

Efficacy of selected Bioagents against *Alternaria* leaf spot of French bean (*Phaseolous vulgaris* L.)

Abstract

French bean (*Phaseolous vulgaris* L.) is a significant food, legume crop utilized as a pulse and green vegetable crop, belongs to family fabaceae. The French bean is a cool-season, day-neutral vegetable that can withstand extreme temperatures. Area, Production and Productivity on French bean in India (2019-20) was 22.1 Mha, 2226 million Tonnes and 7-10 t/ha. They are high in protein and have a similar texture to meat. Green pods have 1.7 g protein, 0.1 g fat, 4.5 g carbohydrate, 1.8 g fibre, and are high in minerals and vitamins in a 100 g serving. Several diseases affect French bean crop majorly and cause yield loss. *Alternaria* leaf spot (*Alternaria alternata*), Angular leaf spot (ALS) (*Phaeoisariopsis griseola*), Bean rust (*Uromyces appendiculatus*), Anthracnose (*Colletotrichum lindemuthianum*), Aschochyta leaf spot (*Ascochyta phaseolorum*), Cercospora leaf spot (*Cercospora canescens*). Among them, *Alternaria* leaf spot of French bean is one of the most severe diseases, which is caused by *Alternaria alternata* in the French bean. An experiment was conducted in the Zaid season to check the efficacy of bioagents against *Alternaria alternata* on field conditions. Bioagents viz., *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* as seed treatment. An untreated replication served as control. Among the treatments, the maximum germination percentage, maximum plant height (cm) at 90 DAS, maximum no. of primary branches and secondary branches at 60 DAS was recorded in T₄ – *Trichoderma viride* @2.5% + *Pseudomonas fluorescens* @ 2.5% followed by T₁ – *Trichoderma viride* @ 5% as compared to untreated check control T₀. The minimum disease intensity (%) at 75 DAS was recorded in T₄ – *Trichoderma viride* @ 2.5% + *Pseudomonas fluorescens* @ 2.5 %, followed by T₁ – *Trichoderma viride* @ 5% as compared to untreated check control T₀.

Keywords: *Alternaria alternata*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*.

Introduction

In India, the French bean (*Phaseolous vulgaris* L.) is a significant food, legume crop utilized as a pulse and green vegetable crop, belongs to family fabaceae. The most important species of *Phaseolus* is the French bean, which originated in Central and South America. Bush bean is said to be a Central and South American native (Swaider *et al.*, 1992). It is a diploid ($2n=2x=22$) annual species (Galvan *et al.*, 2003). The French bean is mostly grown in North America and Europe, including the United States, England, Poland, Brazil, Mexico, Myanmar, China, and India. Green bean production in 2019 was 23.6 million tonnes, with China accounting for 79 percent of the total. In 2019, the world produced 26.8 million tonnes of dry beans, with Myanmar, India, and Brazil leading the way. Because the French bean is a short-season crop, producers may make more money in a shorter amount of time. French bean is sown on the plains of North India during two seasons: July-September and January-February. Sowing takes place in the highlands from March through May. The French bean is a cool-season, day-neutral vegetable that can withstand extreme temperatures. For maximum growth and pod yield, the French bean recommends a temperature range of 15 to 25 degrees Celsius (Rashid, 1999). They are high in protein and have a similar texture to meat. Green pods have 1.7 g protein, 0.1 g fat, 4.5 g carbohydrate, 1.8 g fibre, and are high in minerals and vitamins in a 100 g serving. According to reports, it contains both carminative and reparative characteristics that help with constipation and diarrhoea (Duke 1981). In India, ten states, namely Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Karnataka, Andhra Pradesh, Gujarat, Jharkhand, Tamil Nadu, and Telangana, account for more than 90% of total pulse output. The major states of Maharashtra, Punjab, and Andhra Pradesh provide roughly 2-2.5 tonnes ha⁻¹ of grain/dry seed productivity in India. Furthermore, the current seed yield of French bean is between 1250 and 1500 kg/ha.

