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RESEARCH **P**APER

Biological control of onion basal rot caused by *Fusarium oxysporum* f. sp. *cepae*

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Basal rot disease of onion is caused by *Fusarium oxysporum* Schlechtend: Fr. f. sp. *cepae* (Hans.). Biocontrol agents were isolated from the rhizosphere soil of onion cultivated in different places of Tamil Nadu, India. 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* sp., *T. harzianum* (TH 3) gave the greatest (83%) inhibition and *Pseudomonas* sp. (Pf 12) exerted significantly the greatest (75%) reduction of mycelial growth of *F. oxysporum* f. sp. *cepae.* Based on the laboratory analysis, effective biocontrol agents were evaluated in glass house and field conditions. Among the thirteen treatments tested in the field, the combination of bacterial and fungal biocontrol agents (Pf12 +Pf27+ TH3) gave significantly the greatest (85%) disease reduction. These biocontrol agents were useful as an alternative to chemical control of the onion basal rot and to enhanced growth and yield of onion.

Key words : *Fusarium oxysporum*. f. sp. *cepae*, Onion basal rot, *Pseudomonas* sp., *Trichoderma* sp

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INTRODUCTION

Onion (*Allium cepa* var. *aggregatum* G. Don) is one of the most important vegetable crops. China ranks first in world onion production followed by India, USA, Turkey, Pakistan, Russia, Indonesia, Vietnam, and Myanmar. China represents 28 per cent of the world area and 32 per cent of the production, India ranks second with 17 and 10 per cent of world area and production, respectively. In India, the area and production of onion was 1.04 million ha and 15.75 million t, respectively, in 2011-12. Tamil Nadu produced about 338,900 t of onion from 33,800 ha in 2011-12 (Anonymous, 2012).

Basal rot is the most destructive disease of onion and causes yield loss in all growing areas of the world (Coskuntuna and Ozer, 2008). Yield loss up to 50 per cent has been recorded in susceptible cultivars (Everts *et al.*, 1985) with 90 per cent losses during the seedling stage (Davis and Reddy, 1932). In India, the incidence of basal rot was first reported by Mathur and Shukla (1963). In Tamil Nadu, this disease was first observed by Ramakrishnan and Eswaramoorthy (1982) from Coimbatore district.

Management of the disease through chemicals and the use of resistant varieties is possible to some extent. But the hazardous impact of agrochemicals on the environment, development of resistant mutants, escalating cost of pesticides and frequent breakdown of resistant varieties leads to demand for a sustainable, alternative management approach to the disease. Biocontrol is an important component of integrated disease management (IDM), which 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 to, unlikely to harm other beneficial micro-organisms and generally improving soil

health and sustainability of agriculture (Sheo Raj *et al.*, 2004).

Rhizobacteria such as *Pseudomonas fluorescens* and *Bacillus* strains could provide significant levels of disease suppression and substantially enhance plant growth and yield. Antagonistic bacteria are considered as ideal biological control agents owing to their rapid growth, easy handling and aggressive colonization of the rhizosphere (Gnanamanickam *et al.*, 2002). *Trichoderma* spp. are fungi with the ability to tolerate the antagonistic activities of competing organisms in soil leading to extremely rapid growth and abundant production of spores, appropriate enzymes and antibiotics (Parke *et al.*, 1991). Hydrolytic enzymes produced by *Trichoderma* spp. play an important role in destruction of plant pathogens (Chet *et al.*, 1981).

An ecofriendly approach should ensure the maximum suppression of the disease without any adverse impact on the ecosystem. These requirements were considered in this study undertaken to develop a suitable ecofriendly management system against basal rot onion.

RESEARCH METHODOLOGY

Fungal culture :

The pathogen was isolated from the diseased tissues of onion by the tissue segment method (Rangaswami, 1958). The infected portions of onion bulbs were cut into small pieces using a sterilized scalpel, surface sterilized with 0.1 per cent mercuric chloride for 1 min, washed in three changes of sterile distilled water and then placed on previously poured and solidified potato dextrose agar (PDA) medium in a Petri dish. These plates were incubated at (28 ± 2^0C) for 5 days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture.

