G. mosseae*pot culture experiment was conducted in a glass house.The sand maize culture of the pathogen *F. oxysporum*f.sp.*cepae* were added into 15×30cm diameter earthern pots @ 1.5% W/W of soil. The pots were planted with five bulbs per pot. The talc based formulation of the antagonistics (*Tv5* @ 5g/kg of soil + *Pf2* @ 5g/kg of soil+ *Glomusmosseae* @ 50g/pot) were delivered as basal and soil application at 30and 45 days after sowing. The pathogen alone inoculated pots served as control and carbendazim (0.1%) was used for comparison. The observations on the per cent disease incidence of basal rot were recorded up to harvest. In addition growth parameters like shoot length, root length and number of bulblets were recorded. Each treatment was replicated thrice.

Statistical analysis

The data collected were subjected to statistical analysis using computer aided IRRISTAT software developed by the International Rice Research Institute, Philippines.

RESULTS AND DISCUSSION

Antifungal activity of *Trichoderma* sp. against mycelial growth of *F.oxysporum*f.sp.*cepae* in *in vitro* (Dual culture technique)

In general all the native *Trichoderma* spp. tested significantly inhibited the mycelial growth of *F.oxysporum*f.sp.*cepae* (Table 1). However, among the isolates, the isolate Tv_5, showed maximum inhibition and significantly inhibited the growth of *F.oxysporum*f.sp.*cepae* (15.42 mm), which was 82.86 per cent reduction on the growth of the pathogen when compared to control. This was followed by the isolates Tv_3 and Tv_9 in the decreasing order of merit, which inhibited the growth of *F.oxysporum*f.sp.*cepae* by 81.98 and 79.86 per cent respectively over control. The least growth inhibition of the pathogen was exhibited by the isolate Tv_7.

Table 1. Antifungal activity of *Trichodermaviride* against mycelial growth of *F. oxysporum*f. sp. *cepae*in *in vitro* (Dual culture technique)

Isolates	Mycelial growth of *F. oxysporum* f. sp. *cepae* (mm)	Per cent (%) inhibition over control
Tv_1	35.78^{g}	60.24
Tv_2	47.12^{i}	47.64
Tv_3	16.21^{b}	81.98
Tv_4	38.21^{h}	57.54
Tv_5	15.42^{a}	82.86
Tv_6	23.72^{e}	73.64
Tv_7	50.24^{j}	44.17
Tv_8	28.82^{f}	67.97
Tv_9	18.12^{c}	79.86
Tv_{10}	20.21^{d}	77.54
Control	90.00^{k}	-

*Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

The results of the present study correspond with those of Ilhe (2013), who observed that *T. viride* was found to be most effective in ininhibiting the growth of *F. oxysporum*f. sp. *cepae*. Hacer Handan and Oktay (2015) observed that *T. harzianum* 16 and 23 strains showed significant inhibition of mycelial growth of the pathogenic strains of *F. oxysporum*. Both *T. harzianum* strains produced volatile and non-volatile metabolites that inhibited growth of *F. oxysporum* strains. Narayan Prasad Verma *et al.* (2018) showed variation in the antagonistic activities of *Trichoderma* sp. isolates against the tested *Fusarium*sp and also, inhibition of the pathogen may be attributed to the production of secondary metabolites (such as glioviridin, viridin and gliotoxin) the antagonists (Inbar *et al.,* 1994). Several studies (Jayalakshmi *et al.,* 2009; Muhammad and Amusa, 2003; Shabir *et al.,* 2013) reported that inhibition of some soil borne pathogens, including *Fusarium* spp. by

Trichoderma species could probably be due to the secretion of extracellular cell wall degrading enzymes such as chitinase, β-1, 3-glucanase, β-1, 6-glucanase, protease, cellulase and lectin, which help mycoparasites to colonize their host.

Efficacy of native bacterial isolates against *F.oxysporum*f. sp. *cepae* (Dual culture)

The results presented in Table 2 reveal varying degrees of antagonism by the isolate of *Pseudomonas* against *F. oxysporum*f. sp .*cepae*. Among the *Pseudomonas* isolates, Pf_2 produced significantly the minimum mycelial growth (23.32 mm) accounting for 80.82 per cent reduction on the mycelial growth of *F.oxysporum*f. sp .*cepae*over control.

