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Molecular Characterization of *Sclerotinia sclerotiorum* Sacc. Initiating White Mold Disease in French Bean (*Phaseolus vulgaris* L.) and its Biological Management

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ABSTRACT

Background: White mold disease caused by *S. sclerotiorum* is a serious peril to the cultivation of french bean in India. The disease primarily spreads by spores and normally in forms of sclerotia, which can remain viable in soil for several years and under appropriate environmental conditions, germinate to form mycelium, leading to infectious hyphae or producing apothecia.

Methods: In the present study, antagonistic activity of locally isolated bio-inoculants including four fungi and three bacteria were evaluated against white mold disease of french bean. *In vitro* efficacy of bio-inoculants was tested by dual culture method against *S. sclerotiorum.*

Result: The molecular characterization of isolated pathogen was performed using ITS sequencing. The sequence length of the pathogen was 516 bp and approximately identical to publicly available *S. sclerotiorum* sequences on NCBI data base. *In vitro* study showed that, *P. fluorescens* isolate Pf008 was best causing 95.55 per cent mycelium growth reduction. In field experiment, the bio-inoculants were tested by various inoculation methods. *Sclerotinia sclerotiorum* infested soil treated with bio-inoculants as seed treatment and soil application. Results revealed that, seed treatment with formulation of *T. harzianum* isolate TS004 was best and enhance the growth promotion and increase the grain yield *i.e.* 13.93 q/ha and 12.10 q/ha during the year 2018-19 and 2019-20 respectively. Soil application with formulation of *T. viride* isolate TS006 was the utmost effective in reducing disease incidence by 83.91% during the year 2018-19 and seed treatment with formulation of *T. viride* isolate TS006 reducing disease incidence by 74.83% in the year 2019-20.

Key words: Bio-inoculants, Disease, French bean, Isolate.

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is an important legume crop belongs to family Fabaceae. It is also known as common bean, green bean, dry bean, rajma in North India and among food legumes the french bean has been the third most important worldwide famous crop, superseded only by soybean and peanut. There are two major commercial classes of french bean varieties, snap and dry beans (Jhanavi *et al.,* 2018). It is esteemed for its protein rich (23%) seeds. It is a cold weather crop and highly sensitive to frost, high temperature and excessive soil moisture (Sabate *et al.,* 2018). In India, the crop is grown mainly in the states of Maharashtra, Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Tamil Nadu, Kerala, Karnataka and West Bengal.

French bean suffers from a number of fungal diseases. However, among the fungal diseases, white mold caused by the fungus *Sclerotinia sclerotiorum* Lib. is a very detrimental disease affecting the french bean in the province of India. The disease is also known as sclerotinia wilt or stalk rot. The fungus is favored by temperate climates, moderate temperatures and high relative humidity (Mamani Gonzales *et al*., 2015). *S. sclerotiorum* primarily spreads by spores and normally in forms of sclerotia, which may infect stems, leaves and blooms and even spread to neighboring plants (Zhou and Boland, 1998). Sclerotia of the pathogen can remain viable in soil for several years and under appropriate environmental conditions, germinate to form mycelium, leading to infectious hyphae or producing apothecia, which release millions of airborne ascospores (Bardin and Huang, 2001). One of the major problems in managing this disease, that the pathogen produces large numbers of sclerotia which could stay viable for a long time

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in the soil (Alsum *et al.,* 2017). This fungus can cause devastating economic losses in the harvests; therefore, its management is of regional importance. As for the last strategy, intensive use of chemical substances in crop management has led to microbial pathogen resistance to fungicides and has also caused serious problems for human health and the quality of the environment (Sabate *et al.,* 2018). Therefore, it is necessary to explore the alternative of chemical management of disease. The study's goal was to assess the efficacy of some locally available bio-inoculants (fungi and bacteria) in managing white mold on French beans.

MATERIALS AND METHODS

The *in vitro* experiments were conducted to manage white mold disease of french bean caused by *Sclerotinia sclerotiorum* with isolates of different bio-inoculants at Centre of Excellence for Sanitary and Phytosanitary (SPS), Department of Plant Pathology and Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, U.P.

