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

Abstract

Frenchbean(*Phaseolousvulgaris*L.)isasignificantfood,legumecroputilizedasapulseand green vegetable crop, belongs to family fabaceae.The French bean is a cool-season, day- neutral vegetable that can withstand extreme temperatures. Area, ~~Production~~ production and ~~P~~Productivity onFrenchbeaninIndia(2019-20)~~was~~were22.1Mha,2226millionTonnesand7-10t/ha.Theyare highinproteinandhaveasimilartexturetomeat. Greenpodshave1.7gprotein,0.1gfat,4.5 g carbohydrate, 1.8 g fibre, and are high in minerals and vitamins in a 100 g serving. Several diseases affects 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*~~),~~) andCercospora leaf spot (*Cercospora canescens)*. Among them, Alternaria leaf spot of French bean is one of the most severe disease, which is caused by ~~*Alternaria*~~ *A.alternata* in the French bean. An experiment was conducted in the *Zaid* season to check the efficacy of bioagents against ~~*Alternaria*~~ *A.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 T_4 – ~~*Trichoderma*~~ *T.viride* @2.5%+ ~~*Pseudomonas*~~ *P.fluorescens* @ 2.5% followed by T_1 – ~~*Trichoderma*~~ *T.viride* @ 5% as compared to untreated check control T_0 . The minimum disease intensity (%) at 75 DAS was recorded in T_4 – ~~*Trichoderma*~~ *T.viride* @ 2.5% + ~~*Pseudomonas*~~ *P.fluorescens* @ 2.5 %, followed by T_1 – ~~*Trichoderma*~~ *T.viride* @ 5% as compared to untreated check control$T_{0.}$

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 speciesof*Phaseolus*istheFrenchbean,whichoriginatedinCentralandSouthAmerica.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 AmericaandEurope,includingtheUnitedStates,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 podshave 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).ThefirsttoreportsymptomsofAlternarialeafspotofFrenchbean**(SamiandHagedorn 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 frequentlymerged,resultinginhugenecroticareas.Partiallydefoliation,commencingwiththe lowest leaves, was also seen on occasion, resulting in a loss of plant vigour. This disease is causedbythe*Alternariaalternata*fungusandotherAlternariaspecies.Wind,rain,insects,and seed easily propagate spores formed on sickplants.

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*~~A.

Comment [u1]: Cite recent references in introduction

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Comment [u3]: add: Biosecurity for reducing ochratoxin A productivity and their impact on germination and ultrastructures of germinated wheat grains. EM El-Taher, AEG TM, MS Ashour Journal of Microbiology, Biotechnology and Food Sciences 10 (1), 135-151 *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,SamHigginbottomUniversityofAgriculture,TechnologyAndSciences,Prayagraj during *ZAID* season2021.

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,themicroscopewasusedfortheexaminationofmorphologicalcharacteristics 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:

Comment [u4]: add : Efficacy of fungal rust disease on willow plant in Egypt
TM Abd El-Ghany, MET Eman, HH El-Sheikh
Australian Journal of Basic and Applied Sciences 3 (3), 1527-1539

Comment [u5]: Add: Entomopathogenic fungi and their role in biological control
TM AbdelGhany
El-Ghany, TMA, Ed, 1-42

Comment [u6]: Add: Effectiveness of a biological agent (Trichoderma harzianum and its culture filtrate) and a fungicide (methyl benzimacold-2-ylcarbamate) on the tomato rotting activity (growth …
TM Abdel-Ghany, MM Bakri
Bioresources 14 (1), 1591-1602

Evaluation of natural sources for repress cytotoxic Trichothecenes and Zearalenone production with using Enzyme-linked immunosorbent assay
TM Abd El-Ghany, MA Ganash, MM Bakri, AMH Al-Rajhi, MA Al Abboud
Life Sci. J 13 (8), 74-86

Mycoparasitic nature of Egyptian Trichoderma isolates and their impact on suppression Fusarium wilt of tomato
AM Nofal, MA El-Rahman, TM Abdelghany, A El-Mongy
Egyptian Journal of Biological Pest Control 31 (1), 1-8

Significance of Local Trichoderma Isolates in Controlling Pythium ultimum and Rhizoctonia solani on Bean in Egypt
AM Nofal, TM Abdelghany, WFM Abd-EL-Hamed
Egyptian Journal of Phytopathology 49 (2), 131-140

Comment [u7]: Fungal leaf spot of maize: pathogen isolation, identification and host biochemical characterization
TM Ghany
Mycopath 10 (2)