Table 2. Antifungal activity of *Pseudomonas fluorescens* against mycelia growth of *F.oxysporum*f. sp .*cepae*in *in vitro* (Dual culture technique)

Isolates	Mycelial growth of *F. oxysporum*f.sp. *cepae* (mm)	Percent (%) inhibition over control
Pf_1	35.73[d]	60.10
Pf_2	23.32[a]	80.82
Pf_3	48.55[i]	46.05
Pf_4	29.72[b]	78.22
Pf_5	49.62[j]	44.86
Pf_6	41.81[f]	53.54
Pf_7	31.75[c]	64.72
Pf_8	38.98[e]	56.68
Pf_9	46.12[h]	48.75
Pf_{10}	42.35[g]	52.94
Control	90.00	-

*Mean of three replications*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

This was followed by isolate Pf_4 which recorded 78.22 per cent reduction on the mycelial growth over control. The isolate Pf_{10} was the least effective among *Pseudomonas* isolates as it recorded the minimum per cent inhibition control. Boukerma *et al.* (2017) reported that the potential of *P. fluorescens* PF15 and *P. putida* PP27 showed significant inhibition of the *F.oxysporum*f.sp. *lycopersici* in tomato. Malathi (2015) reported that Pf 12 and Pf 27 of the *Pseudomonas* isolates were found to be the most effective in inhibiting the growth of *F. oxysporum*f. sp. *cepae*. Many strains of *Pseudomonas* have been found to produce broad spectrum antibiotics including phenazine, pyrrolnitrin, pyoverdine and 2,4 diacetyl phloroglucinol (Gardener *et al.,* 2000), lytic enzymes such as chitinases and β-1,3-glucanases which degrade fungal chitin (Velazhahan *et al.,* 1999), siderophore (Loper, 1988), HCN (Ahl *et al.,* 1986) and induced systemic resistance (Van Peer *et al.,* 1991). Several earlier workers have suggested a significant role of secondary metabolites such as antibiotics, siderophores of pseudomonads in suppression of fungal pathogens (Vinodkumar *et al.,* 2007; Sreedevi and Charitha Devi, 2012). Such multiplicity of mechanisms exerted by *P. fluorescens* might be the reason for the reduction in the growth of the pathogen.

Effect of combined application of antagonists and *G. mosseae* on basal rot incidence of onion (Pot trial)

The results obtained on the efficacy of combined application of antagonists and *G. mosseae* is furnished in Table 3. Among the treatments, soil application with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_2) plus soil application of *G. mosseae* treatment (T_7) recorded the minimum wilt incidence (4.82 %). This was followed by the treatment (T_4) with soil application of *T. viride* (Tv_5) + *P. fluorescens* (Pf_5) which recorded results (7.05 %) at par results with carbendazim 0.1 % in reducing the basal rot incidence. Control recorded the maximum disease (45.87%) incidence. The observations made in the present study are in accordance with the findings of Tayal *et al.* (2011) and Sathiya sivananthamoorthy (2017). Shiva Yendyo *et al.* (2018) also showed that the combined application of native isolates of *Trichoderma* spp. and *P. fluorescens* reduced the disease incidence of *Ralstonia* wilt of tomato.

Table 3. Effect of combined application of antagonists and *G. mosseae*on basal rot incidence of onion (Pot trial)

Tr. No.	Treatments	Basal rot incidence (%)			% disease increase over control		
		30 DAS	45 DAS	At harvest	30 DAS	45 DAS	At harvest
T_1	SA of *Trichodermaviride*(5) @ 5g/kg of soil	5.23[f]	8.21[f]	9.59[f]	76.42	72.63	79.15
T_2	SA of *Pseudomonas fluorescens*(2)@ 5g/kg of soil	6.52[g]	9.54[g]	12.81[g]	70.60	68.02	72.07
T_3	SA of *Glomusmosseae* @ 50g/kg of soil	7.95[h]	10.67[h]	15.62[h]	64.15	64.43	65.94
T_4	SA of *Trichodermaviride* (5) @ 5g/kg of soil + SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil	2.45[b]	4.12[b]	7.05[b]	88.95	86.26	84.63
T_5	SA of *Trichodermaviride*(5) @ 5g/kg of soil + SA of *Glomusmosseae* @ 50g/kg of soil	4.81[d]	6.05[d]	7.12[d]	85.93	80.06	84.47
T_6	SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil + SA of *Glomusmosseae* @ 50g/kg of soil	4.52[e]	7.28[e]	8.84[e]	78.31	79.83	80.72
T_7	SA of *Trichodermaviride*(5)@ 5g/kg of soil + SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil+ SA of *Glomusmosseae* @ 50g/kg of soil	0.00	2.45[a]	4.82[a]	100.00	91.83	89.49
T_8	Carbendazim 50% WP@0.1%	3.12[c]	5.82[c]	7.50[c]	94.95	87.26	84.63
T_9	Control	10.00[i]	15.34[i]	45.87[i]	---	---	---