Alternaria leaf spot which is the most serious stumbling block to French bean production in the tropics and subtropics, with an estimated annual loss of 0.39 million tonnes (MT). The first to report symptoms of *Alternaria* leaf spot of French bean (Sami and Hagedorn 1969). The disease's symptoms began as small, brown, irregular lesions that grew in size and became dark brown in colour, oval in shape, and with indistinct zonations. The lesions frequently merged, resulting in huge necrotic areas. Partially defoliation, commencing with the lowest leaves, was also seen on occasion, resulting in a loss of plant vigour. This disease is caused by the *Alternaria alternata* fungus and other *Alternaria* species. Wind, rain, insects, and seed easily propagate spores formed on sick plants.

To control *Alternaria alternata*, the causal pathogen of french bean leaf spot disease, various control strategies have been investigated, including chemical, biological, cross protection, farming practices, and resistant cultivars (Hiremath and Sundaresh, 1985). Chemical management of this pathogen has yielded positive effects on occasion, but inappropriate use of fungicides frequently results in environmental pollution and *Alternaria*

alternata resistance. Biological control is considered an important approach for controlling many fungal plant pathogens and exploration for new biological agents is increasing as potential biological control antagonists. (**Deshmukh et al., 2010**). *Trichoderma spp.* is most promising and effective biocontrol agent (**Bendahmane et al., 2012, Tran 2010**).

One such attempt has been made to evaluate the efficacy of selected Bioagents viz., *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis* as seed treatment. against *Alternaria* leaf spot of French bean (*Phaseolous vulgaris* L.) *in vivo*

Material and Methods

Experimental site:

The experiment was conducted at the Central Research Field and Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj during ZAID season 2021.

Methodology:

Collection of disease samples:

Plants showing typical symptoms, in the field of standing crop i.e., the infected plant part of Frenchbean is selected. These disease plant materials were brought to the lab for further investigation.

Identification of the fungus by slide preparation:

Examination of the fungal colony characteristics was done through microscopic examination. Using a sterile needle, a small portion of the infected plant part was taken and placed on a sterile glass slide. It was stained using lactophenol and cotton blue and covered with the coverslip. Then, the microscope was used for the examination of morphological characteristics of fungal structures (**Grahovac et al., 2012**).

Morphological Characters of *Alternaria alternata* :

The conidia were simple, obclavate, pale to dark brown in colour and generally formed in chains. Conidia have both transverse and vertical septa (muriform) conidia. Conidiophores pale to dark brown in colour. Hyphae were brown with light brown conidiophores at the top of each branch (**Meena et al., 2014**).

Evaluation of selected bioagents *in vivo*:

The efficacy of selected bioagents against *Alternaria alternata* was carried out in field condition.

Disease intensity:

Disease intensity (%) formula given by **Wheeler (1969)** was used for the calculation:

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of rating} \times \text{Maximum disease grade}} \times 100$$

Observations recorded:

Observations were recorded during the course of experiment. Observations were plant height, number of Primary and secondary branches per plant and disease intensity.

Results

Table.1 Efficacy of selected bioagents on plant height (cm), number of primary and secondary branches per plant

Tr. No.	Treatments	Plant height (cm)			No. of primary branches/plant	No. of secondary branches/plant
		30 DAS	60 DAS	90 DAS		
T ₀	Control (untreated check)	9.200	15.80	24.83	2.667	2.867
T ₁	<i>Trichoderma viride</i> @ 5%	15.86	20.83	42.76	4.067	4.533
T ₂	<i>Pseudomonas fluorescens</i> @5%	14.86	20.46	41.13	3.133	4.467
T ₃	<i>Bacillus subtilis</i> @5%	11.10	17.66	27.30	3.000	3.533
T ₄	<i>Trichoderma viride</i> @ 2.5%+ <i>Pseudomonas fluorescens</i> @2.5%	16.43	22.16	51.66	4.667	5.800
T ₅	<i>Trichoderma viride</i> @ 2.5% + <i>Bacillus subtilis</i> @ 2.5%	12.16	19.46	37.96	3.103	4.000
S. Ed.(±)		0.087	0.187	0.182	0.210	0.178
CD (5%)		0.195	0.421	0.411	0.093	0.079

