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Original Research Article

EFFICACY OF *TRICHODERMA* SPP. AGAINST *FUSARIUM PALLIDOROSEUM* (COOKE) SACC. CAUSING WILT OF CHILLI (*CAPSICUM ANNUM* L.)

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Abstract: In this project, two-function monomer of bisphenol A-glycidyl (BIS-GMA) was synthesized using diglycidyl ether bisphenolA epoxy resin (DGEBA) and methacrylic acid in the presence of a suitable Antagonistic activity of six species of *Trichoderma* viz., *Trichoderma viride* (S), *Trichoderma viride* (M), *Trichoderma viride* (C), *Trichoderma harzianum* (S) *Trichoderma harzianum* (M) and *Trichoderma harzianum* (C) against *Fusarium pallidoroseum* (Cooke) Sacc inflicting wilt of chilli was studied under both *in vitro* and *in vivo* conditions. Under *in vitro* conditions maximum inhibition of *F. pallidoroseum* was recorded by *T. viride*(S) (60.53%) followed by *T. harzianum* (S) (47.37%) while *T. harzianum* (M) (27.80%) proved least effective in inhibiting mycelial growth. In experimental chilli field with history of wilt incidence evaluation of six species of *Trichoderm*a against *F. pallidoroseum* of chilli was conducted by dipping seedlings separately in spore suspension of (10^6 spores ml^{-1}) of test biocontrol agents prior to transplanting. *T. viride* (S) and *T. harzianum* (S) were most promising followed by *T. viride* (C) and *T. viride* (M). All the bio-inoculants delayed mortality until fruit formation stage, increased plant stand and reduced significantly wilt incidence.

Key words: *Trichoderma, Fusarium pallidoroseum* , Fusarial wilt, Chilli, Efficacy, Inhibition

Introduction: Chilli (*Capsicum annum* L.) is mainly cultivated for its vegetable green fruits and for the dry chilli as the spice of commerce. It is a rich source of Vitamin C, A and B. In India it is an important cash crop, which is grown for the both domestic and export market. India is the largest producer of chillies in the world (8.5 lakh tones) followed by China (4 lakh tonnes), Pakistan (3 lakh tonnes) and Mexico (3 lakh tonnes). Andhra Pradesh ranks first in India both in area and production with 2.04 lakh hectares producing 323 thousand tones (Anonymous, 2010). Plants being sessile organism are exploited as a source of food and shelter by wide range of parasites including bacteria, fungi and viruses (Gachomo et al., 2003). Chilli is no exception and a fungal

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pathogen that invades chilli is *Fusarium spp.* and causes *Fusarium* wilt. The disease is known to be caused by *Fusarium pallidoroseum* (Cooke) Sacc. Recently it has become a serious problem in Kashmir (India) and presents a formidable challenge to chilli producers. The ability of a plant to ward off a pathogenic attack depends upon the coordination of different defence strategies. (Nayeema Jabeen et al. 2009).

Plant diseases are caused mainly by fungi, bacteria, viruses and nematodes. Biocontrol of plant disease involves the use of an organism or organisms to reduce disease, pests and diseases adversely affect crop productivity and the stability of production in the tropics. In India, the annual losses amount of Rs. 45,000 crore (Singh and Singh 2004.). The control of soil borne plant disease including wilt [*F. pallidoroseum* (Cooke) Sacc.] of chilli through fungitoxicants is both a health hazard and uneconomical for various reasons (Naik, 2003). Emphasis laid on biological plant disease management owing to environmental concerns, development of fungicidal resistance in pathogens and lack of reliable and economical chemical control without any effects on beneficial soil microflora and it led to an explosion in research efforts to identify strain and commercialize effective biocontrol agents for a number of soil-borne pathogens (Bruchl, 1985). The modern agriculture mostly depends on chemical pesticides, insecticides, and fungicides to control the pathogens and pests. The wide use of these chemicals to control the disease or insects highly pollute the soil, water air etc. and also decrease the soil born micro organisms thereby decrease the fertility of the soil. An alternative to the use of chemical fertilizers, fungicides and pesticides is the biological control the (partial) replacement of chemical control by biological is becoming increasingly interesting to the crop protection industry, especially as regulations on the use of chemical control become ever more stringent. The maximum residue limit (MRL) applied by legislation and buyers in the food industry is also increasingly strict. Last but not least, there is the risk of plant pathogens developing resistance to chemical control products. Biological control is therefore a useful and necessary part of growers' crop protection programmers. Biological control is based upon the natural enemies of harmful organisms, usually bacteria or fungi. These natural enemies are multiplied by manufacturers and sold as ready to- use control products. Growers can use biological control to replace (part of) their chemical control. Species of *Trichoderma* with considerable potential in controlling a number of soil borne pathogens have been identified (Cook, 1993; Mukhopadhyay, 1996). However, such studies have mainly been conducted under controlled conditions. An attempt was made to isolate *Trichoderma* spp. locally and isolates of *Trichoderma* obtained from different sources were screened for the efficacy in the control of wilt of chilli caused by *F.pallidoroseum* (Cooke) Sacc under both *in vitro* and *in vivo* conditions.

