Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5166

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (4):** 1258-1264 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

In vitro Evaluation of Biocontrol Agents Against *Damping Off Disease* Caused by *Pythium debaryanum* on Tomato

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Received: 7.07.2017 | Revised: 15.07.2017 | Accepted: 16.07.2017

ABSTRACT

Damping-off diseases incited by different species of Pythium are a persistent problem worldwide, often resulting in reduced yields and occasionally resulting in major crop damage. There have been increasing restrictions on the use of chemical fungicides, and the development of disease-suppressive biocontrol agents has become a major goal of horticulture crops. An experiment was conducted with twenty four fungal biocontrol agents and twelve bacterial biocontrol agents were screened for their efficacy against phytopathogenic fungi Pythium debaryanum through dual culture technique. The Trichoderma harzianum -7 and Pseudomonas fluorescence -3 were found effective in inhibition of mycelium (80.03, 58.50) against Pythium debaryanum under in vitro conditions.

Key words: *Pythium debaryanum, Trichoderma harzianum, Pseudomonas fluorescence, Biocontrol agents, Tomato.*

INTRODUCTION

Tomato (*Lycopersicon esculentum*. Mill) is one of the important vegetable crop in India and world. Soil borne fungal pathogens such as *Pythium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* infects the tomato crop causing damping off disease and is becoming a potential threat to its cultivation. *Pythium* damping off is a familiar crop disease caused by a genus of organisms called *Pythium*, which are commonly called water moulds. *Pythium* damping off is a very common problem in fields and greenhouses, where the organism kills newly emerged seedlings. This disease complex usually involves other pathogens such as *Phytophthora* and *Rhizoctonia*. Pre and post-emergence damping-off disease caused by *Pythium* spp. in vegetable crops which are economically very important worldwide[17]. Rapid germination of sporangia of *Pythium* in 1.5–2.5 h after exposure to exudates or volatiles from seeds or roots[12] followed by immediate infection makes management of the pathogen very difficult. Many *Pythium* species, along with their close relatives, *Phytophthora* species are plant pathogens of economic importance in agriculture. *Pythium* spp. tends to be very generalistic and unspecific in their host range, which causes extensive and devastating damping off and is often very difficult to prevent or control[9].

Cite this article: Prasad, M.R., Sagar, B.V., Devi, G.U. and Rao, S.R.K., *In vitro* Evaluation of Biocontrol Agents Against *Damping Off Disease* Caused by *Pythium debaryanum* on Tomato, *Int. J. Pure App. Biosci.* **5(4):** 1258-1264 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5166

Although fungicides have shown promising results in controlling the damping-off disease, phytotoxicity and fungicide residues are serious problems leading to environmental pollution and human health hazards. In this context, the great task now facing scientists is to develop, one such alternative, which has been proposed for biological control of several plant pathogens, involves the introduction of selected microorganisms such as *Trichoderma* spp and bacterial biocontrol agents to the soil. However, while laboratory experiments and biological control field trials document the ability of some *Trichoderma and* bacterial biocontrol strains to reduce *Pythium* Inoculum in soil, a clear answer to the process by which these fungal antagonists contribute to biological control of *Pythium* spp. has not yet emerged, although mechanisms of antagonism, including mycoparasitism, antibiosis and competition have been suggested[3]. The present study addresses the "In vitro evaluation of biocontrol agents against damping off disease caused by *Pythium debaryanum* on tomato".

MATERIAL AND METHODS

Isolation and Identification

The test pathogen *Pythium debaryanum* was isolated from the disease tomato samples and collected from the farmer's field. Based on morphological and cultural characters of the pathogen was identified as *Pythium debaryanum*[2]. Pathogenicity was proved by using susceptible cultivar of tomato Arka Vikas following soil inoculation method. The per cent seed germination was poor in inoculated pots compared to control. The affected seedlings were pale green and a brownish water soaked lesion was seen at the basal portion of the stem (collar region) with profuse white mycelial growth on collar region of the seedlings. The pathogen was re-isolated from the diseased seedlings and fungus obtained resembled the original culture in all aspects.

Isolation of fungal and bacterial bio control agents

About 24 isolates of fungal biocontrol agents and 12 bacterial bio control agents were isolated from the rhizosphere samples of tomato collected in Ranga Reddy district. Particularly *Trichoderma* spp, *Pseudomonas* spp and *Bacillus* spp were isolated. Further morphological characteristics of these isolates were studied and identified based on the key characteristics provided by Rifai[11].