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

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

Comment [u9]: describe the 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	*Trichoderma viride* @ 5%	15.86	20.83	42.76	4.067	4.533
T_2	*Pseudomonas fluroscens*@5%	14.86	20.46	41.13	3.133	4.467
T_3	*Bacillus subtilis*@5%	11.10	17.66	27.30	3.000	3.533
T_4	*Trichoderma viride* @ 2.5%+ *Pseudomonas fluorescens* @2.5%	16.43	22.16	51.66	4.667	5.800
T_5	*Trichoderma viride*@ 2.5% + *Bacillus subtilis*@ 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 (%)			
		45 DAS	60 DAS	75 DAS	MEAN
T_0	Control (untreated check)	16.00	24.53	49.33	29.95
T_1	*Trichoderma viride* @ 5%	6.22	14.93	34.66	18.60
T_2	*Pseudomonas fluroscens*@5%	**7.55**	17.60	38.47	21.21
T_3	*Bacillus subtilis*@5%	12.44	22.40	44.00	26.28
T_4	*Trichoderma viride @ 2.5%+ Pseudomonas fluorescens* @2.5%	5.77	12.00	33.71	17.16
T_5	*Trichoderma viride*@ 2.5% + *Bacillus subtilis*@ 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 stastically significant over control,thetreatments(T_1,T_2)arefoundnon-significanttoeachotherat90DASplantheight**.**

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.Resultshowedthattheminimumsecondarybrancheswasrecordedincontrol(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 T_2,T_5 and T_5,T_3.

Disease intensity (%):

The disease intensity of the French bean ~~*Alternaria* ~~*A.alternata* under different treatments at mean disease .The minimum disease intensity was recorded in the treatment combinationofthe~~*Trichoderma*~~*T.viride*@2.5%+~~*Pseudomonas*~~*P.fluroscens*@2.5%is(17.163) followed by *Trichoderma viride* @ 5% (18.608) followed by *Pseudomonas fluroscens*@5% (21.210) followed by ~~*Trichoderma* ~~*T.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* ~~*T.viride @2.5%* +~~*Pseudomonas* ~~*P.fluroscens* @2.5% (17.163) followed by ~~*Trichoderma*~~*T.viride*@5%18.608arebesttreatmentinthediseasemanagementoftheFrench bean ~~*Alternaria*~~*A.alternata.*

Among the different treatments that all the treatments are statistically significant over control,thetreatmentsT_5,T_2andT_1,T_4arefoundnon-significanttoeachotheratthreemean readings.

Discussion

The results in the increased plant height were observed highest at treatments combinationsofthebacterialandfungalbiocontrolcombinationofthe~~*Trichoderma*~~*T.viride*and *Pseudomonas fluroscens. Pseudomonas fluroscens* is PGPR bacteria the previous findings of **Yadav *et al.*,** **(2014) Abdelghany et al., where** the performance of three rhizosphere competent microbial strains like Rhizobium, *Pseudomonas* and *Trichoderma viride* promoted good better plant growth in both thecropsofchickpeaandrajma.Itwasalsoobservedthatthecombinedapplicationofthe

Comment [u10]: Role of biofertilizers in agriculture: a brief review
MA Al Abboud, TMA Ghany, MM Alawlaqi
Mycopath 11 (2)

Maize (Zea mays L.) growth and metabolic dynamics with plant growth-promoting rhizobacteria under salt stresses
TM Abd El-Ghany, YS Masrahi, A Mohamed, A Abboud, MM Alawlaqi, ...
Journal of Plant Pathology and Microbiology 6 (9), 305

microbes enhanced seed germination and plant growth better than their individualapplication. Thebiopriming oftheseedswiththesuitablebacterialandfungalmicroflorahelpstoproduce moregrowthpromotingfactorsthathelpstherootandshootelongation.Theincreasesoilmicro flora makes the microbial to soluble the micronutrients which help in active uptake of the nutrients and overall development of the plant shootsystem.

The branches are the most important vegetative growrth stages the treatment combination of the both biocontrol and fungal antagonist bioagents *Tichoderma viride* and *Pseudomonas fluroscenes* shows the highest number of the primary and secondary branches. The present findings were supported by the **Negi *et al.*, 2014 as the** antagonisticcombinations of *Trichoderma harzianum* and *Pseudomonas fluorescens* were found to be most effective in promotingtheplantgrowthactivitiesaswellasincreasingyieldparametersofpeacropinfield 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 combinationsof*Trichodermaviride*+*Pseudomonasfluroscens*and*Trichodermaviride*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 lysisof their cell wall,~~and~~ beside **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 *Alternariaalternata*.

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 offinal~~*Trichoderma*~~*T.viride@2.5%+*~~*Pseudomonas*~~*P.fluorescence*@2.5%(17.163)followedby ~~*Trichoderma*~~ *T.viride* @ 5% (18.608) followed by ~~*Pseudomonas*~~ *P.fluroscenes*@5% (21.210) %. The treatments also find most effective in other growth parameters like plant height, germination percentage andbranches.

Theprobablereasonmaybeduetothebioprimingoftheseedwithantagonisticbacteria 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 propogules 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 othertreatment.

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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