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Evaluation of Bio-Control Agents against Fusarium Wilt of Cucumber

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Abstract

The experiment was carried out in Department of Plant Pathology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India during cropping season of May to August in year 2018–2019 to study the efficacy of fungal and bacterial bio-control agents in managing the Fusarium wilt of cucumber. Different fungal and bacterial bio-control agents were thus tested under laboratory conditions for their efficacy in inhibiting the mycelial growth of *Fusarium oxysporum* by using dual culture technique and streak plate method, respectively. Among fungal bio-control agents, *Trichoderma harzianum* was found most effective with 61.08% inhibition of mycelial growth of *F. oxysporum* followed by *Trichoderma virens* with 57.51% mycelial inhibition whereas, among bacterial bio-control agents, maximum mycelial inhibition of 45.69% was observed in *Pseudomonas fluorescens*. During the cropping season, seed, and soil treatment with effective fungal and bacterial bioagents was carried out under field conditions. Seed treatment and soil application of *Trichoderma harzianum* was found effective with disease control of 48.25% followed by *Trichoderma virens* which is proved to be efficacious with 42% disease control against Fusarium wilt of cucumber.

Keywords: *Fusarium oxysporum*, bio-control agents, seed treatment, soil treatment

1. Introduction

Cucumber (*Cucumis sativus* L.) is among the oldest cultivated vegetable crop in India belonging to the family Cucurbitaceae and is known to be one of the oldest cultivated vegetables. Cucumber is a rich source of vitamins, antioxidants, minerals and consumed as salad or in pickles. Among various diseases, Fusarium wilt is the most serious disease caused by *Fusarium oxysporum* in long term monoculture cropping. Damping-off, plant stunting, yellowing, and wilting of the older leaves with brown vascular discoloration are typical symptoms of the disease. Later, infection leads to small and abnormal fruits followed by complete collapse of infected plants. Fusarium wilt of cucumber was firstly reported from Florida (Owen, 1955). In India, the occurrence of Fusarium wilt was reported firstly from Jammu and Kashmir (Pagoch and Raina, 2012). In Himachal Pradesh, its occurrence has been reported from Solan (Shukla and Sharma, 2017; Sharma and Shukla, 2021). It is cultivated as an off-season crop in mid hills of Himachal Pradesh during summer and rainy season under open and protected conditions. For the management of the disease, different practices like crop rotation, sanitation, application of fungicides, seed treatments and use of bio-control agents are envisaged (Harman, 2006; Hu et al., 2010).

Among various management practices, use of bio-control agents is an important component of Integrated Disease Management (IDM) which is non-polluting, bio-degradable, selective in mode of action and unlikely to harm human beings and other beneficial microorganisms. The application of bio-control agents to suppress soil-borne pathogens has been widely used. Soil application of antagonistic microbes with suitable substrate and seed treatment with bio-control agents has been reported to be more effective than the direct application (El-Hassan and Gowen, 2006; Trillas et al., 2006; Kumari et al., 2021). Also, due to the higher costs involved with use of chemical fertilizers and pesticides, the need for application of bio-fertilizers has also risen. Biofertilizers have low-cost input involved and improve the crop growth, yield as well as food quality (Kashyap et al., 2014). Use of *Trichoderma* strains is widely practiced due to their effectiveness against soil borne diseases and promotes quality and yield of crops (Mei et al., 2019; Yedidia et al., 2001; Howell, 2003; Mukhopadhyay and Kumar, 2020). *Trichoderma* belongs to free living fungus group, commonly found in soil ecosystem which is effective in control of soil and seed borne diseases

in several crops. Effectiveness of *Trichoderma* is due to the formation of specialized structures, degradation of the host cell wall and Mycoparasitism (Kripalini et al., 2019). Mycoparasitism involves coiling around the hyphae and its attachment on the host or breaking septa of hyphae or conidia of pathogen.