Identification of fungal and bacterial bio control agents

For isolation of *Trichoderma* strains, a serial dilution technique was followed. For this purpose one millilitre of each solution was pipetted onto a Rose Bengal Agar (RBA) plate and incubated at 28°C for 1 week. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony was reisolated onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification, and the plates were stored at 4°C. Two techniques, visual observation on petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micro-morphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores, their branching pattern, shape, size, angle to main axis, phialide numbers and conidial shape and colour. Species identification was based on the morphological and taxonomic keys. Rhizosphere soil samples were screened for *Pseudomonas* spp and *Bacillus* spp. using dilution method with King's B Agar as semi selective medium. *Pseudomonas* spp and *Bacillus* isolates were estimated by morphological and physiological characteristics based on Bergeys' Manual of Systematic Bacteriology.

Evaluation of fungal and bacterial bio control agents

The rhizosphere microorganisms isolated from tomato plants were screened for their antagonistic activity against the test pathogen *Rhizoctonia solani* by following dual culture technique[5].

Antagonistic activity of fungal bio-control agents

The test antagonists *Trichoderma* spp. were tested against test pathogen *Pythium debaryanum* and they were grown on the same plate to test the antagonistic activity. About 15 to 20 ml of melted and cooled PDA medium was poured in to Petri plates and allowed to solidify. Fungal disc of the antagonist of was placed at one end of media on Petri plate. A 9 mm test pathogen PDA culture disc was placed at the opposite end. Four replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2^0 C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$R = \frac{CD - TD}{CD} \times 10$$

Where,

R = Per cent growth reduction of test pathogen

CD = Radial growth of test pathogen in check (mm)

TD = Radial growth of test pathogen in treatment (mm)

Evaluation antagonistic activity of bacterial bio-control agents

The antagonistic activity of native bacterial isolates spp. were tested against the test pathogen *Pythium debaryanum* by following dual culture technique. A gentle superficial streak was made at four ends of the Petri plate on PDA medium by means of a sterilized inoculation needle. A nine mm PDA culture disc of the pathogen was placed in middle of Petri plate. Three replications along with suitable control were maintained. The plates were incubated in an inverted position at room temperature (25 ± 2^0 C) till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the percentage inhibition was calculated by adopting following formula.

$$R = \frac{CD - TD}{CD} \times 100$$

Where,

R = Per cent growth reduction of test pathogen

CD = Radial growth of test pathogen in check (mm)

TD = Radial growth of test pathogen in treatment (mm)

RESULTS AND DISCUSSION

Evaluation of native fungal bio-control agents on the growth of *Pythium debaryanum*

The data presented in Table-1, Plate-1 and Figure-1 indicated that all the isolates of *Trichoderma* showed antagonistic effect against *P. debaryanum* and significantly reduced the growth. The per cent inhibition of *P. debaryanum* ranged from 80.03 to 24.47 per cent. Maximum per cent inhibition of *P. debaryanum* was observed by *T. harzianum* - 7(80.03) followed by *T. viride*-6(79.93) while it was minimum in case of *T. viride*-2 (24.47). Out of 24 native isolates of *Trichoderma*, isolate 6 recorded more than 70 % of inhibition of *P. debaryanum.*

Similar observations were reported by Sanjay and Kaushik[13], Anitha and Tripathi[1], Sharma *et al*.[14], El-Abbasi *et al*.[7] and Singh[15], while working with *Pythium* spp. Vinit[16] reported that *T. viride*-1433 was found be highly effective against *Pythium debaryanum.*

Kavitha and Nelson[10] reported that the antagonistic activity of *Trichoderma* spp. isolated from rhizosphere of sunflower. Both *T. viride* and *T. koningii* were isolated and found to be effective against *Fusarium oxysporum* and *P. debaryanum*.

Evaluation of native bacterial bio-control agents on the growth of *Pythium debaryanum*

It is evident from the data presented in Table-2, plate-2 and Figure- 2 indicated that the *P. f-*

3 was found to be significantly superior (58.50) in inhibiting *P. debaryanum* followed by *P. f* -1 (49.56) while a minimum of 9.61 per cent inhibition was recorded by *B. subtilis* -1. The bacterial isolates of *B. subtilis* -1, 2, 3, 4, 5, 6 and *P. f*-4 was on par with each other in inhibiting *P. debaryanum*. It was found that *Pseudomonas fluorescence*-3, was most effective against *P. debaryanum* which inhibited 58.50 per cent growth of mycelium.