Table.2 Effect of selected botanicals on disease intensity of frenchbean at 45, 60, and 75 DAS:

Tr. No.	Treatments	Disease Intensity (%)			MEAN
		45 DAS	60 DAS	75 DAS	
T ₀	Control (untreated check)	16.00	24.53	49.33	29.95
T ₁	<i>Trichoderma viride</i> @ 5%	6.22	14.93	34.66	18.60
T ₂	<i>Pseudomonas</i> <i>fluorescens</i> @5%	7.55	17.60	38.47	21.21
T ₃	<i>Bacillus</i> <i>subtilis</i> @5%	12.44	22.40	44.00	26.28
T ₄	<i>Trichoderma viride</i> @ 2.5%+ <i>Pseudomonas</i> <i>fluorescens</i> @2.5%	5.77	12.00	33.71	17.16
T ₅	<i>Trichoderma</i> <i>viride</i> @ 2.5% + <i>Bacillus</i> <i>subtilis</i> @ 2.5%	8.44	18.40	37.52	21.45
S.Ed.(±)		0.650	0.76	0.683	0.763
CD (5%)		1.466	1.70	1.542	2.436

Plant height (cm):

The statistical analysis of data showed that all treatments were found significantly effective. Result showed that the minimum height was recorded in control (24.833) and the maximum height was observed in the combination treatment of *Trichoderma viride* @ 2.5+ *Pseudomonas fluorescens* @2.5%(51.677), followed by *Trichoderma viride* @ 5% (42.767) followed by *Pseudomonas fluorescens* @ 5% (41.133) followed by *Trichoderma viride* @ 2.5%+ *Bacillus subtilis*@ 2.5% (37.967), and finally *Bacillus subtilis*@ 5% (27.300).

Among the different treatments that all the treatments are statically significant over control, the treatments (T₁,T₂) are found non -significant to each other at 90 DAS plant height.

Number of primary and secondary branches per plant:

The statistical analysis of data showed that all treatments were found significantly effective. Result showed that the minimum primary branches was recorded in control (2.667) and the maximum primary branches was observed in the combination treatment of *Trichoderma viride* @ 2.5%+ *Pseudomonas fluorescens* @2.5% (4.667), followed by *Trichoderma viride* @ 5% (4.067) followed by *Pseudomonas fluorescens* @ 5% (3.133) followed by *Trichoderma viride* @ 2.5%+ *Bacillus subtilis*@ 2.5% (3.103), and finally *Bacillus subtilis*@ 5% (3.000).

The statistical analysis of data showed that all treatments were found significantly effective. Result showed that the minimum secondary branches was recorded in control (2.867) and the maximum secondary branches was observed in the combination treatment of *Trichoderma viride* @ 2.5%+ *Pseudomonas fluorescens* @2.5%(5.800), followed by *Trichoderma viride* @ 5% (4.533) followed by *Pseudomonas fluorescens* @ 5% (4.467) followed by *Trichoderma viride* @ 2.5%+ *Bacillus subtilis*@ 2.5% (4.000), and finally *Bacillus subtilis*@ 5% (3.533).

Among the different treatments that all the treatments are statistically significant over control, the treatments are found non -significant to each other are T₂,T₅ and T₅,T₃.

