In vitro antagonistic activity of *Trichoderma viride* isolates against *Sclerotinia sclerotiorum* and their role in growth promotion of common bean

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ABSTRACT

A study was done to investigate the antagonistic effects of three *Trichoderma viride* isolates (TV1, TV2 and TV3) on *Sclerotinia sclerotiorum*. The results revealed that the isolate TV1 proved to be the best in suppressing the mycelial growth by 77% after 7 days of incubation. However 74.91% and 50% inhibition were recorded on 5th and 3rd days of incubation, respectively. The isolate TV2 caused inhibition of 74.77% on 7th day, and 72.66% and 48.33% after 5th and 3rd days of incubation, respectively. 72.54%, 70.41%, and 51.66% inhibition in mycelial growth of the pathogenic fungus was caused by TV3 isolate after 7th, 5th, and 3rd days of incubation respectively. All the test isolates of *T. viride* showed a significant effect on the vigour index of common bean. The maximum germination percentage was observed on treatment with TV1 isolate (85%) followed by TV2 (83%) and TV3 (81%) respectively, in comparison to untreated seeds which showed a germination percentage of 73%. Maximum vigour index was shown by those plants whose seeds were treated with TV1 (11560), followed by those treated with isolate TV2 (10707) and TV3 (9234), whereas the control plants showed significantly lower vigour index of 7446 in comparison to treated plants .

Key words: White mould, vigour index, Identification, Germination percentage

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INTRODUCTION

White mould, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary is pathogenic to more than 400 species of plants worldwide (Garg *et al*., 2010). Beans, soybean, cotton, sunflower, tomato, and potato are among the most important crops affected by the fungus. It is regarded as the most dangerous disease to many plants because it can infect all plant parts, and has a wide host range (Boland and Hall, 1994). The green pods, stalks, pedicels, young branches, and petiole bases of common bean (*Phaseolus vulgaris* L.) remain coated by dense cottony white mycelia when grown at a temperature of 16-20°C with wet aerial parts and high soil moisture. In *Phaseolus* germplasm, field resistance or tolerance has recently been discovered (Abawi *et al.,* 1975; Coyne *et al.,* 1977). This disease has not been effectively controlled by chemical management or cultural techniques such as crop rotation (Coyne *et al*., 1974). Furthermore, chemical control is unreliable because fungicide-resistant pathogen strains may emerge (Li *et al*., 2008). All these factors are motivating scientists around the world to develop alternative eco-friendly methods. Presently, *Trichoderma* species have got a lot of attention as prospective fungal biocontrol agents for a variety of plant infections. In addition to their antagonistic activity, treatment of crop seeds with different *Trichoderma* strains have also been reported to possess growth promoting properties, increasing

seed germination percentage, root and shoot length and vigour index of different plants.

The current study was aimed to study the effect of different isolates of *Trichoderma viride* on the mycelial growth of *Sclerotinia sclerotiorum* causing white mould of common bean and its effect on seed germination and vigour index of common bean in order to develop integrated disease management module for the white mould.

MATERIALS AND METHODS

Isolation and identification of microbes

The pathogen was isolated from infected pods of bean collected from different localities of Kashmir valley which displayed typical disease symptoms. The infected pods were rinsed with water and surface sterilized by immersing them for 30 seconds in 0.1% aqueous mercuric chloride solution, followed by 3-4 times washing with sterilized distilled water. These were put aseptically upon sterile Petri plates containing Potato Dextrose Agar (PDA) medium and cultured for 7 days at 18±2^{o}C. The single spore method was used to obtain pure cultures. The fungus was identified on the basis of cultural and microscopic characteristics (Hanlin, 1998). Among the microscopic characteristics shape, size, branching, and septation of mycelium and conidia, whereas in cultural characteristics, colony colour, shape, and texture, were taken into consideration for identification. Isolates of *Trichoderma viride* (TVl, TV2 and TV3) were isolated from soil of *Pisum sativum* by serial dilution technique (Waksman, 1922) and were identified according to Park *et al.* (2005).