Isolation and purification of the pathogen

The isolation and purification of the *S. sclerotiorum* was done by using the methodology of Jia (2009). Fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer (Alsum *et al.,* 2017).

Molecular characterization of the pathogen

Molecular characterization of the isolate pathogen involved partial amplification of the ITS region (Freeman *et al.,* 2002), followed by nucleotide sequencing. For this, ITS1 and ITS4 primers were used, following the conditions and procedure reported by Rahman *et al.* (2015). Phylogenetic analysis of the generated sequences was carried out using the MEGA6 program, with the sequences of *Sclerotinia sclerotiorum* showing the highest homologies in Gen Bank.

Collection of bio-inoculants

The isolates of different bio-inoculants (Table 1) were collected from the Centre of Excellence for Sanitary and Phytosanitary, Department of Plant Pathology, SVPUAT, Meerut, Uttar Pradesh (India).

Table 1: List of different bio-inoculants used against *Sclerotinia sclerotiorum*.

Bio-Inoculants	Accession number
Trichoderma harzianum isolate-TS001	MW015937.1
Trichoderma harzianum isolate-TS004	MW015938.1
Trichoderma viride isolate-TS006	MW015940.1
Trichoderma koningi isolate-TS007	MW015939.1
Pseudomonas fluorescens isolate-Pf008	MW199141.1
Pseudomonas fluorescens isolate-Pf024	MW199125.1
Bacillus subtilis isolate-B005	MW199117.1

Screening of bio-inoculants against pathogen suppression *in-vitro* condition

Antagonistic activities of microorganisms were tested against the soil borne plant pathogen *Sclerotinia sclerotiorum* by employing dual culture techniques of Rahman *et al.* (2009) on PDA.

Test for seed germination by roll paper towel method

The seeds were treated with the formulation of each bio inoculant and placed on moist paper towels (50-100) at equal distance and covered with another moist paper towel and rolled carefully without disturbing the already arranged seeds. Tie the rolled paper towel with a rubber band at both the ends. To avoid water loss, rolled towels containing seeds was wrapped with polythene and incubated for four to five days at room temperature. Examined germinated and ungerminated seeds by naked eyes (Kesharwani *et al*., 2018).

Screening of bio-inoculants on growth promotion and white mold disease suppression of french bean under pot condition

On the basis of *in vitro* result the selected bio-inoculants were evaluated for growth promotion of french bean. The selected fungal bio-inoculants were mass multiplied on Bajra seed and bacterial bio-inoculants were multiplied in nutrient broth medium. The soil was treated with selected bio-inoculants (at the rate of 5 g/kg of soil for fungal and 50 ml/kg of soil for bacterial bio-inoculants) and seed were sown in 8 inch and 16 cm diameter plastic pot at 2 cm depth. Soot length and root length were recorded at 45 days and 90 days after sowing.

$$\text{Disease incidence \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Evaluation of formulated bio-inoculants against white mold diseases suppression and growth promotion in french bean under field condition

The bio-inoculants were evaluated for growth promotion and management of white mold disease of french bean under field conditions at Crop Research Centre, SVPUAT, Meerut, Uttar Pradesh. Seeds were sown on November, 2018-19 and 2019-20 during *Rabi* season. The experiment was carried out following RBD with three replications. Size of the plots was kept 4.0 m ×3.0 m and plant spacing was kept 45 × 10 cm for french bean.

Experimental plots were made sick by soil inoculating @ 50 g/m^2 with inoculum of *Sclerotinia sclerotiorum*. Fifteen treatments (including one control) were evaluated using four *Trichoderma* spp. isolates *i.e.* TS001, TS004, TS006, TS007, two *Pseudomonas fluorescence* isolates *i.e.* Pf008, Pf024 and *Bacillus* spp. isolate B005 used as seed treatment and soil application with FYM. The seeds were treated with formulated bio-inoculants @ 10 g/kg of seed (2×10^6 cfu) of *Trichoderma* spp., *Pseudomonas fluorescence* (2×10^8 cfu) and *Bacillus* spp, (2×10^8 cfu) and 6 kg/ha for soil application with FYM. During the growing period the plots were inspected

regularly to record the incidence of disease from seedling to maturity stage of the crop. Data were recorded on percent disease incidence, shoot length, root length, number of branches, number of pods/plant and yield. The per cent disease incidence was calculated by the following formula:

$$\text{Disease incidence } \% = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

