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Antagonistic Potential of Native Isolates of *Trichoderma longibrachiatum* and *Pseudomonas fluorescens* against *Alternaria porri* (Ellis) Cif.

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The aim of the study was to access the anti-bacterial and anti-fungal activity on the growth of *Alternaria porri* under *in vitro* condition. The antagonistic activity of bacterial and fungal antagonists (*Pseudomonas* and *Trichoderma spp*.) was determined by the dual culture technique both on King's B and PDA media respectively, Paper disc assay and Agar well mathod. Plates were incubated at 28ºC for 7 days and 2 days. The antagonistic activity of these antagonists was evaluated by mycelial growth and mycelial dry weight of test fungus (*Alternaria porri*). Among the bacterial antagonists (*P. fluorescens, B. subtilis, B. cereus, Serratia marescens* and *P. putida) and fungal antagonists (T. viride, T. harzianum, T. longibrachiatum and T. virens)* tested, *P. fluorescens* and *T. longibrachiatum* were found to be effective to *A. porri* as they recorded the maximum percent inhibition compared to others.

Introduction

Onion (*Allium cepa* L.) a bulbous, biennial herb, rightly called as "Queen of kitchen" is one of the most important vegetable crops grown throughout the world. It belongs to the family Alliaceae. The genus *Allium* contains 300 species.

The primary Centre of origin of onion is Central Asia (Vavilov, 1951) and Near East Asia. Onion considered as poor man's staple spice. It contains several medicinal properties and it cures many chronic diseases. Chemical composition of onion is anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties such as quercetin (Slimestad *et al.*, 2007). India ranks first in area under onion cultivation. In the world it occupies the total area of 11.5 Lakh ha, Production of 187.36 Lakh MT and Productivity of 16.29 t/ha (NHRDF, Nashik, 2015).

In India, area under onion is 1173.36000 ha, production of 187.36 Lakh MT and productivity of 16.13 t/ha (NHRDF, Nashik, 2015). Onion is affected by 66 diseases including 10 bacterial, 38 fungal, six nematode, three viral, one mycoplasma, one parasitic plant and seven miscellaneous diseases and disorders (Schwartz and Mohan, 2010). Of these, purple blotch of onion caused by *Alternaria porri* (Ellis) Cif, is the most destructive foliar disease, prevalent in almost

all onion growing areas of the world. The disease causes a significant reduction in seed and bulb yield, with seed losses up to 100 % (Abo-Elyousr *et al.,* 2014).

Now a days, different chemicals including systemic and contact fungicides have been used for the management of this disease (Rahman *et al.,* 2003). Certain fungicides such as chlorothalonil, zineb, mancozeb and propineb are effective on *Alternaria porri* but are not considered as a safe approach to gain more yield. Accumulation of chemicals in the soil result in soil pollution, nutrient loss, killing of non-target beneficial microbes. Intensive use of fungicides has not only resulted in the accumulation of toxic compounds but is potentially hazardous to human and environment (Reshu and Khan, 2012).

Recent studies include use of antagonistic micro-organisms which has been considered a more natural and environmentally acceptable alternative to the existing chemicals (Eziashi *et al.,* 2007). In recent years, interest in the use of bacteria for biological control of plant pathogenic fungi has increased (Haas and Keel, 2003; Ravicharan *et al.,* 2011), especially the use of plant growth promoting rhizobacteria (Raaijmakers and Weller, 2001; Weller *et al.,* 2002).

Among the various antagonistic bacteria used for the management of plant diseases, PGPR play a vital role and it has been reported to improve plant growth either through direct stimulation of the plant or by suppression of pathogens (Osorio *et al.,* 2012).

The virulent culture of *P.fluorescens* was obtained from the culture collection centre of Department of Agricultural Plant Pathology, Annamalai University were taken for the study. Fungal antagonist *T. longibrachiatum* was isolated from rhizosphere soil.