Evaluation of biocontrol agents against basal rot of onion:

Screening of the fungal and bacterial biocontrol agents against *Fusarium oxysporum f.sp. cepae in vitro* Fungal and bacterial cultures were isolated from the rhizosphere soil of onion cultivated in different places of Tamil Nadu, India. Thirteen isolates of *Trichoderma* spp. and sixty two isolates of *Pseudomonas* sp. were isolated and screened against *F. oxysporum* f. sp. *cepae* by the dual culture method (Dennis and Webster, 1971). A 9 mm mycelial disc of *F. oxysporum* f. sp. *cepae* was placed on one side of the Petri plate and the *Trichoderma* sp. or *Pseudomonas* sp. were placed on the opposite side near the periphery of the Petri plate which was then incubated at 28 ± 2^0C. After 4 days of incubation, mycelial growth of the pathogen and the inhibition zone were measured in treatment and in control plates, with only the pathogen, and the percentage inhibition (PI) of mycelial growth was calculated.

Pot culture experiment :

A pot culture experiment was conducted to study the biocontrol efficacy of *Trichoderma* sp., *Pseudomonas* sp. and organic amendments. The isolates of *F. oxysporum* f. sp. *cepae* were multiplied on sand-maize medium (Riker and Riker, 1936). The medium containing sand and maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1} and then packed in polypropylene bags. The bags were sterilized at 120°C for 20 min. for two consecutive days and each bag was inoculated with two 9 mm PDA culture discs of actively growing *F. oxysporum* f. sp. *cepae*. Bags were incubated at 28 ± 2^0C for 15 days and used as the inoculum source. Earthen pots (30 cm diam x 60 cm height) were filled with 5 kg of soil pot^{-1}. Organic amendments Mahua cake and Neem cake @ 150 kg ha^{-1}, vesicular arbuscular mycorrhiza (VAM) @ 2 kg ha^{-1} and vermicompost @ 1.25 t ha^{-1} were applied 10 days before sowing. The talc based formulation of the *Pseudomonas* sp. (containing 10^9 cfu ml^{-1}) and *Trichoderma* spp. (10^6 cfu ml^{-1}) were delivered @ 5 g kg^{-1} of soil. In this experiment, carbendazim (0.1%) was used as a chemical control. Five onion bulbs (cv. Co 5) were planted in each pot with 14 treatments and three replications. All pots, except the uninoculated control, were challenge inoculated with *F. oxysporum* f. sp. *cepae* (10 g sand maize medium) after 15 days of sowing. The per cent disease incidence was recorded periodically at 15 day intervals up to harvest.

Field experiment :

A field experiments was conducted to evaluate the efficacy of biocontrol agents against onion basal rot in Rajakkalpatti, Madurai district, Tamil Nadu, India. A Randomized Block Design (RBD) was used with plot size 5 × 4 m and spacing 15 × 10 cm. Mahua cake and Neem cake @ 150 kg ha^{-1}, VAM @ 2 kg ha^{-1} and vermicompost @ 1.25 t ha^{-1} were applied 10 days before sowing. Onion bulbs (cv. Co 5) were planted in each plot

with 13 treatments and three replications. The talc based formulation of the *Pseudomonas* sp. (containing 10^9 cfu ml^{-1}) and *Trichoderma* sp. (10^6 cfu ml^{-1}) were applied @ 2.5 kg ha^{-1}. The percentage disease incidence was recorded periodically at 15 day intervals up to harvest. All normal agronomical practices including irrigation, weeding and fertilizer (NPK 100:50:50) application were followed at regular intervals. Natural incidence of basal rot of onion was recorded and growth parameters, shoot and root length, number of bulblets and yield were recorded.

Statistical analysis :

The data were statistically analysed by using AGRES software package developed by Tamil Nadu Agricultural University, Coimbatore, India.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Dual culture experiment :

The basal rot pathogen *F. oxysporum* f. sp. *cepae* was isolated from the diseased bulbs of onion collected from different places of Tamil Nadu. Nine isolates of *T. viride* and six isolates of *T. harzianum* were tested for their antagonistic activity against *F. oxysporum* f. sp. *cepae* by the dual culture technique. Among the tested isolates, *T. harzianum* (TH3) recorded 83 per cent inhibition on the mycelial growth of pathogen followed by *T. viride* (TV5) which caused 79 per cent inhibition (Fig. 1). Native strains of sixty two *Pseudomonas* sp. isolates were tested for their antagonistic activity against *F. oxysporum* f. sp. *cepae* by dual culture. Pf 12 exerted the greatest (75%) reduction of mycelial growth followed by Pf 27 with 66 per cent reduction.