Molecular characterization of *Sclerotinia sclerotiorum* through ITS sequencing and phylogenetic analysis

The molecular characterization of an isolated pathogen was performed using ITS sequencing. The nucleotide sequence generated in this study was assigned GenBank accession number MW173486.1. The isolate (svpuat) was attributed to *Sclerotinia sclerotiorum*. The length of the ITS sequence of isolate was 516 bp and almost identical to publicly available *Sclerotinia sclerotiorum* sequences (Fig 1 and 2). The phylogenetic relationship among the isolate and the related species, including *S. sclerotiorum* (*e.g.,* GenBank accession numbers MW342187, KT595416 and LC318707 *etc.*), is shown in Fig 1. The results revealed that the isolates belong to a similar group of the type strain. This observation was similar to previously reported descriptions of Rahman *et al.,* (2020). The study of Ciesniewska *et al.* (2017) revealed that, internal transcribed spacer ITS1/ITS4 PCR primers are effective for rapidly and accurately differentiating between the two species of *Sclerotinia*, they resulted a 540-bp product with all isolates tested (24 *S. sclerotiorum* and 26 *S. trifoliorum*).

Screening of bio-inoculants against pathogen suppression *in vitro*

A total of seven bio-inoculants tested against the *Sclerotinia sclerotiorum*. On the basis of their antagonistic efficiency against pathogen (Line Fig 1), it was observed that all isolates inhibit the mycelial growth of *Sclerotinia sclerotiorum.* The maximum percent inhibition of pathogen growth 95.55% was observed in *Pseudomonas fluorescens* isolate-Pf008 against *Sclerotinia sclerotiorum* (Fig 3 and 4). The study of DeMelo and Faull (2000) reported that, *T. harzianum* and *T. koningii* were effective in inhibiting the mycelium growth of *R. solani*. A Similar result was obtained by Dutta *et al.* (2008). They studied the *in vitro* efficacy of bio-inoculants by dual culture method against *S. sclerotiorum.* Results revealed that, the best mycelium growth reduction was found to be caused by *Pseudomonas fluorescens*, which caused 64.93% mycelium growth reduction, followed by *Bacillus subtilis* (62.86%) and *Trichoderma harzianum* (59.08%). However, Sharma *et al.* (2016) recorded maximum

Table 2: Effect of different inoculation techniques of bio-inoculants on growth promotion and suppression of white mold disease of french bean under pot condition.

Treatment	Treatment details	Germination %	Growth parameter		% Disease Incidence	% Disease control
			Shoot length (cm)	Root length (cm)		
T_1	Seed treatment with *Trichoderma harzianum* isolate TS001	93.33	48.35	15.58	7.06	87.29
T_2	Seed treatment with *Pseudomonas fluorescence* isolate-Pf008	86.66	44.24	12.89	7.69	86.16
T_3	Seed treatment with *Trichoderma harzianum* isolate TS001+ *Pseudomonas fluorescence* isolate-Pf008	86.66	45.69	11.76	15.38	72.31
T_4	Soil application of *Trichoderma harzianum* isolate TS001 enriching with FYM	93.33	42.45	13.46	8.33	85.00
T_5	Soil application of *Pseudomonas fluorescence* isolate-Pf008 enriching with FYM	86.66	39.13	12.38	7.69	86.16
T_6	Soil application of *Trichoderma harzianum* isolate TS001+ *Pseudomonas fluorescence* isolate enriching with FYM	80.00	36.32	9.94	8.33	85.00
T_7	Seed bio-priming with *Trichoderma harzianum* isolate TS001	86.66	38.38	13.76	7.69	86.16
T_8	Seed bio-priming with *Pseudomonas fluorescence* isolate-Pf008	80.00	42.29	14.59	8.83	85.00
T_9	Seed bio-priming with *Trichoderma harzianum* isolate TS001+ *Pseudomonas fluorescence* isolate isolate-Pf008	66.66	40.28	11.76	10.00	81.99
T_{10}	Control (without treated)	60.00	32.56	8.28	55.55	0.00
	CD at 5%	3.54	4.31	1.31	2.654	-

69.8 % inhibition of *S. sclerotiorum* with *T. viride* and minimum by *Bacillus subtilis* (42.2%).

