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Trichoderma harzianum (Th-3) a Potential Strain to Manage the Purple Blotch of Onion (*Allium cepa* L.) Caused by *Alternaria porri* under North Indian Plains

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Abstract

Purple blotch of onion by *Alternaria porri* (Ellis) Neerg. causes heavy yield loss in both bulb and seed crops. Using biocontrol agent to manage the disease is one of the most important approaches for successful disease management and sustainable onion production. For the development of bioagents based strategy seventeen isolates of fungal and six isolates of bacterial bioagents obtained from different sources were tested for their bioefficacy against *A. porri*. Effective isolates (Th-3, Th-30, Tv-12, Tv-15, Pf-3 and Bs-2) were selected and tested for volatile and non-volatile metabolites production and vigour induction under *in vitro*. *Trichoderma harzianum* isolate (Th-3) expressed high level of disease reduction and growth promotion in susceptible onion (cv. Pusa Red) when different methods *viz*., seed treatment, seedling dip and three foliar sprays were evaluated on onion bulb crop under glass house and field conditions. Field experiments (2008-2010) on onion seed crop also confirmed the potential of Th-3 isolate on disease suppression and growth promotion.

Keywords: Onion, *Alternaria porri*, *Trichoderma harzianum*, bioagents

1. Introduction

Onion is a vegetable crop of global importance and is known as protective food because of its special nutritive value. It also owns potent medicinal value in ayurvedic and homeopathic therapy. India ranks second in onion production after China. The area under onion cultivation was 4.78 lakh ha, with a production of 66.7 lakh mt. bulbs and having an average productivity of 10.38 (t/ha). Among the diseases, purple blotch caused *Alternaria porri* (Ellis) Neerg. is one of the destructive diseases, hence it causes frequent epidemic in most of the onion growing region of the country. Initial symptoms appear on leaves and inflorescence as small (2-3 mm in dia.) water soaked lesions that quickly develop purple centre with yellow margin under favorable conditions (Verma & Sharma, 1999). The yield losses of bulb and seed crop in India due to this disease under favorable conditions are 96 % (Gupta & Pathak, 1998) and 97% (Lokra, 1999), respectively. This disease has become a great menace to onion growers in India, which was confirmed by 70% of onion yield losses in Maharashtra by this pathogen during 2010. Lack of resistant variety is one of the reasons for perpetuation of pathogen throughout the year and cause epidemic and farmers are forced to spray very high amount of pesticide on both onion bulb and seed crop. Due to health risk and pollution hazards by use of chemical fungicides in plant disease control, it is considered appropriate to minimize their use. Biological control of plant pathogens through antagonistic microorganisms is eco-friendly and a sustainable approach apart from the best alternative to the use of fungicides. Inhibitory effect of *Trichoderma* species *viz., T. harzianum, T. pseudokoningii* and *T. virens* on mycelial growth and spore germination of *A. porri* by using liquid cultural filtrates was extensively studied by (Imtiaj & Lee, 2008; Tyagi et al., 1990) evaluated species of *Penicillium, Aureobasidium pullulans, Sporobolomyces roseus* and *Crytococcus luteolus* against *Alternaria porri* and found them effective in inhabiting growth of the pathogen. *P. fluorescens* (Pf1), *Bacillus subtilis* and *T. viride* were tested alone and in combination for suppression of onion leaf blight (*Alternaria palandui*) disease under glasshouse and field conditions. In addition to disease suppression, treatment with a

mixture of antagonists promoted plant growth in terms of increased plant height and ultimately bulb yield (Karthikeyan et al., 2006). With this background, in the present investigation efficacy of antagonistic microorganisms in managing purple blotch *in vitro* and *in vivo* has been determined and the results are discussed.

2. Materials and Methods

Seeds and bulbs of onion (cv. Pusa Red) susceptible to *Alternaria* blight were obtained from the Division of Vegetable Science, IARI, New Delhi. The virulent isolate *Alternaria porri* isolated from the infected onion leaf samples from New Delhi region was used as test pathogen. Ten days old culture of *A. porri* was used in each experiment. Isolates of *T. harzianum, T. viride, T. atroviridae, Pseudomonas fluorescens* and *Bacillus subtilis* were obtained from Biocontrol laboratory and *Cladosporium herborum* was obtained from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi (Table 1).