Effect of combined application of antagonists and *G. mosseae* on plant growth promotion of onion (Pot trial)

The efficacy of combined application of antagonists and *G. mosseae* is furnished in Table 4. Among the treatments, soil application with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_{52}) and soil application of *G. mosseae* treatment (T_7) recorded the maximum parameters such as shoot length (35.42 cm), root length (5.0 cm) and bulbs (6.0). This was followed by the treatment (T_4) with combination of *T. viride* (Tv_5) and *P. fluorescens* (Pf_2) which recorded at par results. Control recorded the minimum growth parameters such as shoot length (18.62 cm), root length (2.5 cm) and number of bulbs per plant (1.0). Similar observations were made by Ngullie and Daiho (2013) who recorded reduced incidence of seedling rot in both greenhouse and field condition from the combination of *T. viride* + *P. fluorescens*.Malathi (2015) reported increase in the growth factors like shoot length, root length and number of bulbs with Pf 12 + Pf 27 + TH 3 in the field experiment.

Table 4. Effect of combined application of antagonist and *G. mosseae*on biometrics of onion (Pot trial

Tr. No.	Treatments	Shoot length (cm)	Root length (cm)	Number of bulbs /plant
T_1	SA of *Trichodermaviride*(5) @ 5g/kg of soil	32.52^{e}	4.2^{c}	4.0^{c}
T_2	SA of *Pseudomonas fluorescens*(2)@ 5g/kg of soil	30.18^{f}	4.0^{e}	4.0^{c}
T_3	SA of *Glomusmosseae* @ 50g/kg of soil	29.15^{g}	3.8^{f}	3.0^{d}
T_4	SA of *Trichodermaviride* (5) @ 5g/kg of soil + SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil	34.28^{b}	4.5^{b}	5.0^{b}
T_5	SA of *Trichodermaviride*(5) @ 5g/kg of soil + SA of *Glomusmosseae* @ 50g/kg of soil	33.72^{c}	4.2^{c}	4.0^{c}
T_6	SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil + SA of *Glomusmosseae* @ 50g/kg of soil	32.18^{d}	4.3^{d}	4.0^{c}
T_7	SA of *Trichodermaviride*(5)@ 5g/kg of soil + SA of *Pseudomonas fluorescens*(2) @ 5g/kg of soil+ SA of *Glomusmosseae* @ 50g/kg of soil	35.42^{a}	5.0^{a}	6.0^{a}
T_8	Carbendazim 50% WP@0.1%	32.15^{e}	4.2^{c}	4.0^{c}
T_9	Control	18.62^{h}	2.5^{g}	1.0^{e}

*Mean of three replications *In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT

Enhanced plant growth by the siderophore producing strains of fluorescent *Pseudomonas* was reported (Dutta *et al*., 2005). *P. fluorescens* might have stimulated plant growth by improving uptake of minerals into the host plants particularly phosphate (Kloepper *et al.,* 1980), siderophore mediated iron uptake (Jurkevitch *et al.,* 1988), association with N_2 fixation (Hong *et al*., 1991), production of IAA (Dubeikovsky *et al.,* 1993), promotion of mycorrhizal function and solubilizing nutrients such as phosphorous. *Trichoderma* spp. are known to produce large quantities of fungistatic metabolites such as trichodermin, dermin, trichoviridin, trichobrachin, chitinase, β-1,3 glucanase, protease etc. (Elad *et al*., 1982; Bruchkner *et al*., 1990) which were active against many soil borne pathogens. Thus, the results of the present study and the earlier reports have confirmed that the growth promoting substances produced by *P. fluorescens*, *T. viride* (Tv_3+Pf_5) and *G. mosseae* would have exerted a synergism in promoting the growth parameters of onion.

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