Similar findings were obtained by Howell and Stipanovic[8] while working on biological control of *Pythium* and *Rhizoctonia solani* who reported that fluorescent *Pseudomonas* significantly controlled the disease. Elad and Chet[6] while working on damping off disease in tomato, cucumber, melon, beans and cotton plants also noted that competition for nutrients between germinating oospores of *Pythium* spp and bacterial isolates of *Pseudomonas putida* and *P. cepacia* was common.

Table 1: Antagonistic activity of native isolated fungal bio-agents against *Pythium debaryanum*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control
Trichoderma viride-1	22.05	76.5 (-61.042)
Trichoderma viride-2	68	24.47 (-29.62)
Trichoderma viride-3	22.8	75.37 (-60.287)
Trichoderma viride-4	22.2	75.57 (-60.375)
Trichoderma viride-5	22.9	75.9 (-60.647)
Trichoderma viride-6	19.06	79.93 (-63.438)
Trichoderma viride -7	22.6	74.77 (-59.85)
Trichoderma viride-8	62	32.73 (-34.8)
Trichoderma viride -9	23	76.73 (-61.3)
Trichoderma viride-10	26	70.33 (-56.99)
Trichoderma viride -11	26.6	62.1 (-51.987)
Trichoderma viride -12	20.13	77.3 (-61.66)
Trichoderma harzianum -1	19.07	78.6 (-62.47)
Trichoderma harzianum -2	41.2	54 (-47.281)
Trichoderma harzianum -3	42.6	52.53 (-46.439)
Trichoderma harzianum -4	22	75.23 (-60.15)
Trichoderma harzianum -5	53.2	40.7 (-39.616)
Trichoderma harzianum -6	28.6	70.3 (-57)
Trichoderma harzianum -7	18.2	80.03 (-63.43)
Trichoderma harzianum -8	19.2	76.03 (-60.68)
Trichoderma harzianum -9	19.12	77.37 (-61.724)

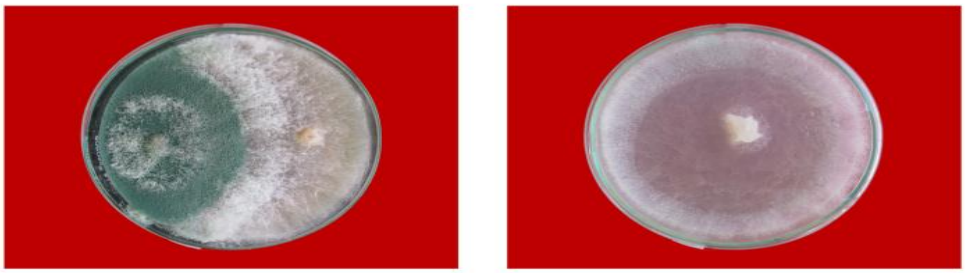
Trichoderma harzianum -10	58.6	34.77 (-36.072)
Trichoderma harzianum -11	58.6	34.77 (-36.072)
Trichoderma harzianum -12	17.2	74.03 (-59.381)
Control	90	
CD at 5% SE(d) SE(m)	4.785 2.372 1.678	

***** Mean of three replications ***** Figures in parentheses are angular transformed values

Table 2: Antagonistic activity of native isolates of bacterial bio-agents against *Pythium debaryanum*

Treatment	*Radial growth in (mm)	*Per cent inhibition over control (%)
Bacillus subtilis -1	81.2	9.61 (-17.88)
Bacillus subtilis -2	78	13.29 (-21.19)
Bacillus subtilis -3	79.2	11.83 (-19.98)
Bacillus subtilis -4	77.2	14.3 (-21.9)
Bacillus subtilis -5	68.2	23.66 (-29.03)
Bacillus subtilis -6	72	19.97 (-26.15)
Pseudomonas fluorescence-1	45.2	49.56 (-44.73)
Pseudomonas fluorescence -2	62.6	30.3 (-33.37)
Pseudomonas fluorescence -3	18.6	58.5 (-49.9)
Pseudomonas fluorescence-4	78.6	13.6 (-21.62)
Pseudomonas fluorescence -5	56	37.73 (-37.85)
Pseudomonas fluorescence -6	64	28.8 (-32.45)
Control	90	-
CD at 5% SE(d) SE(m)	5.66 2.72 1.92	