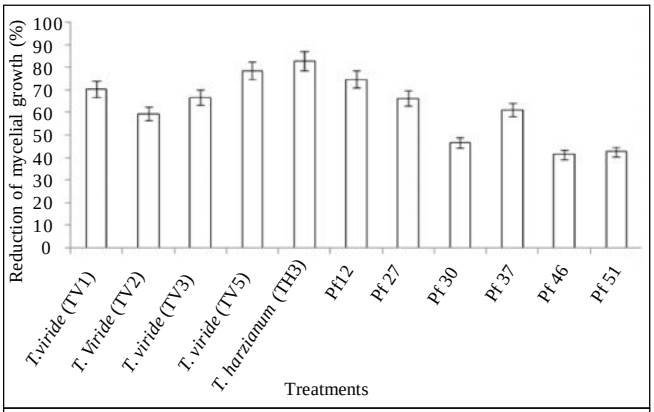

Fig. 1 : Efficacy of biocontrol agents against *Fusarium oxysporum* f. sp. *cepae in vitro*

Pot culture experiment :

A pot culture experiment was conducted to test the efficacy of talc formulations of biocontrol agents (*Pseudomonas* sp., *Trichoderma* sp.) for the control of basal rot of onion plants. Among the fourteen treatments tested, carbendazim (T_{12}) resulted in least disease incidence (5.8%) and 89 per cent disease reduction over control. Here carbendazim was used as chemical control. Among the biocontrol agents tested T_7-treatment (Pf 12 + Pf 27 + TH 3) resulted 7.4 per cent disease incidence (Table 1), which represented an 85 per cent disease reduction followed by T_6, T_5 and T_4 recorded 10.1, 10.4 and 10.33 per cent disease incidence, respectively. The least disease reduction (67%) was observed with vermicompost.

Table 1 : Effect of antagonists, organic amendments, biofertilizer and their combination on basal rot incidence of onion plants in a pot culture experiment

Treatments	Disease incidence (%)	Reduction over control (%)
T_1-TH3	11.5	77
T_2 -Pf12	10.94	79
T_3 -Pf27	11.1	78
T_4 -T_1+T_2	10.33	80
T_5 -T_1+T_3	10.4	80
T_6 -T_2+T_3	10.1	80
T_7 -T_1+T_2+T_3	7.4	85
T_8 -Mahua cake	13.1	74
T_9 -Neem cake	14.2	72
T_{10} -VAM	15.8	69
T_{11} -Vermicompost	16.5	67
T_{12} - Carbendazim	5.8	89
T_{13} -Control (inoculated)	50.7	-
T_{14} - Control (un inoculated)	0.0	-
C.D. (P=0.05)	0.51	

Field experiment :

In this experiment, carbendazim (T_{12}) resulted in least disease incidence (5.5%) and 88 per cent disease reduction over control. Among the biocontrol agents tested T_7 (Pf 12 + Pf 27 + TH 3) significantly recorded the greatest (85%) disease reduction (Table 2) followed by T_6, T_4 and T_5 gave 81, 80 and 80 per cent reduction of

the disease, respectively. The highest yield was recorded in T_7 treatment (9625 kg ha^{-1}) followed by T_6 treatment (8460 kg ha^{-1}). The growth factors, shoot length of 37.5 cm and root length (7.20 cm) with more bulbs (6.00) were highest (T_7) in field experiments (Table 3).

Table 2 : Effect of antagonists, organic amendments, biofertilizer and their combination on basal rot incidence and yield of onion plants in the field

Treatments	Disease incidence (%)	Reduction over control (%)	Yield (kg ha^{-1})
T_1 - TH3	10.5	77	7875
T_2 - Pf12	9.8	79	8110
T_3 - Pf27	10.0	78	7960
T_4 - T_1+T_2	9.2	80	8215
T_5 - T_1+T_3	9.2	80	8170
T_6 - T_2+T_3	9.0	81	8460
T_7 - T_1+T_2+T_3	6.8	85	9625
T_8 - Mahua cake	13.6	70	7620
T_9 - Neem cake	14.0	70	7170
T_{10} - Vermicompost	16.3	65	7100
T_{11} - VAM	15.5	66	7085
T_{12} - Carbendazim	5.5	88	8475
T_{13}-Control	46.1	-	7005
C.D. (P=0.05)	0.41	-	62.3

Table 3 : Effects of biocontrol agents and their combination on growth parameter of onion in the field