Table 1. List of biocontrol agents used for *in vitro* screening against *A. porri*

S. No.	Bio-agents	Isolates/Strains	Sources
1	*Trichoderma harzianum*	Th-3, Th-8, Th-10, Th-17 &Th-Ag	Soil
		Th-7	Rhizophere of cabbage
		Th-30	Rhizosphere of cauliflower
2	*Trichoderma viride*	Tv-2 ,Tv-4 & Tv-17	Soil
		Tv-12	Phylloplane of ground nut
		Tv-15	Phylloplane of mustard
		Tv-18	Rhizosphere of wheat
		Tv-32	Rhizosphere of ground nut
3	*Trichoderma atroviridae*	Tav-1	Soil
4	*Cladosporium herbarum**	Ch-1016	Palm oil mill effluent
		Ch-3137	Crocus sativus
5	*Pseudomonas fluorescens*	Pf -1 & Pf -2	Soil
		Pf -3	Phylloplane of soybean
		Pf -4	Phylloplane of mustard
6	*Bacillus subtilis*	Bs-1 & Bs-2	Soil

*Isolates collected from Indian type cultural collection, others from Biocontrol lab, IARI, New Delhi.

Assay of biocontrol agents (BCAs) *in vitro* was conducted to select an effective biocontrol agents (BCAs) against *A. porri,* 17 fungal and six bacterial isolates were screened by dual culture method as described by Dennis and Webster (1971) using PDA medium. The PDA medium inoculated with the pathogen alone served as control. The inoculated plates were incubated at 27±1^{o}C with three replications for seven days. Both antagonist and pathogen was measured when the test pathogen attained maximum radial growth in control treatment. Radial growths of *A. porri* isolate were recorded and percent inhibition was calculated using the formula (Vincent, 1927).

$$I = \frac{C}{C - I} \times 100$$

I = Percent growth inhibition, C = Colony diameter of pathogen in control, T = Colony diameter / radial growth of pathogen in treatment.

The onion bulb crop trial was conducted during winters in 2008-09 at Glass house, Division of Plant Pathology, IARI, New Delhi. To evaluate the bioefficacy of BCAs *viz., T. harzianum* (Th-3 and Th-30) *T. viride* (Tv-12 and Tv-15), *P. fluorescens* (Pf-3) and *B. subtilis* (Bs-1) were selected based on dual culture method and mode of action was confirmed to volatile and non-volatile compounds production (Pandey & Upadhyay, 1997). Onion seeds (cv. Pusa Red) were treated @ 2 x10^{10} spore suspension per ml for fungal and 2 x10^{9} cfu for bacterial bioagents for half an hour and shade dried for two hours. Seeds treated with mancozeb75 EC @ 0.25% were used for comparison and sterile water used as a control. The treated seeds were sown in pots. The same treatments were followed for 60 days old seedlings for root dipping. For foliar application the liquid formulation (developed in Biocontrol Laboratory) was sprayed on 45, 55 and 65 days after transplanting and challenge inoculation with spray *A. porri* were done two days after first spray of bioagents. The purple blotch incidence and intensity were recorded at 30 days after first BCAs spray.

The field trials were conducted to determine the effectiveness of the above mentioned BCAs. The onion bulb crop trial was conducted during winters in 2008-09 at Experimental field, Division of Plant Pathology, IARI, New Delhi. The treatments were followed as in glass house trial. Treated seedlings were planted with 20x10 cm spacing in the plots size of 2x3 m^2. The onion seed crop trial was conducted during winters in 2009-10 at seed production unit, Division of Vegetable Science, IARI, New Delhi where this disease is endemic in nature as monoculturing of this crop facilitated continuous survival of *A. porri* in the form of spores and dormant mycelium in plant debris and soil. The onion bulb was treated with BCAs followed by three foliar sprays of BCAs at ten day intervals 130 days after planting. The treated bulbs were in plot size of 1.5x5 m^2 with the spacing of 75x60 cm. The purple blotch intensity and incidence were recorded 30 days after first spray of the BCAs described earlier. The disease severity was measured by using 0-5 rating scale and incidence was expressed in percentage (Borker & Patil, 1993).

The standard statistical method used in *in vitro* studies was Completely Randomized Design (CRD). The field experiments were laid out in Randomized Block Design (RBD) with 8 treatments including controls and replicated 3 times. The plot size was 2 x 6 sq m and 1.5 x 6 sq m onion bulb and seed crop, respectively.