Disease intensity (%):

The disease intensity of the French bean *Alternaria alternata* under different treatments at mean disease .The minimum disease intensity was recorded in the treatment combination of the *Trichoderma viride* @2.5% +*Pseudomonas fluroscens* @2.5% is (17.163) followed by *Trichoderma viride* @ 5% (18.608) followed by *Pseudomonas fluroscens*@5% (21.210) followed by *Trichoderma viride* @2.5%+ *Bacillus subtilis*@ 2.5 % (21.455) and finally by *Bacillus subtilis*@ 5% (26.281).

The treatment combinations of mean value of disease intensity shows that the *Trichoderma viride* @2.5% +*Pseudomonas fluroscens* @2.5% (17.163) followed by *Trichoderma viride* @ 5% 18.608 are best treatment in the disease management of the French bean *Alternaria alternata*.

Among the different treatments that all the treatments are statistically significant over control , the treatments T₅,T₂ and T₁,T₄ are found non -significant to each other at three mean readings.

Discussion

The results in the increased plant height were observed highest at treatments combinations of the bacterial and fungal biocontrol combination of the *Trichoderma viride* and *Pseudomonas fluroscens*. *Pseudomonas fluroscens* is PGPR bacteria the previous findings of **Yadav et al., 2014** the performance of three rhizosphere competent microbial strains like *Rhizobium*, *Pseudomonas* and *Trichoderma viride* promoted good better plant growth in both the crops of chickpea and rajma. It was also observed that the combined application of the

microbes enhanced seed germination and plant growth better than their individual application. The bio priming of the seeds with the suitable bacterial and fungal micro flora helps to produce more growth promoting factors that helps the root and shoot elongation. The increase soil micro flora makes the microbial to soluble the micronutrients which help in active uptake of the nutrients and overall development of the plant shoot system.

The branches are the most important vegetative growth stages the treatment combination of the both biocontrol and fungal antagonist bioagents *Trichoderma viride* and *Pseudomonas fluorescens* shows the highest number of the primary and secondary branches. The present findings were supported by the **Negi et al., 2014** as the antagonistic combinations of *Trichoderma harzianum* and *Pseudomonas fluorescens* were found to be most effective in promoting the plant growth activities as well as increasing yield parameters of pea crop in field conditions.

The present research findings were obtained and the comparison of the present investigation with the previous findings with various researches reveals that the treatment combinations of *Trichoderma viride* + *Pseudomonas fluorescens* and *Trichoderma viride* shows most effective in the management of foliar disease of *Alternaria alternata* of cow pea. The present research findings have been supported by the **Thakur (2017), Kayim et al. (2018)** the biocontrol agents have the capability of suppressing the growth of the pathogen by the lysis of their cell wall and **Li et al ., (2015)** states that the bacterial biocontrol have the capability of the regulating the growth of mycelium and germ tubes of the fungal pathogens ,therefore the combination of the both the bacterial and fungal bioagents plays a dual role in the inhibiting the sporulation and their management of the *Alternaria alternata*.

Conclusions

From the above results and summary it has been concluded that the seed treatment for the management of the alternaria leafspot in French bean shows that the combination of the seed treatment show very effective in management the disease intensity with the mean value of final *Trichoderma viride* @ 2.5%+ *Pseudomonas fluorescens* @2.5%(17.163) followed by *Trichoderma viride* @ 5% (18.608) followed by *Pseudomonas fluorescens*@5% (21.210) %. The treatments also find most effective in other growth parameters like plant height, germination percentage and branches.

The probable reason may be due to the bioprimering of the seed with antagonistic bacteria and fungi flora bio-agents might have triggered the host cells for the increased synthesis of phytohormones, which may have appreciably involved in the enhanced growth of the plants. These putative bio-agents might have also produced gluconases, chitinases etc, which might have played a role in the degradation of fungal propagules present in the seed tissues. The reduced germination and growth with respect *Bacillus* and *Trichoderma viride* + *Bacillus subtilis* treatment might be due to the high dose of the inoculums or incompatibility compare to other treatments. Due to nutritional competence, this treatment might have remained less promising over other treatment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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