Potential of green approaches has been found to mitigate biotic stresses in plants. These involves the use of PGPRs, among which fluorescent Pseudomonas is considered important due to their mechanism of production of antibiotics and other antimicrobial compounds (Naik, 2016; Sarvani and Reddy, 2013; Debnath and Bhattacharya, 2002). *Trichoderma* includes important features like niche colonization, high adaptability, antibiotic spectrum etc. *Bacillus* strains has also been reported to be an effective bio-control agent to manage Fusarium wilt as they have the capacity to form spores which survive and remain metabolically active even under harsh environmental conditions and been formulated as biofertilizers (Li et al., 2012; Ling et al., 2011; Liu et al., 1995; Singh et al., 1999). Due to environmental and health concerns, non chemical practices are encouraged over the large use of pesticides and chemicals. Thus, biological control represents an alternative safe and potential option of plant disease management (Rashad et al., 2017; Allay and Chakraborty, 2013; Yang et al., 2014; Anusha et al., 2019). Keeping this in mind, the present studies were conducted to evaluate bio control agents effective against *Fusarium oxysporum* inciting Fusarium wilt of cucumber.

2. Materials and Methods

Efficacy of fungal bio-control agents (*Trichoderma harzianum*, *T. viride*, *T. virens*) and bacterial bio-control agents (*Pseudomonas fluorescens*, *Bacillus* sp.) was evaluated against *Fusarium oxysporum* causing Fusarium wilt of cucumber. The experiment was carried out in Department of Plant Pathology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni Solan (30.8645° N, 77.1695° E) during 2018-2019. Evaluation of bio-control agents was done under *in vitro* conditions and pot house conditions in the research farm of KVK Kandaghat, Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP).

2.1. Evaluation of bio-control agents under in vitro conditions

The fungal bio-control agents were screened under *in vitro* conditions against *Fusarium oxysporum* for their antagonistic activity by using dual culture method (Huang and Hoes, 1976) while, bacterial antagonists were evaluated by using streak plate method. Each treatment was replicated four times. Colony diameter of the test fungus as well as each antagonist up to the zone of inhibition was recorded and the per cent growth inhibition of the test pathogen over control was calculated according to Vincent (1947) as under:

$$I=(C-T)/C\times100$$

Where,

I - Per cent inhibition in mycelial growth

C - Linear mycelial growth in control (mm)

T - Linear mycelial growth in treatment (mm)

2.2. *Mass multiplication of fungal bio-control agents for seed treatment*

Spores from well sporulated cultures of fungal antagonists raised on potato dextrose agar (PDA) medium were harvested and suspension (10^7 conidia ml^{-1}). was made in sterilized distilled water with the addition of carboxy methyl cellulose (CMC 1%) according to the method suggested by Mukopadhyay et al. (1992).

2.3. *Mass culture of fungal bio-control agents for soil inoculation*

The mass culture of fungal antagonists was prepared on wheat bran: sawdust: tap water (3:1:1 w/w/v) + 2 per cent jiggery medium and autoclaved at 1.05 kg cm^{-2} for 1 hr on two consecutive days. Polypropylene bags containing autoclaved medium were aseptically inoculated with mycelial discs (5mm diameter) taken from their actively growing 3 days old cultures. Inoculated polypropylene bags were incubated at 25±2°C for 15 days. The bags were shaken regularly after 3 days so that fungus grows uniformly.

2.4. Mass culture of bacterial bio-control agents

Bacterial bio-control agents were multiplied on nutrient broth. Two loopful of bacterial culture (48 hour old) was added to the nutrient broth medium and incubated at 28±2°C for three days. Concentration of the medium was maintained at 10^6–10^8 cfu ml^{-1} by serial dilution method.

2.5. Seed and soil treatment

Cucumber seeds of the hybrid "KH-1" were surface sterilized with 0.1% sodium hypochlorite solution for 30 seconds and rinsed in four successive changes of sterilized distilled water and thereafter, seeds were soaked in spore suspension of fungal bio-control agents with the addition of carboxy methyl cellulose (CMC @1%) according to the method suggested by Mukhopadhyay et al. (1992).

The experiment was replicated four times having three seedlings per replication. The pots were kept in the glasshouse and observed for appearance of disease symptoms. The experiment was conducted in completely randomized design with four replications each and a control maintained. Data in terms of number of wilted plants in each treatment and per cent disease incidence was recorded.

Disease incidence (%) = (Total number of plants infected/Total number of plants observed)×100

3. Results and Discussion

3.1. Evaluation of bio-control agents under in vitro conditions

Fungal and bacterial bio-control agents were evaluated against Test fungus causing Fusarium wilt of cucumber. Data

on mycelial growth of the pathogen were recorded and per cent mycelial inhibition and inhibition zone was calculated and presented in Table 1.