Materials and Methods

Six fungal antagonists viz., *T. viride* (S) and *T. harzianum* (S) were isolated from the experimental chilli field with history of wilt incidence of Division of Plant Pathology Shere-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar, Srinagar and cultures of *T. viride* (C) and *T. harzianum* (C), *T. viride* (M) and *T. harzianum* (M) procured from *Central*. The Petri plates in which only pathogen discs were inoculated on PDA served as control*Institute* of Temperate Horticulture (CITH), Srinagar and Division of Sericulture Mirgund, Baramulla, Kashmir were studied by dual culture method' (Denis and Webster, 1971) and "poisoned food technique" on solid medium. 5 mm discs of seven days old cultures of test pathogen (*Fusarium pallidoroseum*) as well as bio control agents were taken with the help of a cork borer and placed on the fresh PDA containing Petri-plate at the opposite corner near the periphery about 60 mm apart in 90 mm petri-plate and incubated at 25±2^{o}C in

BOD incubator. Antagonistic activity was assessed after 7 days of incubation by measuring the radius of the *Fusarium pallidoroseum* colony in the direction of the antagonistic colony and the radius of the *Fusarium pallidoroseum* colony in the control plate.

A field trial using *Trichoderma* spp. was conducted in experimental chilli field having history of wilt incidence in Division of Plant pathology Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir Shalimar Srinagar. A total of 7 treatments (T1 = seed dip treatment with *Trichoderma viride* (S) @ 10^6 spores per ml, T2 = seed dip treatment with *Trichoderma harzianum* (S) @ 10^6 spores per ml, T3 = seed dip treatment with *Trichoderma viride* (C) @ 10^6 spores per ml, T4 = seed dip treatment with *Trichoderma harzianum* (C) @ 10^6 spores per ml ,T5 = seed dip treatment with *Trichoderma viride* (M) @ 10^6 spores per ml, T6 = seed dip treatment with *Trichoderma harzianum* (M) @ 10^6 spores per ml, and T7 = untreated check) were evaluated under field conditions. The experiment was laid out in randomized block design with three replications for each treatment (Table-2). The forty five days old seedlings of chilli were dipped for 2 hours in *Trichoderma* spp. at propagule concentration of 10^6 spores or c.f.u ml^{-1} prior to transplanting in raised beds of 2 x 2 m^2 plot size at 30-25 cm spacing (Anonymous, 2004). Observations on wilt incidence were recorded by counting the plants showing wilting out of the total number of plants examined.

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Number of plants examined}} \times 100$$

Wilt incidence was recorded at flowering, fruit formation and ripening stages. Fruit weight was recorded at each picking and total yield till harvest for each treatment was recorded and expressed in q ha^{-1}. The data was subjected to statistical analysis as per the methods described by Panse and Sukhatame (1985). The software used for analysis was Minitab.

Results and Discussion

All the tested bio control agents significantly inhibited the mycelial growth of the test pathogen when compared to control. Significantly higher mycelial growth inhibition of *F.pallidoroseum* was recorded in case of *T.viride* (S) (60.53%) followed by *T. harzianum* (S) (47.37%) and *T. viride* (C) (35.00%) while *T.harzianum* (M) (27.80%) proved least effective in inhibiting the mycelial growth of *F.pallidoroseum* (Table-1). Jun and Kim (2004) reported that the antifungal activity of *Trichoderma virens* and *Trichoderma harzianum* to *Pythium spp*. was stronger than that of *Trichoderma koningii*. Dharmaputra et al. (1994) tested two isolates of *T. harzianum* and one isolates of *T. viride* against three isolates of *Ganoderma* from oil palms. All three *Trichoderma* isolates inhibited the mycelial growth of the pathogen but *Trichoderma harzianum* (isolate B10-1) showed the best performance among the three isolates. Due to the variable antagonistic potential of individual isolates, the first screening is to select the most active antagonist against that particular pathogen before a species or particular isolate of *Trichoderma* can be considered as a biocontrol agent (Roiger and Jeffers, 1997). Jinantara (1995) reported that the three isolates of *T. harzianum* possessed different ability to attack *sclerotium rolfsii* and this result was in agreement with Henis et al. (1983) who found different isolates of *T. harzianum* could parasitise sclerotia of S. *rolfsii* at varying percentage inhibition. These findings are also in conformity with Muhammad and Amusa (2003) and Shabir and Rubina (2010) who reported that the inhibition of *R. solani* by *Trichoderma* species could probably be due to the secretion of extracellular cell degrading enzymes such as

chitinase B-1, 3- glucanase, cellulose and lectin, which help mycoparasites in the colonization of their host. The inhibition of pathogen may also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridin and gliotoxin.