Dual culture technique

The antifungal activity of three *T. viride* isolates was tested against the isolated pathogenic fungus *Sclerotinia sclerotiorum* using a dual culture approach (Prince *et al*., 2011). PDA medium was poured into sterilized Petri plates (having 9 cm diameter) and allowed to solidify for 15 minutes at room temperature. On opposite sides of Petri plates containing PDA medium, antagonistic fungus and pathogenic fungus were introduced. Culture plates without the antagonistic fungus (*T. viride*) served as control. The experiment was repeated thrice for each treatment. All the inoculated Petri plates were kept at 18±2°C in an incubator. After 3rd, 5t^{h} and 7th day of incubation, the mycelial growth inhibition was measured and calculated using the following formula:

$$\text{Growth inhibition} = \frac{C-T}{C} \times 100$$

Where "C" denotes colony diameter of pathogen in control and "T" represents pathogen colony diameter in dual culture (Wonglom *et al*., 2019).

Effect of *Trichoderma viride* on vigour index

Under greenhouse conditions, the growth promoting ability of the *T. viride* isolates (TV1, TV2 and TV3) on common bean seeds was tested. A new approach developed by Mukhtar *et al.* (2012) was employed to coat 60 common bean seeds with suspensions of each isolate of *T. viride* while as sterile distilled water served as a control. Haemocytometer was used to adjust the spore suspension concentrations of *T. viride* to 1x10^{6} conidia/mL. Common bean seeds were sterilized before being coated in seed covering suspensions for about 30 minutes followed by drying in air for 24 hours on filter paper. In Classman substrate 2, seeds coated with *T. viride* were sown in 4 replications (5 seeds per pot). Seed germination percentages, and root and shoot lengths were measured after seven days of sowing. The formula given by Abdul-Baki and Anderson (1973) was used to calculate the vigour index:

Vigour index = [Mean of root length (mm) + Mean of shoot length (mm)] x percentages of seed germination.

RESULTS

Identification of the pathogen

The colonies of *S. sclerotiorum* were white in colour with thin mycelium. The mycelium grew very rapidly and covered the whole Petri plate within 4-5 days of incubation at 18±2^{0}C. Small, cream to brownish sclerotia developed over the mycelium after 10 days of incubation which then enlarged and turned black at the time of maturation. Mycelium was branched and septate. Spores were hyaline, ellipsoid to ovoid, and measured 10-17×5-8µm.

Antifungal activity of *T. viride* isolates

The antifungal activity of *T. viride* isolates TV1, TV2 and TV3 and the suppression of mycelial

growth was recorded and compared to the control (Table 1). Significant inhibition in pathogen mycelial growth was observed at third, fifth and seventh day of incubation in comparison to control, where the pathogen grew luxuriantly. The *T. viride* isolate TV1 was highly effective and significantly suppressed the mycelial growth by 77% after 7 days of incubation. However, 74.91% and 50% inhibition was also recorded on 5th and 3rd days of incubation, respectively.

Table 1. Antifungal activity of *T. viride* isolates on the mycelial growth of *Sclerotinia sclerotiorum*. Values are represented as mean±SD. Values followed by same alphabets are not statistically different ($p<0.05$) by Duncan's test.

***Trichoderma viride* isolate (TV)**	**Days after inoculation**	**Mycelial diameter of pathogen in treatment (mm)**	**Mycelial diameter of pathogen in control (mm)**	**Mycelial inhibition (%)**
TVI	3	30.00±2.00b	60.00±1.00c	50.00
TV2		31.00±2.00b	60.00±1.00c	48.33
TV3		29.00±2.00b	60.00±1.00c	51.66
TV1	5	22.33±2.51a	89.00±1.00d	74.91
TV2		24.33±2.51a	89.00±1.00d	72.66
TV3		26.33±2.51ab	89.00±1.00d	70.41
TV1	7	20.66±2.08a	89.66±0.57d	77.00
TV2		22.66±2.08a	89.66±0.57d	74.77
TV3		24.66±2.08a	89.66±0.57d	72.54

Isolate TV2 caused inhibition of 74.77% on 7th day of incubation and 72.66% and 48.33% after 5th and 3rd days of incubation, respectively. Similarly, 72.54%, 70.41%, and 51.66% inhibition in pathogen mycelial growth was caused by TV3 isolate on 7th, 5th, and 3rd days of incubation, respectively. While TV1 was the most effective in inhibiting the mycelial growth of pathogenic fungus quantitatively however, the lowest inhibition in mycelial growth was caused by isolate TV2 on day 3rd of incubation. The pathogenic fungus without antagonistic fungus (control) grew and sporulated vigorously and covered the whole surface of the Petri plates within 4-5 days.