3. Results

3.1 Effects of Antagonist on Radial Growth of the Pathogen

To screen out the effective bioagents against *A. porri*, 17 fungal and six bacterial BCAs were tested by using dual culture technique in *in-vitro*. Almost all BCAs inhibited the mycelial growth of the pathogen significantly over control (Table 2) *T. harzianum* (Th-3) inhibited the growth of pathogen upto 61.5 per cent which was significantly superior to all other isolates, followed by the other *Trichoderma* isolates Tv-12 (56%), Th-30 (53%) and Tv-15 (52%), while *C. herbarum* (Ch-1016) 15% showed the lowest inhibition. Among the bacterial strains *P. fluorescens* (Pf-3) showed highest inhibition growth of 49% followed by *B. subtilis* Bs-1 (48%) on *A. porri*, while Bs-2 (33%) showed the lowest inhibition (Figure 2). The bioefficacy of *T. harzianum* (Th-3 and Th-30), *T. viride* (Tv-12 and Tv-15), *Pseudomonas fluorescence* (Pf-3) and *Bacillus subtilis* (BS-1) was confirmed with volatile and non-volatile compound production. *In vitro* study showed that *Cladosporium herbarum* was not effective against *A. porri* thus, contradicting the report of 66.6% reduction in *A. porri* infection with *cladosporium herbarum* associated with the phyllosphere of the onion plant (Tyagi et al., 1990). The *Trichoderma* species revealed the pathogen on PDA medium may be through diffusible antibiotics production, mycoparasitism, siderophore formation (Park, 1960). Protease and fungal cell wall degrading enzymes make the fungi an attractive biocontrol agent (Dennis & Webster, 1971; Elad, 2000).

Table 2. Mode of action of isolates of fungal antagonist against *A. porri*

S.No.	Name of fungal antagonist	Radial growth of pathogen (mm)	Percent inhibition over control	Mode of action
1	Th-3	15.33	61.54	Inhibition
2	Th-7	31.00	22.25	Mycelial over growth
3	Th-8	29.00	27.27	Mycelial over growth
4	Th-10	32.00	19.74	Inhibition
5	Th-17	28.00	29.77	Mycelial over growth
6	Th-30	18.67	53.20	Inhibition
7	Th-Ag	22.67	43.15	Inhibitiom
8	Tv-2	32.33	18.91	Inhibition
9	Tv-4	24.67	38.15	Inhibition
10	Tv-12	17.67	55.71	Inhibition
11	Tv-15	19.33	51.51	Mycelial over growth
12	Tv-17	23.66	40.66	Inhibition
13	Tv-18	27.00	32.28	Mycelial over growth
14	Tv-32	29.00	27.27	Inhibition
15	Av-1	32.33	18.91	Mycelial over growth
16	Ch-1016	34.00	14.73	Mycelial over growth
17	Ch-3137	27.00	32.27	Mycelial over growth
18	Control	39.87	-	
CD (*P=0.05*)		5.06		

Table 3. Mode of action of isolates of bacterial antagonist against *A. porri*

S.No.	Name of bacterial antagonist	Radial growth of pathogen (mm)	Percent inhibition over control	Mode of action
1	Pf-1	24.67	35.85	Inhibition
2	Pf-2	23.00	40.18	Inhibition
3	Pf-3	19.67	48.86	Inhibition
4	Pf-4	24.00	37.58	Inhibition
5	Bs-1	20.00	47.93	Inhibition
6	Bs-2	25.67	33.45	Inhibition
7	Control	38.45	-------	
	CD (P=0.05)	2.90		

3.2 Glass House Trial

The six BCAs (Th-2, Th-30, Tv-12, Tv-15, Pf-3 and Bs-2) were studied against purple blotch disease in pot culture under glass house conditions. The bulb onion crop result revealed that the Th-3 treatment showed lesser disease severity of 13.3% as against 41.4% disease incidence in control. The next best treatment was Pf-3 (17.8%) followed by Tv-15 (18.2%). Isolate Th-3 also induced the plant growth parameters such as number of leaves and plant height followed by other isolates (Table 4). Onion seed germination and growth of onion seedlings by stimulation of growth may be due to regulators such as IAA (Glick et al., 1998).

Table 4. Effect of bioagents against *A. porri* in onion bulb crop under glass house conditions during 2007-08

S.No.	Treatments	Plant disease incidence		Plant growth promotion	
		Per cent disease index (PDI)	Disease reduction over control (%)	Number of leaves	Plant height (cm)
1	Th-3	13.37 (22.25)	67.66	12.4	54.00
2	Th-30	26.60 (31.00)	35.64	9.4	39.40
3	Tv -12	21.18 (27.38)	48.78	9.4	46.60
4	Tv-15	18.19 (26.69)	56.15	8.8	48.20
5	Pf-3	17.79 (25.68)	57.04	9.6	44.60
6	Bs-1	19.32 (26.06)	53.28	10.0	49.40
7	Mancozeb	11.28 (19.49)	72.72	7.0	42.00
8	Control	41.36 (34.61)	-	6.0	36.60
	CD (0.05)	(3.75)		2.83	6.53

*Value in the parenthesis is arc sin transformed.