Seed germination test

The results showed that (Fig 5) *Trichoderma harzianum* isolate TS004 was best which showed 93.33% seed germination followed by formulation of *Trichoderma koningi* isolate TS007 with 90.00% seed germination. Similarly, Kumar *et al.* (2018) reported that maximum (85.82%) seed germination was observed in *Trichoderma viride* and minimum seed germination was found in *Pseudomonas fluorescens* (82.32%) as compared to control (70.96%). The study of Dubey *et al.* (2017) showed that seeds treated with Pusa 5SD (*T. harzianum*-IARI P4) in combination with *P. fluorescens* gave the highest (97.9%) seed germination.

Effect of different inoculation techniques of bio-inoculants on growth promotion and suppression of white mold disease of french bean under pot condition

The result form the Table 2 indicated that all the inoculation techniques of bio-inoculants were found effective in growth promotion and suppression of disease. The maximum germination (93.33%), growth promotion *viz.* shoot length (48.35 cm), root length (15.58 cm) and minimum disease incidence (7.06%) with reduction of disease (87.29%) over control was recorded in seed treatment with *Trichoderma harzianum* isolate TS001. While, in control germination per cent (60.00%), growth of plant *viz.* shoot length (32.56 cm), root length (8.28 cm) and disease incidence (55.55%) was recorded. The study of Zhang *et al.* (2016); Elsheshtawi

Table 3: Effect of formulated bio-inoculants on growth promotion of french bean under field conditions during year 2018-19 and 2019-20.

	2018-19						2019-20					
Treatment	Shoot length	Root length	No. of branches /plant	No. of pods /plant	Yield	% Increase in yield	Shoot length	Root length	No. of branches /Plant	No. of pods /Plant	Yield	% increase in yield
T_1	42.53	12.66	5.46	7.33	10.58	28.55	39.35	11.56	6.56	8.58	9.64	33.70
T_2	48.46	18.40	8.02	11.80	13.93	69.25	46.56	15.23	9.26	10.40	12.10	67.82
T_3	45.73	16.60	6.60	8.40	11.56	40.46	44.21	13.65	5.60	7.86	10.14	40.63
T_4	44.93	15.33	4.33	9.80	12.64	53.58	38.43	12.44	5.52	7.46	9.62	33.42
T_5	39.80	12.00	6.66	5.60	9.93	20.65	36.52	11.80	6.86	6.64	8.15	13.03
T_6	36.13	9.96	7.06	7.86	9.53	15.79	29.24	8.67	5.23	5.80	7.68	6.51
T_7	38.40	13.80	5.26	6.40	8.81	7.04	32.40	11.42	6.82	6.85	7.75	7.04
T_8	42.86	14.20	5.86	7.16	10.58	28.55	38.85	12.56	5.26	7.36	8.80	7.48
T_9	48.20	17.26	7.60	8.90	11.64	41.43	45.20	14.15	8.54	9.84	11.64	61.44
T_{10}	42.33	12.83	6.53	7.46	10.56	28.31	43.36	12.86	6.84	8.32	9.62	33.42
T_{11}	36.86	10.44	4.46	10.26	11.22	36.33	35.28	10.64	5.21	6.84	8.53	18.30
T_{12}	39.44	11.20	5.53	9.33	9.81	19.19	32.43	9.88	5.86	5.52	8.10	12.34
T_{13}	38.06	12.26	6.66	10.40	9.64	17.13	29.16	8.64	5.52	5.58	7.92	9.84
T_{14}	37.20	9.16	4.60	7.60	9.50	15.30	31.28	7.56	4.28	6.64	8.16	13.17
T_{15}	26.60	7.40	3.26	4.33	8.23	0.00	28.30	7.26	2.86	3.86	7.21	0.00
CD at 5%	4.32	1.40	0.62	0.90	1.10	-	3.87	1.19	0.64	0.76	0.95	-

*Details of the treatments are given in Table 4.