Treatments	Shoot length (cm)	Root length (cm)	Number of bulblets per plant
T_1 - TH3	32.2	6.79	4.0
T_2 - Pf12	33.4	6.22	4.3
T_3 - Pf27	33.2	6.42	4.0
T_4 - T_1+T_2	34.3	6.34	5.3
T_5 - T_1+T_3	35.4	6.54	5.0
T_6 - T_2+T_3	36.2	6.65	5.3
T_7 - T_1+T_2+T_3	37.5	7.20	6.0
T_8 - Mahua cake	29.9	5.92	3.7
T_9 - Neem cake	29.7	5.27	3.3
T_{10}-Vermicompost	24.9	5.15	3.0
T_{11} - VAM	27.2	5.20	3.3
T_{12} - Carbendazim	25.2	5.15	3.0
T_{13}-Control	24.4	4.98	2.7
C.D. (P=0.05)	0.38	0.05	0.03

Fungal and bacterial biocontrol agents were tested against *F. oxysporum* f. sp. *cepae in vitro*. Among these, *T. harzianum* (TH3) recorded 83 per cent inhibition on the mycelial growth of pathogen followed by *T. viride* (TV5) and *Pseudomonas* sp. (Pf 12) which showed 79 and 75 per cent inhibition, respectively. *Trichoderma harzianum* and *T. viride* (Pers.) have been reported antagonistic to *F. oxysporum* in onion under *in vitro* and *in vivo* conditions (Rod, 1984). Fungal antagonists *T. viride, T. harzianum, T. hamatum, T. koningii, T. pseudokoningii* were effective against *F. oxysporum* f. sp. *cepae* infecting onion under *in vitro* conditions (Rajendran and Ranganathan, 1996). *T. harzianum* and *T. viride* reduced onion basal rot caused by *F. oxysporum* f. sp. *cepae* to an extent of 89 and 77 per cent, respectively (Flori and Roberti, 1993). Coskuntuna and Ozer (2008), Malathi (2013) and Sudhasha *et al.* (2008) reported that seed treatment with *T. harzianum* gave significant reduction in basal rot incidence on onion under pot and field conditions.

In the present study, 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.* This might be due to the production of antibiotics, volatile compounds and lytic enzymes. Many strains of *Pseudomonas* have been found to produce broad spectrum antibiotics including phenazine, pyrrolnitrin, pyoverdine and 2,4-diacetylphloroglucinol (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).

In the present investigation, carbendazim (T_{12}) resulted least disease incidence (5.5%) and 88 per cent disease reduction over control. Among the biocontrol agents T_7 treatment (Pf 12 + Pf 27 + TH 3) resulted 85 per cent disease reduction in field condition. The growth factors shoot length, root length and number of bulbs was high with this treatment in the field experiment. Treatment (T_7) also gave a yield of 9625 kg ha^{-1} which was significantly higher than all other treatments. There is current interest in gradually replacing synthetic chemicals with biological and other safe approach to disease management. For this, the use of biocontrol agents need to be exploited more and these products made available for use as agro products for the management of plant disease.

In addition to disease control, bioformulations and their mixtures are reported to enhance the plant growth by increasing seedling emergence (Dunne *et al.,* 1998), plant height (Raupach and Kloepper, 1998) and yield (Loganathan, 2002). Many studies have been carried out under field conditions, using Plant Growth Promoting Rhizobacteria (PGPR) strains against various pathogens

(Dagmar *et al.,* 1989; Umesha *et al.,* 1998; Singh *et al.,* 1999). Marimuthu *et al.* (2002) reported that there is synergistic effect of combined application of *Azospirillum* and *P. fluorescens* Pf1 on the reduction of root rot incidence and enhanced plant growth and yield of cotton under field conditions. The yield increase under field conditions might be due to the growth-promoting compounds such as gibberellin, cytokinins or auxin from tryptophan which is produced by biocontrol agents that increased the growth rate and yield of groundnut under field condition (Pal *et al.,* 2000 and Ilhe *et al.,* 2013).

Soil application of *P. fluorescens* increases the rhizosphere population of this organism. Fluorescent pseudomonads, which constitute 20 per cent of the total bacterial population, are expected to give better protection against soil-borne pathogens. They had high affinity for amino acid exudates and probably this might contribute to their high rhizosphere competence (Bakker and Chet, 1982). Association of fluorescent pseudomonads with reduction in survival and activity of *Macrophomina phaseolina* has been reported by Arora *et al.* (1992).

One of the emerging strategies for managing plant diseases is the use of microbial biocontrol agents, with the aim of reducing pesticide usage, providing non-polluted harvested produce and eventually to safeguarding human health and the environment. The present study emphasizes that well-defined goal, giving ample evidence of successful field application of bioformulations in onion to manage basal rot, an economically important disease and enhance the plant growth and yield of onion.

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