3.3 Field Trial on Onion Bulb Crop

Experimental results on onion bulb crop indicated that the liquid based formulation of BCAs (Th-3, Th-30, Tv-12, Tv-15, Pf-3 and Bs-2) significantly reduced the leaf blight incidence. The magnitude of disease reduction varied between 64.8 % (Th-3) and 27.1 % (Th-30) among the bioagents (Table 4). The highest disease reduction was observed in seed treatment, seedling dip and 0.25% spray of mancozeb75 EC after disease incidence. However, among BCAs treatment the highest disease reduction (64.8 %) was noticed in seed treatment, seedling dip and three foliar spray of Th-3 isolate and followed by Tv-15 (52.8). The ancillary characters like number of leaves, plant height bulb diameter were increased and ultimately yield (10.23 tons/acre) was increased over control plot (7.65tons/acre) (Table 6). It indicated that Th-3 has protective as well as growth promoting activity to the onion growth and bulb development. Bioagents such as *Chaetomium globosum*, *T. harzianum*, *T. koningii* and *Fusarium*

sp. increased the number of healthy plants in both radish samples tested, against *A. raphani* and *A. brassicicola* disease (Vannacci & Harman, 1987).

3.4 Field Trial on Onion Seed Crop

To validate the effect of BCAs on onion bulb crop under glass house and field conditions, studies were applied to onion seed crop through bulb treatment and foliar spray of BCAs. Disease incidence in *T. harzianum* (Th-3) treated plots was very less (27. 36 %) compared with all other treatments but lower than Mancozeb 75 EC (0.25%) treated plants (15.2%). The percent plant disease reduction by the bioagent tested was between 78.3 and 52.7 (Table 5). Plants treated with Th-3 showed significant difference among other treatments with respect to number of leaves, plant height, and seed weight per plant at 42.2g over control at 30.6g and seed yield 210.2 kg/acre over control plot at 152.9 kg/acre; even though Th-3 showed low disease reduction (61.0%) potential than mancozeb (78.4%). It indicated that *T. harzianum* (Th-3) isolate not only suppress the pathogen infection and invasion but also induced the growth and yield parameter in onion seed crop.

Table 5. Effect of bioagents against purple blotch of onion bulb crop under field conditions during 2008-09

S.No	Treatments	Plant disease incidence		Plant growth and yield promotion				
		Percent disease index (PDI)	Disease reduction over control (%)	Number of leaves	Plant height (cm)	Diameter of bulb (mm)	Yield/plot (6 sq m) in kg	Bulb yield (tons/acre)
1	Th-3	18.17 (25.19)	64.77	8	59.6	75.25	15.35	10.23
2	Th-30	37.59 (37.81)	27.10	6.4	44.8	57.37	9.37	6.25
3	Tv-12	30.28 (33.38)	41.29	6.8	54.0	68.87	13.12	8.75
4	Tv-15	24.32 (29.51)	52.84	7.6	56.2	71.12	14.47	9.65
5	Pf-3	30.75 (33.68)	40.37	7.2	44.6	63.00	11.62	7.74
6	Bs-1	27.31 (31.50)	47.04	7.4	55.6	68.50	13.59	9.06
7	Mancozeb	11.70 (23.14)	77.31	7.4	44.8	66.00	12.00	8.00
8	Control	51.58 (45.91)	-	6.8	41.2	59.50	11.47	7.65
CD (*P=0.05*)		(4.31)		NS	7.39	3.896	1.7	

*Value in the parenthesis is arc sin transformed.

Table 6. Effect of biocontrol agents against purple blotch of onion seed crop under field conditions during 2009-10

S.No	Treatments	Plant disease incidence		Plant growth and yield promotion			
		Percent disease index (PDI)	Disease reduction over control	Number of flowering stock	Flowering stalks height (cm)	Average seed weight per plant (gms)	Seed yield (kg/acre)
1	Th-3	27.36(30.34)	61.06	12.90	120.83	42.16	210.20
2	Th-30	33.19(35.16)	52.76	8.067	103.50	35.42	177.10
3	Tv-12	30.90(33.43)	56.02	10.60	109.90	38.28	191.40
4	Tv-15	29.30(30.83)	59.30	11.53	115.97	39.76	198.00
5	Pf-3	33.14(33.29)	52.83	10.00	110.90	34.61	172.80
6	Bs-1	28.48(32.20)	58.41	10.30	114.97	38.35	191.75
7	Mancozeb	15.20(25.20)	78.36	10.97	114.23	36.36	181.80
8	Control	70.27(57.01)	-	8.50	97.17	30.59	152.95
CD (*P=0.05*)		(3.77)		0.93	9.13	2.57	