Table 1: *In vitro* evaluation of bio-control agents against *Fusarium oxysporum* causing wilt of cucumber

Sr. No.	Treatment	Mycelial growth (mm)	Per cent inhibition	Inhibition zone (mm)
Fungal antagonists				
1.	*Trichoderma harzianum*	35.02	61.08 (51.38)	-
2.	*Trichoderma viride*	54.50	39.44 (38.33)	-
3.	*Trichoderma virens*	38.10	57.51 (49.41)	-
Bacterial antagonists				
4.	*Bacillus* sp.	59.75	33.60 (35.41)	11.37
5.	*Pseudomonas fluorescens*	48.87	45.69 (42.51)	14.32
6.	Control	90.00	0.00 (0.00)	-
	CD (*p*=0.05)	1.26	(0.84)	-

Figures in the parentheses are arc sine transformed values

It is evident from the data (Table 1) that all the bio-control agents were able to check the mycelial growth of *F. oxysporum* either by over growing or by exhibiting inhibition zones. In general, fungal bio-control agents were more effective in inhibiting mycelial growth of the test pathogen as compared to bacterial bio-control agents. Minimum radial mycelial growth of pathogen (35.02 mm) was observed in *Trichoderma harzianum* followed by *T. virens* (38.10 mm). *T. viride* was found to be the least effective exhibiting radial mycelial growth of 54.50 mm (Figure 1). Likewise, maximum per cent mycelial inhibition of the pathogen was observed in *T. harzianum* (61.08) followed by *T. virens* (57.51).

Among bacterial bio-control agents, minimum radial mycelial growth (48.87 mm) and maximum per cent mycelial inhibition (45.69) of pathogen was observed in *Pseudomonas fluorescens* while, in *Bacillus* sp., 59.75 mm radial mycelial growth of pathogen was observed (Figure 1). Similarly, maximum inhibition zone of 14.32 mm was observed in *Pseudomonas fluorescens* while, in *Bacillus* sp., an inhibition zone of 11.37 mm was recorded.

Efficacy of *Trichoderma* spp., *P. fluorescens* and *Bacillus* sp. against *F. oxysporum* have been very well documented in literature (Lifshitz et al., 1986; Leeman et al., 1995; Larkin and Fravel, 1998; Prasad et al., 2002). These workers

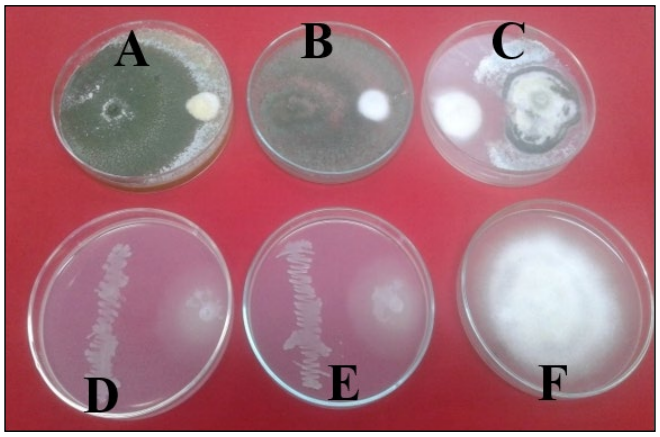

Figure 1: *In vitro* evaluation of bio control agents against *Fusarium oxysporum* causing wilt of cucumber; A) *Trichoderma harzianum*; B) *Trichoderma virens*; C) *Trichoderma viride*; D) *Pseudomonas fluorescens*; E) *Bacillus* sp.; F) Control

reported that the mode of mycoparasitism of *Trichoderma* isolates appeared to be coiling around and its attachment on the host or penetration into the host hyphae or breaking septa of both the hyphae and the conidia. Results of present investigation are in conformity the findings of Aparna et al (2022), who revealed that *Trichoderma harzianum* was most effective in inhibiting the growth of *Fusarium oxysporum* f sp. *cucumerinum* followed by *Trichoderma viride* and *Trichoderma asperellum*. Ramesh and Singh (2017) carried out extensive work on efficacy of 27 *Trichoderma* cultures and revealed that these effectively inhibited the pathogen growth under dual culture studies. Mycoparasitic mechanisms such as coiling, penetration, overgrowing, lysis, and antibiosis by antagonistic fungi like *Trichoderma harzianum*, *T. viride* appears to be effective in management of soil borne pathogens. Results agree with finding of workers which tested the efficacy of Trichoderma against Fusarium wilt (Hussein, 2016; John et al., 2010; Akrami, 2015). Bacterial bio-control agents are promising as they possess many traits like production of bioactive metabolites like antibiotics, siderophores, volatiles and other attributes like colonization ability and better adaptation to environmental stresses.