Under *in vivo* conditions all the biocontrol agents significantly reduced wilt incidence with corresponding increase in growth and fruit yield of plants in comparison to check (Table-2). *T. viride* (S) proved most effective followed by *T. harzianum* (S) in reducing wilt incidence of (2.00, 13.12, 33.56 and 3.75, 24.76,48.25% respectively) at flowering, fruit formation and ripening stage with corresponding increase in fruit yield of (73.25-61.31 q ha^{-1}) as compared to check (42.50 q ha^{-1}). *T. harzianum* (M) proved least effective in reducing wilt incidence (6.90, 30.90 and 55.70%) respectively at flowering, fruit formation and ripening stage with fruit yield of 53.15 qha^{-1}. Maximum shoot and root length was observed with *T. viride* (S) as 96.5 and 16.5 cm respectively. This treatment was followed by *T. harzianum* (S) 93.5 and 14.5 cm as compared to check 80.5 and 9.5 cm. *T. harzianum* (M) proved least effective in improving the plant growth characters. *T. viride* was reported as an important species by Mukhopadhyay (1987) and Dwivedi *et al.* (1993). Mukherjee and Tripathi (2000) also reported sufficient antagonism against *Fusarium* spp. in soil by *T. viride and T. harzianum.* Wilt disease of chilli (*F. pallidoroseum*) was also controlled by seed and seedling dip treatment with *gliocladium* spp. and *Paecilomyces* spp. (10^8 spores ml^{-1}) (Masoodi, 2000).A strain of *Aspergillus niger* (AN27) has been found to show broad spectrum against *Pythium* spp., *Fusarium* spp., *Macrophomina* spp., and *Sclerotinia* spp., (Sen *et al*.,1992).Similarly sett dip treatment of artificially inoculated highly susceptible cane variety (COC671) with *T. harzianum*, *T. viride* and culture filtrate of *Pseudomonas fluorescence* in King's B medium effectively reduced disease incidence (Bhat and Sabalpara, 2001). *Trichoderma* spp. have been reported to produce antibiotic compounds (Trichodermin), extracellular enzymes (chitinase, cellulase) unsaturated monobasic acids (Dermadine), and polypeptides (Alamethicine, Suzukacillin) that either damage plant pathogen or enhance their population in biota. Furthermore, Rini and Sulochana (2006), Shabir et al., (2012) reports that application of *Trichoderma* sp.*;* reduces the pathogen population in soil by means of mycoparasitism and production of antibiotic which may reduce the soil borne pathogens in soil. Similar findings have been reported by Inbar et al. *(*1994), Marnoranjitham et al. *(*2001), Champawat and Sharma (2003), Srivastava (2004) and Shabir et al. (2012), and they suggested several possible mechanisms to explain this phenomenon including control of minor pathogens, production of plant hormones, production of vitamins, conversion of non utilizable materials into a form that can be utilized by the plant and increased uptake and translocation of minerals, increases the efficiency of nutrient uptake solubilizing certain insoluble nutrient elements like rock phosphate.

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Table-1 : Growth inhibition of *Fusarium pallidoroseum* (Cooke) Sacc. by different species of *Trichoderma*

Isolates	Per cent inhibition over control	*Zone of inhibition
Trichoderma viride (S)	60.53 (29.97)	-
Trichoderma harzianum (S)	47.37 (28.83)	+
Trichoderma viride (C)	35.00 (27.75)	+
Trichoderma harzianum (C)	33.68 (26.72)	+
Trichoderma viride (M)	34.21 (27.71)	+
Trichoderma harzianum (M)	27.80 (19.15)	+
CD (P = 0.05)	**1.12**	

*, + shows zone of inhibition is present
- shows zone of inhibition is absent
Figure in parenthesis are arc sine transformed values

Table-2 : Effect of *Trichoderma* spp. on growth, yield and suppression of fusarial wilt of chilli (*Capsicum annum* L.)

Isolate doses	Length of plant (cm)		Wilt incidence			Fruit yield	
	Shoot	Root	Flowering	Fruit formation	Ripening stage	kg plot^{-1}	q ha^{-1}
T. viride (S) 10^6 spore ml^{-1}	96.5	16.5	2.00+ (1.41)	13.12** (21.22)	33.56* (35.37)	2.73	73.25
T. harzianum (S) 10^6 spore ml^{-1}	93.5	14.5	3.75 (1.93)	24.76 (29.82)	48.25 (43.98)	2.45	61.31
T. viride (C) 10^6 spore ml^{-1}	91.2	14.2	5.35 (2.31)	28.43 (32.2)	50.00 (44.98)	2.29	57.25
T. harzianum (C) 10^6 spore ml^{-1}	88.9	13.7	6.30 (2.75)	29.53 (39.31)	53.50 (45.45)	2.20	54.35
T. viride (M) 10^6 spore ml^{-1}	90.4	12.6	5.75 (2.34)	29.21 (33.21)	51.00 (45.23)	2.25	55.45
T. harzianum (M) 10^6 spore ml^{-1}	84.2	13.7	6.90 (2.95)	30.90 (40.10)	55.70 (46.55)	2.15	53.15
Check	80.5	9.5	10.20 (3.19)	46.36 (42.89)	68.75 (56.07)	1.70	42.50
CD (P = 0.05)	4.75	0.62	0.20	2.05	2.98	0.13	0.79

* Means of three replications

+ Figures in parenthesis are square root transformed values

** Figures in parenthesis are arc sine transformed values