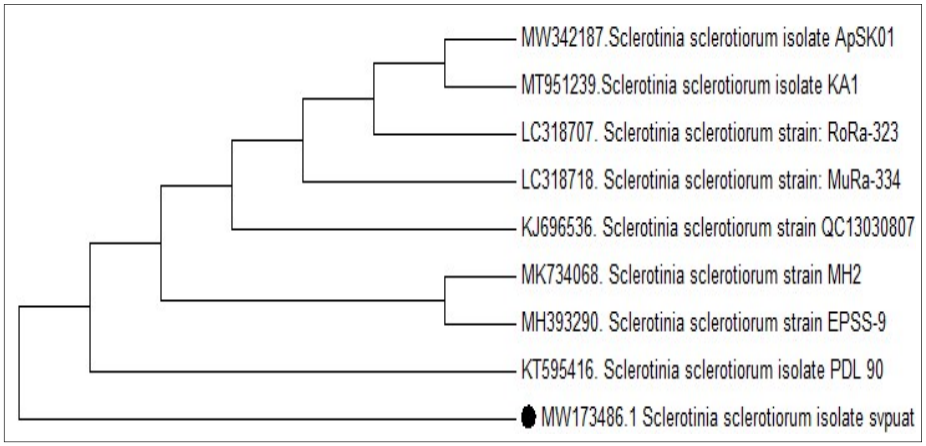

Fig 1: A phylogenetic tree generated using the neighbor-joining method showing the genetic relationship between the *Sclerotinia sclerotiorum* isolate svpuat and other *Sclerotinia sclerotiorum* isolates available in GenBank (NCBI).

et al. (2017) and Sumida *et al.* (2018) evinced that fungal bio-inoculants, such as *Trichoderma asperelloides*, *Trichoderma harzianum* and *Coniothyrium minitans* have been considered to be a viable alternative for the treatment of soybean seed. The result was also concordance with the findings of Dubey *et al.* (2017) they, treated seeds with Pusa 5SD (*T. harzianum*-IARI P4) in combination with *P. fluorescens* gave the seed germination (97.9%), pod yield (26.7 g/pot) and lowest disease incidence (4.50%). Soil application of Pusa bio-pellets of *T. harzianum* (IARI P4) 4.5% and *T. viride* (IBSD T-20) 9.00% also reduced disease incidence.

Evaluation of formulated bio-inoculants against white mold disease suppression and growth promotion in french bean under field conditions

Growth promotion

All the bio-inoculants were found to enhance the growth promotion and increase the yield as compared to control (Table 3). Data recorded during the year 2018-19 showed that seed treatment with formulation of *Trichoderma harzianum* isolate TS004 was best followed by soil application with formulation of *Trichoderma harzianum* isolate TS004 for growth promotion and increased the yield of french bean. The maximum shoot length (48.46 cm), root length (18.40 cm), number of branches/plant (7.02), number of pod/plant (11.80), yield 13.93 q/ha and increase in the yield 69.25% over control was recorded in seed treatment with formulation of *Trichoderma harzianum* isolate TS004. While, in case of control shoot length (26.60 cm), root length (7.40 cm), number of branches/plant (3.26), number of pods/ plant (4.33) and yield (8.23 q/ha) were recorded.

Similarly, in the year 2019-20, data showed that, seed treatment with the formulation of *Trichoderma harzianum isolate* TS004 was best followed by seed treatment with the formulation of *Trichoderma viride* isolate TS006 for growth promotion and increased the yield of French bean. The maximum shoot length (46.56 cm), root length (15.23 cm), number of branches/plant (9.26), number of pods/plant (10.40), yield 12.10 q/ha and increase in yield 67.82% over control were recorded in seed treatment with the formulation of *Trichoderma harzianum isolate* TS004. However, in case of control shoot length (28.30 cm), root length (7.26 cm), number of branches/plant (2.86), number of pod/plant (3.86) and yield (7.21/ha) was recorded. The study of Dutta and Das, (1999) revealed that seed treatment with *Trichoderma harzianum* provided the highest yield (69.51 q/ha), followed by *Bacillus subtilis* and *Pseudomonas fluorescens*. The lowest yield (41.00 q/ha) was recorded in control plot where *S. sclerotiorum* was applied alone. Increased shoot length with higher yield observed in this study might be associated with a reduction in white mold incidence. Likewise, Dubey *et al.* (2017) reported that seeds treated with Pusa 5SD (*T. harzianum*-IARI P4) in combination with *P. fluorescens* gave the highest shoot (27.1 cm), root length (10 cm) and dry plant weight (1538.9 mg/plant). Soil application of Pusa bio-pellets of *T. harzianum* (IARI P4) and *T. viride* (IBSD T-20) also enhanced the shoot, respectively root lengths, dry plant weight.