3.2. Evaluation of bio-control agents as seed and soil treatment

Efficacy of various fungal and bacterial bio-control agents was evaluated against *Fusarium oxysporum* under pot house conditions. Data on per cent disease incidence was recorded and presented below in Table 2.

Perusal of the data (Table 2) revealed that all the bio-agents were effective in reducing the disease incidence significantly over control. Among different fungal bio-control agents tested against *Fusarium oxysporum*, *Trichoderma harzianum* proved most effective with minimum disease incidence of 20.83 per cent and provided disease control of 48.25 but was statistically at par with *T. virens* with disease incidence

Table 2: Efficacy of seed treatment and soil amendment with bio control agents against *Fusarium* wilt of cucumber

Bio control agents / Bioformulations	Disease incidence (%)	Disease control (%)
Trichoderma harzianum	20.83 (27.03)	48.25
Trichoderma viride	33.33 (35.24)	32.54
Trichoderma virens	22.91 (28.51)	45.42
Pseudomonas fluorescens	37.49 (37.71)	27.81
Bacillus sp.	45.83 (42.58)	18.49
Control	62.49 (52.24)	-
SEm±	-	-
CD (*p*=0.05)	(4.06)	

Figures in the parentheses are arc sine transformed values

of 22.91 and disease control of 45.42% (Figure 2). Among the bacterial bio-control agents, *Pseudomonas fluorescens* exhibited significantly lower disease incidence (37.49%) as compared to *Bacillus* sp. (45.83%).

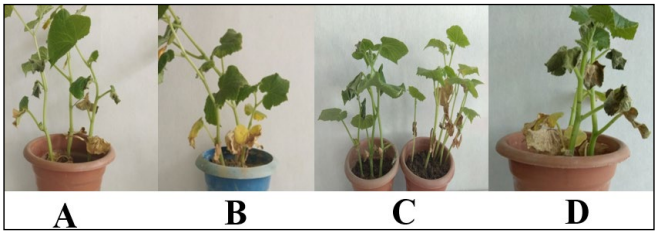

Figure 2: Evaluation of bio-control agents as seed and soil treatment against *Fusarium oxysporum* causing wilt of cucumber; A) *Trichoderma harzianum*; B) *Trichoderma virens*; C) *Pseudomonas fluorescens*; D) Control

Results obtained in the present studies are in agreement with Chang et al. (1986) who reported that soil application of *Trichoderma harzianum* resulted in increased dry weight of tomato, pepper and cucumber seedlings against *Fusarium* sp. while, Prasad et al. (2002) reported that both seed and soil amendment with *Trichoderma harzianum* were effective against *Fusarium* sp. Soil amendment @ 20 g kg^{-1} alone resulted in 61.5% disease control while, 19.9% disease control was recorded in seed treatment alone.

In another study, Bhat et al. (2016) reported that the isolates of *Trichoderma harzianum* and *T viride* completely overgrew *Fusarium solani* and thus wilt incidence significantly. Srivastava et al. (2010) studied the potential of *Pseudomonas*, *Trichoderma harzianum* and AM fungi in management of fusarium wilt of tomato. *Trichoderma* is among the most promising bio control fungi against many fungal plant pathogens. Bharat and Thakur (2014) reported that *Trichoderma* strains have role in management of Fusarium wilt of tomato, as disease severity of wilt was recorded to be lower in tomato plants grown with *Trichoderma harzianum*. Efficacy of *Trichoderma* spp. could be due to their higher competitive ability by different mechanisms viz., mycoparasitism, antibiosis and siderophore production.

4. Conclusion

Application of fungal and bacterial bio-control agents along with bio-formulations as seed treatment and soil amendment has potential in disease management. These fungal bio-control agents play very important role in managing the Fusarium wilt under *in vitro* as well as pot culture conditions.

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