Effect of *T. viride* isolates on vigour index

All the three test isolates of *T. viride* showed a significant effect on the seed germination of common bean (Table 2). The maximum germination percentage was caused by the treatment of TV1 isolate (85%) followed by TV2 (83%) and TV3 (81%) respectively in comparison to untreated seed which showed a lower germination percentage (73%). The shoot length of common bean plants did not show significant variation as compared to the control, however, a significant increasing effect was observed on the root length. The vigour index varied significantly among the three isolates of *T. viride*. Maximum vigour index was shown by those common bean plants whose seeds were treated with TV1 followed by those treated with TV2 and TV3, while as the control plants showed lower vigour index (7446).

Table 2. Effect of *T. viride* isolates on seed germination and vigour index of common bean. Each value is mean of four replicates

***Trichoderma viride* isolate (TV)**	**Germination percentage (%)**	**Root length (mm)**	**Shoot length (mm)**	**Vigour index**
TVI	85	58	78	11560
TV2	83	54	75	10707
TV3	81	42	72	9234
Control	73	36	66	7446

DISCUSSION

In the current study, the effect of *T. viride* isolates on *Sclerotinia sclerotiorum* mycelial growth was studied and a significant effect was observed. *Trichoderma* species are mostly effective antagonists of soil fungal pathogens like *S. sclerotiorum* (Matroudi *et al*., 2009; Ibarra-Mediná *et al*., 2010). Mishra (2010) reported antagonistic effect of the *Trichoderma viride* isolates against *Pythium aphanidermatum* in a dual culture test and reported maximum inhibition (72%) of pathogen mycelial growth. Our results corroborate with Larralde-Corona *et al.* (2008) and Ilyas *et al.* (1985) who reported mycelial growth inhibition of 50% and 47% by *Trichoderma* spp. against charcoal-rot causing fungus, *Macrophomina phaseolina.* Similarly, 33.29- 97.86% mycelial growth inhibition of *Phytophtora palmivora* by *Trichoderma* isolates has been reported by Mpica *et al.* (2009). *T. harzianum* and *T. viride* showed a major inhibition in mycelial growth of *Bipolaris oryzae* causing brown spot disease in rice (Gomathinayagam, 2012). The mycelial growth of various pathogenic fungi isolated from infected samples of brinjal was found to be inhibited by *Trichoderma* spp. (Koka *et al.*, 2017). *Macrophomina phaseolina* isolated from black gram underwent maximum mycelial growth inhibition of 81.55% by an isolate of *T. viride* (Suthin Raj *et al.,* 2020). Similarly another isolate of *T. viride* caused a mycelial growth inhibition of 73.74% on *Macrophomina phaseolina* isolated from black gram (Kannan, 2019).

The effect of *T. viride* isolates on the growth promotion of common bean was also studied during the present study. It was observed that seed germination, and root and shoot length were significantly enhanced by coating the seeds with isolates of *T. viride*. *Trichoderma* isolates were also proven to promote growth in other cultivated plants, including, rice, sunflower, maize, safflower, pepper, radish, chilli, cucumber, mustard, tomato, etc. as has been reported by Ilyas *et al*. (1985), Singh *et al.* (2008) and Joshi *et al.* (2010). Our results are in conformity with Okoth *et al.* (2010) who observed a substantial influence of *Trichoderma* isolates on the vigour index and seed germination of *Phaseolus vulgaris*. Plant growth promotion by *Trichoderma* isolates may be due to antibiotic synthesis, parasitization of other fungus, and competition with harmful plant microbes (Harman *et al.,* 2004). These characteristics have been proposed to form the basis for *Trichoderma's* favourable effects on plant growth and development until recently. However, it is becoming clear that specific strains have a noteworthy impact on plant development and crop productivity (Harman, 2006).

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