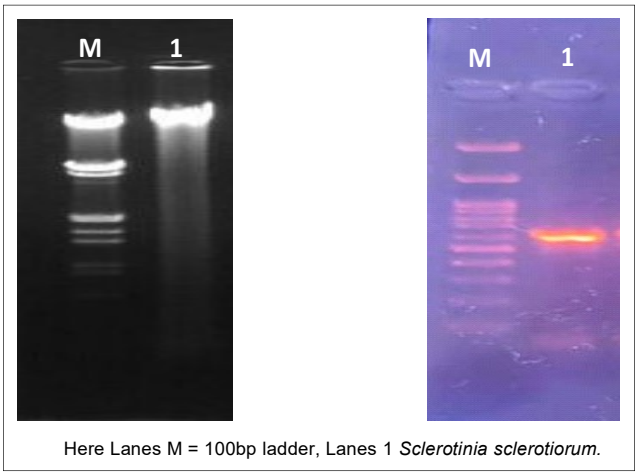

Fig 2: Agarose gel electrophoresis. A) Extracted genomic DNA. B) PCR products of ITS region approximately 600 bp.

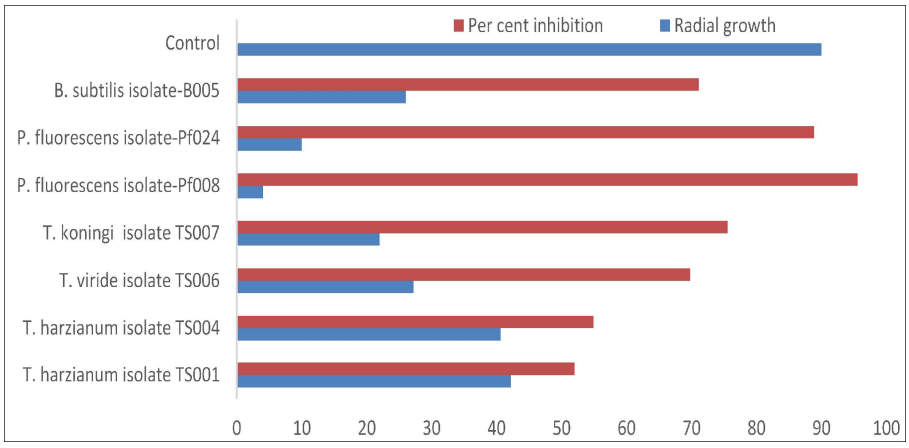

Fig 3: Efficacy of different bio-inoculants against *Sclerotinia sclerotiorum.*

Disease reduction

The bio-inoculants which were inoculated as seed treatment and soil application showed better results for reduction of disease incidence against white mold disease in french bean during 2018-19 (Table 4, Fig 6). The finding evinced that soil application with formulation of *Trichoderma viride* isolate TS006 was recorded best among all the treatments with minimum per cent disease incidence (8.59%) was recorded at 90 days after sowing with an reduction 83.91% of disease incidence over control in this treatment, followed by (8.69%) disease incidence reduction 83.72% of disease incidence over control was recorded in seed treatment with formulation of *Trichoderma harzianum* isolate TS004 at 90 days after sowing. However, disease incidence 53.40% was recorded in control (without treated).