***** Mean of three replications ***** Figures in parentheses are angular transformed values



a) Antagonistic effect of *Trichoderma* isolate-1 against *Pythium debaryanum*

Plate-1

a) Antagonistic effect of Bacterial isolate-6 against *Pythium debaryanum*

Plate-2

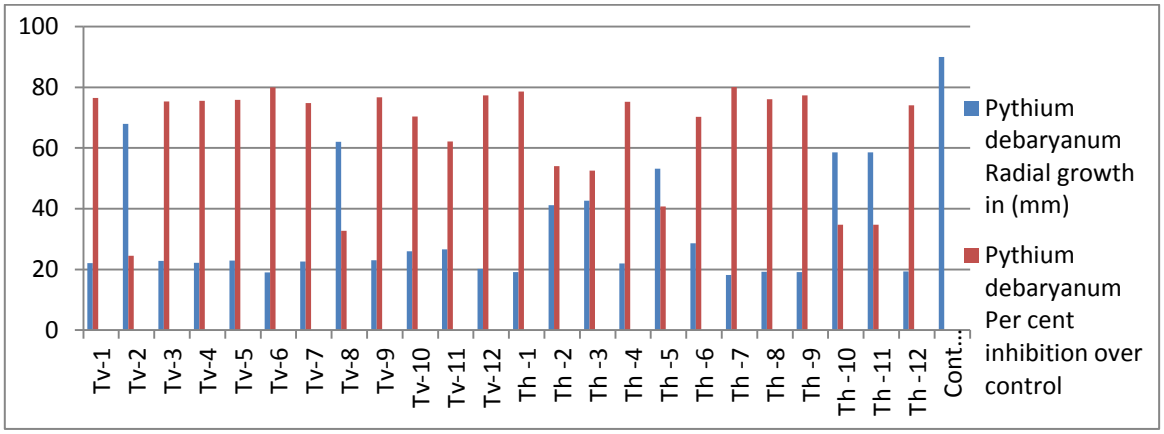


Fig. 1: Antagonistic activity of native *Trichoderma* isolates against *Pythium debaryanum*
(T.v Means -*Trichoderma viride,* Th- *Trichoderma harzianum)*

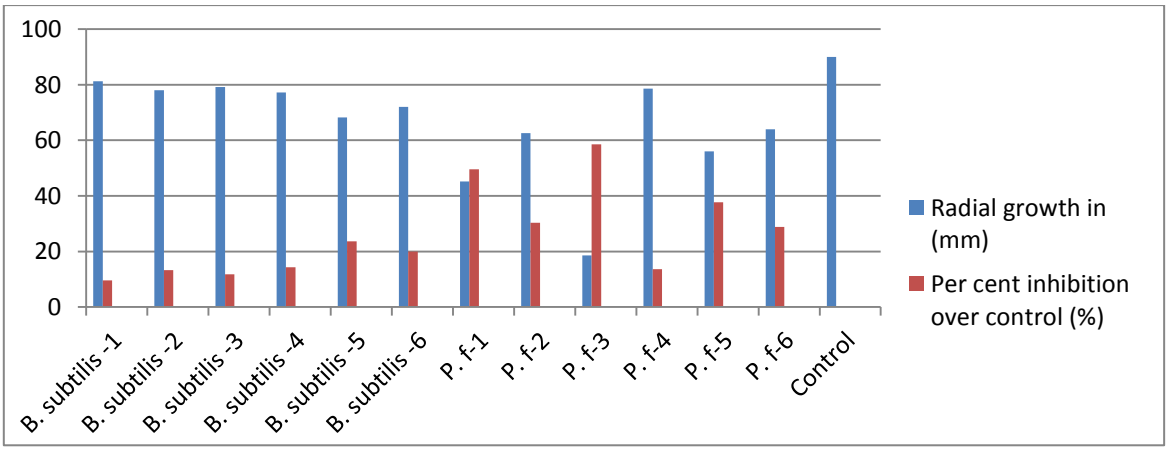


Fig. 2: Effect of native isolates of bacterial bio-agents on radial growth of *Pythium debaryanum*
(B.S- Means *Bacillus subtilis*) (P.f-*Pseudomonas fluorescence)* isolates

CONCLUSION

Among the 24 fungal and 12 bacterial biocontrol agents were tested for their antagonistic activity against *Pythium debaryanum* and the *T. harzianum* -7 (80.03) and *P. f*-3(62.20) per cent recorded maximum inhibition of mycelium against *Pythium debaryanum*.

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