Materials and Methods

The virulent culture of *P.fluorescens* was obtained from the culture collection centre of Department of Agricultural Plant Pathology, Annamalai University were taken for the study. Fungal antagonist *T. longibrachiatum* was isolated from rhizosphere soil. *Trichoderma spp.* was isolated from rhizosphere soil collected from Sivapuri village. The soil particles loosely adhering to the roots were gently teased out and used for the isolation of *Trichoderma spp.* following serial dilution plate technique with *Trichoderma* selective medium. A soil suspension was prepared from rhizosphere sample by shaking 1 g of soil sample in 10 ml of sterile distilled water and serial dilutions were made. From the 10^{-5} soil dilution 1 ml was transferred into sterile Petri dish under aseptic condition in which 15 ml of sterile *Trichoderma* selective medium was poured, gently rotated for uniform mixing of the soil dilution with the medium and incubated at room temperature (28±2^{o}C) for 5-7 days. The isolate was purified by single spore method. The fungus was identified on the basis of morphological and reproductive characters and the pure culture of *Trichoderma spp.* was maintained on *Trichoderma* selective medium and stored at 4˚C.

Effect of culture filtrates of *P. fluorescens* and *T. longibrachiatum* on the mycelial growth of *A. porri* by poisoned food Technique-Grover, R.K. and Moore. R.D. (1962)

The culture filtrate of *P. fluorescens* and *T. longibrachiatum* were separately incorporated into sterilized PDA medium at 10, 20, 30, 40 and 50 per cent by adding the calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred

to sterile Petri dishes separately @ 15 ml and allowed to solidify. Each plate was inoculated at the centre with seven days old (9 mm) PDA culture disc of *A. porri*. Mancozeb 0.2 per cent conc. was used for comparison. The diameter of the mycelial growth (in mm) of *A. porri* was measured when the mycelial growth fully covered the control plates.

Effect of culture filtrates of *P. fluorescens* and *T. longibrachiatum* on the mycelial growth of *A. porri* by Paper disc assay (Saha *et al.*, 1995) and Agar well method (Gupta *et al.*, 2001 a)

In Poisoned food technique, various concentrations of CMFF 136 fungicidal solution and various concentrations of *T. longibrachiatum* were prepared separately. 20 ml of PDA medium was poured into the sterilized Petri plate and allowed to solidify for 10-15 min. Sterile filter paper discs (9 mm) were dipped separately at known concentration of treatments and placed equidistantly over the medium.

Three replications were maintained with individual concentration of both chemical and culture filtrate of the antagonist. The plates were incubated at 28 ± 2^0 C for 48 h. The inhibition zone of the fungal growth around the treated paper discs was measured and recorded. The plates without any chemical and bio control agent were served as control.

In Agar well method, four wells (5 mm dia) were prepared with the help of a sterile cork borer on potato dextrose agar plates two cm just opposite to each other. The culture filtrate (50µl) of different antagonists was pipetted out into each well. The actively growing mycelial disc of *A. porri* was inoculated at the center of each plate. The plates were incubated and inhibition zone formed was recorded after seven days and per cent inhibition on growth was calculated.

***In vitro* efficacy of biocontrol agents against *A. porri* by Dual culture Technique (Dennis and Webster, 1971)**

Fifteen ml of sterilized PDA medium was poured into sterile Petri dishes under aseptic conditions and allowed to solidify. After solidification, nine mm culture disc of *A.porri* (10 days old) was inoculated at 1.5 cm away from the edge of the Petri dish. Similarly, culture disc of *T. longibrachiatum* was placed at equidistance away from the other edge of the Petri dish. In case of bacterial antagonists one cm long streak was gently made onto the medium using two days old culture just opposite to the pathogenic culture at equidistance. Petri dishes inoculated with pathogen alone served as control. Three replications were maintained for each treatment. The inoculated Petri dishes were incubated in a BOD incubator at 28 ± 2^oC for seven days. The zone of inhibition (in mm) and the M09Omycelial growth of *A.porri* were recorded after the incubation period. The effective antagonists were selected based on the inhibition of pathogen. The per cent inhibition of mycelial growth was calculated according to Vincent (1927) as follows.

Per cent inhibition (I) = (C-T / C) x 100

Where, I = inhibition per cent; C = radial growth of the pathogen in control and T = radial growth in treatment.