*Value in the parenthesis is arc sin transform

4. Discussion

Effects of biocontrol agents on onion bulb crop were studied under glasshouse (2007-08) and field condition (2008-09) and (2009-10) field trials on onion seed crop found that disease reduction was higher in Th-3 treated plate. This showed that *T. harzianum* (Th-3) was most effective biocontrol strain when compared to others such as Th-30, Tv-12, Tv-15, Pf-3 and Bs-1, which was next to fungicide Mancozeb 75 EC at 0.25%. In addition to the suppression of diseases the antagonistic treatment greatly induces plant growth. The present study on onion bulb

crop and seed crop glasshouse as well as field condition showed that Th-3 increased the growth and yield parameter such as bulb and seed. Our experimental results were more similar to that of Coskutuna and Ozer (2008). Researcher revealed that *T. harzianum* decreased the onion basal rot caused by *Fusarium oxysporum f. cepae* comparable to fungicide in both pot and field experiments. Authors also reported that bulb diameter was increased and found presence of anti-fungal compounds on onion bulb grown from *Trichoderma* treatment by chromatography. The diversity of mechanism available to *Trichoderma* sp for pathogen suppression through broad range of antifungal metabolites production, mycoparasitism, competition with pathogen of nutrient and occupation of infection court, induced resistance (Elad, 2000). Imtiaz and Lee (2008) reported that *T. virens* was most effective against *A.porri* in *in vivo* compare to other fungal BCAs but in our results *T. horzianum* showed potential bio-control activity against *A. porri* when compared to fungal and bacterial BCAs used for present study. Seed treatment with *T. harzianum*-22, *T. harzianum*-50, increased emergence and improved health of the seedlings, and the antagonistic effect of these *T. harzianm* on *A. brassicicola* on seed coats and seedling roots of cabbage was also proved by Wu and Lu (1984). Meena et al. (2004) identified a natural tool, that is isolate of *T.harzianum* could be ecofriendly viable substitutes for chemical fungicide mancozeb in management of *Alternaria* blight of mustard. *T. harzianum* (T-39) living cells applied to the roots and dead cells applied to the leaves of cucumber plants controls the foliar pathogens such as *Botrytis cinerea*, *Pseudoperonospora cubensis*, *Sclerotinia sclerotiorum* and *Sphaerotheca fusca* under commercial greenhouse conditions (Elad, 2000). Many recent studies have been demonstrated the effect of *T. harzimum* on post harvest diseases which cause fruit rot, for example, significant curative and preventive effect was provided by the antagonistic strain Th-1 of *T. harzianum* against *A. alternata* causing black fruit spot on persimmon fruits (Batta, 2004).The efficacy of *T. harzianum* isolate Th-3 has been most extensively studied on horticulture crops such as cauliflower, cabbage, onion, garlic, chilly, rose and gladiolus against a large number of foliar and soil borne fungi such as *Pythium aphanidermatum, Sclerotinia* spp, *Rhizoctonia solani*, *Colletotrichum* spp and *Alternaria* spp. under *in vitro* and field condition different agro-climatic regions (Northern plain, Western and North western) (Sharma & Sain, 2005; Sharma et al., 2005; Sharma et al., 2004; Sharma et al., 2001).

Results of the present study revealed the potential of Th-3 in increasing growth and development of onion bulb and seed crop. It effectively suppresses the disease of purple blotch on onion leaf and flower stalk caused by *A. porri.* Use Th-3 could be promoted as an active component of bio-intensive integrated pest management programme in Chilli (Sharma et al., 2004). This would increase the success rate of these potential candidates for plant growth promotion and biocontrol activity much to the relevance of agriculturally dependent organic studies.

The current study assures the efficiency of *T. harzianum* (Th-3) as a potential biocontrol agent against *A. porri* pathogen and indicates the need of production and development of *T. harzianum* (Th-3) based BCAs to serve as model for environment friendly biocontrol agent. Th-3 effectively managed the pathogen and simultaneously increased the growth of plants and proved as yield increase in both onion bulb and seed crop. These experimental findings could be very useful to North and North Western plain of India where onion bulb and seed crop is more prevalent and also where ever purple blotch disease is endemic in nature and a menace to commercial seed production.

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