Similarly in the year 2019-20 the minimum per cent disease incidence (6.69%) was recorded at 90 days after sowing with reduction of disease incidence (74.83%) over control in the seed treatment with formulation of *Trichoderma viride* isolate TS006, followed by 7.26% percent disease incidence reduction of disease incidence (72.68%) over control was recorded in seed treatment with formulation of *Trichoderma harzianum* isolate TS004 at 90 days after sowing. While, 26.58% disease incidence was recorded in control (without treated). The study was concordance with the finding of Sharma *et al*. (2016) they reported that minimum disease intensity (26.0%) was recorded with seed + soil application of *T. viride* followed by soil application of *T. viride* (35.7%) as compared to control (68.3%). Similarly, Kumar *et al.* (2018) evinced that the minimum pre-emergence (13.93%) and post-emergence (17.00%) root rot disease incidence was recorded in seed treatment with *T. viride* followed by seed treatment with *T. harzianum*. Maximum

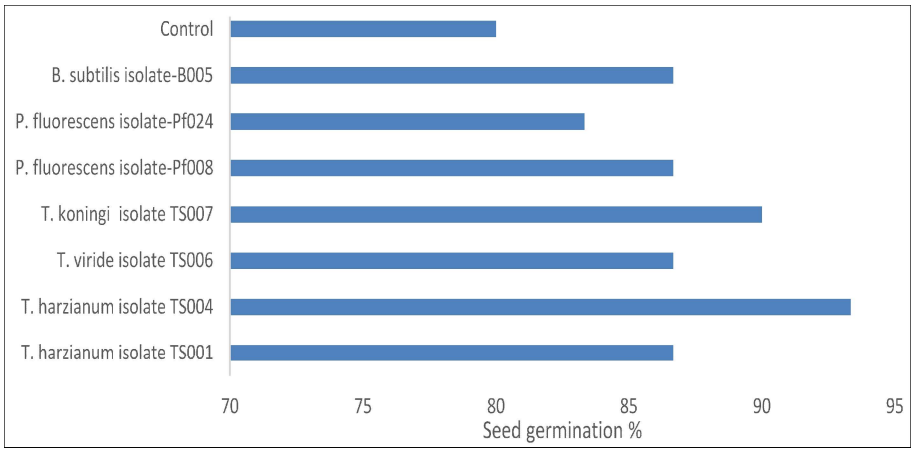

Fig 4: Effect of different bio-inoculants on seed germination.

Table 4: Effect of formulated bio-inoculants on suppression of white mold disease in french bean under field conditions during year 2018-19 and 2019-20.

Treatment	Treatment details	% Disease Incidence after 90 days		% Disease reduction over control	
		2018-19	2019-20	2018-19	2019-20
T_1	Seed treatment with formulation of *T. harzianum* isolate TS001	11.38	9.36	78.68	64.78
T_2	Seed treatment with formulation of *T. harzianum* isolate TS004	9.40	7.26	82.39	72.68
T_3	Seed treatment with formulation of *T. viride* isolate TS006	8.69	6.69	83.72	74.83
T_4	Seed treatment with formulation of *T. koningi* isolate TS007	11.69	12.48	78.10	53.04
T_5	Seed treatment with formulation of *P. fluorescens* isolate -Pf008	15.00	13.43	71.91	49.47
T_6	Seed treatment with formulation of *P. fluorescens* isolate Pf024	15.38	15.58	71.19	41.38
T_7	Seed treatment with formulation of *B. subtilis* isolate B005	20.00	17.36	62.54	34.68
T_8	Soil application with formulation of *T. harzianum* isolate TS001	13.04	10.56	75.58	60.27
T_9	Soil application with formulation of *T. harzianum* isolate TS004	9.69	8.92	81.85	66.44
T_{10}	Soil application with formulation of *T. viride* isolate TS006	8.59	7.54	83.91	71.63
T_{11}	Soil application with formulation of *T. koningi* isolate TS007	12.67	12.67	76.27	52.15
T_{12}	Soil application with formulation of *P. fluorescens* isolate -Pf008	15.67	14.56	70.65	45.22
T_{13}	Soil application with formulation of *P. fluorescens* isolate Pf024	20.55	12.45	61.51	53.16
T_{14}	Soil application with formulation of *B. subtilis* isolate B005	25.69	14.68	51.89	44.77
T_{15}	Control (Without treated)	53.40	26.58	-	-
	CD at 5%	1.93	1.34	-	-