Results and Discussion

Efficacy of certain bacterial antagonists against *A. porri* by Dual Culture Technique

The results of the dual culture technique indicated that all the antagonists inhibited the growth of test fungus significantly when compared to control (Table 1). Among the antagonists *P.fluorescens* was found to be more

Table.1 Efficacy of certain bacterial biocontrol agents against *A. porri* by Dual culture Technique

S. No	Antagonistic organisms	Mycelial growth (mm)	Percent inhibition over control (%)
1	*Pseudomonas fluorescens*	19.43	78.42
2	*Bacillus subtilis*	25.39	75.78
3	*Serratia marescens*	22.42	74.08
4	*Bacillus cereus*	27.45	69.50
5	*Pseudomonas putida*	23.86	73.48
6	Control	90.00	-
	SEd	0.55	0.49
	CD = (p=0.05)	1.12	0.97

Table.2 Efficacy of different species of *Trichoderma* against *A. porri* by Dual culture Technique

S. No	Antagonistic organisms *Trichoderma sp*	Mycelial growth (mm)	Percent inhibition over control (%)
1	*Trichoderma viride*	21.72	75.87
2	*Trichoderma harzianum*	23.22	74.20
3	*Trichoderma longibrachiatum*	20.51	77.22
4	*Trichoderma virens*	24.64	72.62
5	Control	90.00	-

Table.3 Evaluation of *P. fluorescens* against *A. porri* under *in vitro* condition by Paper disc assay and Agar well method

Tr. No.	Conc. of the antagonist (%)	Inhibition Zone (mm)	
		Paper disc assay	Agar well method
1	10	6.14	6.87
2	20	10.26	7.25
3	30	13.59	10.63
4	40	14.68	15.16
5	Control	16.64	16.20
	SEd	0.13	0.15
	CD (p=0.05)	0.29	0.32

Table.4 Evaluation of *T. longibrachiatum* against *A. porri* under in vitro condition By Paper disc assay and Agar well method

Tr. No.	Conc. of culture filtrate of antagonist (%)	Inhibition Zone (mm)	
		Paper disc assay	Agar well method
1	10	7.11	7.84
2	20	11.22	8.23
3	30	14.55	11.60
4	40	15.64	16.12
5	Control	16.64	16.20

Antagonistic to *A. porri* as it recorded the maximum percent inhibition (77.22%) which was followed by *T. viride* (75.87%) and *T. harzianum* (74.20%).The minimum growth inhibition was recorded by *T. virens* (72.62%). Certain bacterial antagonists like *P. fluorescens, B. subtilis, Serratia marescens, B. cereus* and *P. putida* were taken for comparing their inhibitory effect on the mycelial growth of *A. porri*. Among these, *P. fluorescens* recorded least mycelial growth compared to other bacterial antagonists. The fungistatic activity of *P. fluorescens* based on the inhibition of mycelial growth and mycelia dry weight of several pathogens was well established (Balabaskar, 2006; Sundaramoorthy *et al.,* 2014). Different species of *Trichoderma* viz., *T. viride, T. harzianum, T. longibrachiatum* and *T. virens* were taken for comparing the effect on mycelial growth of *A. porri.* Among these, *T. longibrachiatum* recorded least mycelial growth compared to other species. These results were in agreement with laboratory experiments carried out against several foliar pathogens. The usage *of Trichoderma* as a biocontrol agent against the phytopathogens has been emphasized by Elad *et al.,* (2002) and Pandey *et al.,* (2011). Various conc. of *P. fluorescens* was evaluated by two methods such as Agar well and Paper disc assay and the results are summarized in table 3. Maximum inhibition zones were recorded at 40% concentrtion. At 40% concentration the inhibition zones were 14.68 % & 15.16% in paper disc assay and agar well method respectively. The minimum inhibition zones were found at 10% conc. At 10% conc. the inhibition zones were 6.14 and 6.87 in paper disc assay and agar well method respectively. Various conc. of *T. longibrachiatum* was evaluated by two methods such as Agar well and Paper disc assay and the results are summarized in table 4. Maximum inhibition zones was recorded at 40% concentration. At 40% concentrtion the inhibition zones were 15.64% & 16.12% in paper disc assay and agar well method respectively. The minimum inhibition zone was found at 10% conc. At 10% conc. the inhibition zones were 7.11 & 7.84 in paper disc assay and agar well method respectively.

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