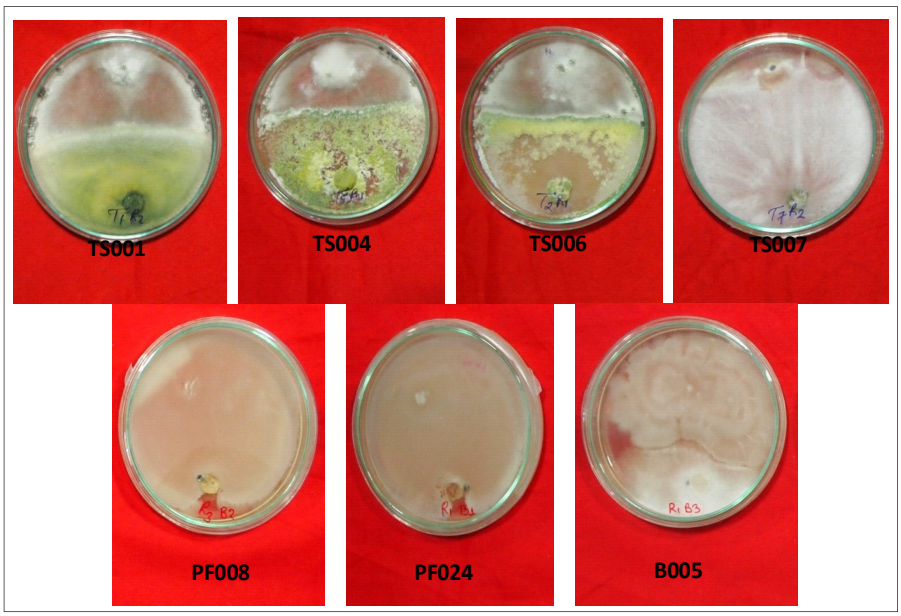

Fig 5: Antagonistic activity of bio-inoculants against *Sclerotinia sclorotiorum.*

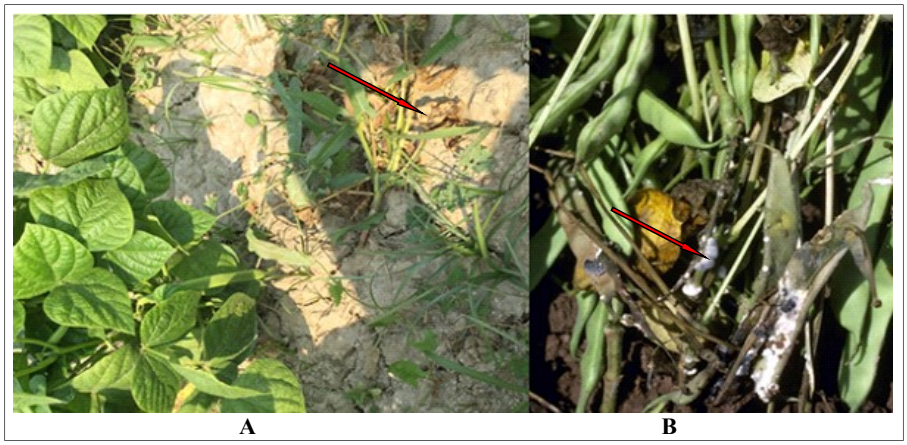

Fig 6: A) Infected plant with *Sclerotinia* stem rot symptom observed in french bean. B) Sclerotia produced by *Sclerotinia sclerotiorum* on pod of french bean.

pre-emergence (26.04 per cent) and post-emergence (31.50 per cent) root rot incidence were recorded in control.

CONCLUSION

Harmless and ecological alternatives to fungicidal management are urgent need to face increasing demand for safe, sustainable and effective management plans to white mold disease of french beans, counting on biocontrol agents and other best disease management tactics. This study demonstrated that the bio-inoculants could be considered a better alternative against *Sclerotinia sclerotiorum* than the fungicides *in vitro* and *in vivo*. The study suggests that all tested bio-inoculants could be used against *S. sclerotiorum* for management of white mold disease. Bacterial bio inoculant *Pseudomonas fluorescens* isolate-Pf008 was found best during *in vitro* study. Instead, during the field study, fungal bio-inoculants, specifically *Trichoderma viride* isolate-TS006 and *Trichoderma harzianum* isolate-TS004, were found to be the most effective for promoting growth and managing white mold disease of french bean.

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Conflict of interest

All authors declared that there is no conflict of interest.

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