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IN VITRO SCREENING OF EFFECTIVE BIOCONTROL AGENTS AGAINST BEAN ANTHRACNOSE PATHOGEN, *COLLETOTRICHUM LINDEMUTHIANUM*

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ABSTRACT

Identification and selection of effective isolate of biocontrol agents is the first and foremost step in biological control. Biocontrol agents were selected based on their antagonistic activity against mycelial growth of the pathogen by following the dual culture technique. Evaluation of *Trichoderma viride* isolates against *C. lindemuthianum* growth under *In vitro* condition showed that all the isolates were effective in inhibiting the pathogen growth. Among the seven isolates (TVMGLT1, TV21GL, TVMNT7, *T. harzianum*, TV15, TVUV10 and TVMG5) tested, isolate TVMGLT1 recorded highest mycelial inhibition of 75.00% reduction over control and highest inhibition zone of 18.00mm. The increased antagonist overgrowth on the *C. lindemuthianum* was observed in TVMGLT1 (65.00mm) and TV21GL (63.00mm). Though all the isolates showed mycelial inhibition, the isolate TVMGLT1 recorded greater antagonistic activity against the growth of *C. lindemuthianum*. The bacterial antagonists like, *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were evaluated against *C. lindemuthianum* growth. Among five *Pseudomonas fluorescens* isolates (PFSR, PF1, PFHS, PF8 and PFOOTY1) tested, PFSR showed higher inhibitory effect (33.33%) and highest inhibition zone of 20.00mm. Similarly, among four *Bacillus subtilis* isolates (BSG3, M2, CBE 4 and G1) tested, BSG3 recorded higher inhibition in mycelial growth (14.28%) and inhibition zone of 5.00mm.

key words: Bean anthracnose, *In vitro* screening, *Trichoderma viride*, *Pseudomonas fluorescens*, *Bacillus subtilis*.

INTRODUCTION

Anthracnose disease caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara is a major limiting factor in field grown beans (*Phaseolus vulgaris* L.) in subtropical and temperate regions. Anthracnose is mainly a seed-borne disease caused by a fungus which has a wide host range on many legume species. This disease can cause serious losses in bean crops in temperate and subtropical zones. Leaves, stems and pods of bean plants are susceptible to infection. Small reddish-brown, slightly-sunken spots form on the pods and rapidly develop into large, dark-sunken lesions. In moist weather, masses of pink spores develop on these lesions. Black-sunken spots, similar to those on the pods, are produced on the stems and the leaf stalks. Infection of the leaves causes blackening along the veins, particularly on the undersurface. Yield losses can reach or exceed 90%, especially in cases of susceptible bean varieties grown under favorable conditions for the pathogen [1, 2].

The fungus can survive in plant debris [3] and by the occasional formation of sclerotia [4], but infected seed is the primary source of inoculums [5]. Young seedlings often suffer from damping-off. The fungus produces globular infection vesicles and then large-diameter primary hyphae that develop inside host cells, feed biotrophically and produce systemic infection [6]. Symptoms on bean include black sunken cankers in the pods containing a salmon-colored center consisting of conidia. The pathogen also affects petioles, leaf veins, stems, and seeds producing

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characteristic anthracnose sunken lesions filled with many spores.

Management of *C. lindemuthianum* in beans is limited by the use of hazardous chemical fungicides. The biocontrol agents are the viable alternates for the management of many plant pathogenic microbes. Besides the effective and economical management of the diseases, the biocontrol agents reduce the risk of hazardous chemical residues. Plant growth promoting rhizobacteria (PGPR) can improve the growth of certain plants and be potential agents for biological control of plant pathogens resulting in disease suppression by antagonism and competition for space and nutrients, production of antifungal compounds, and induction of systemic resistance [7-9].

C. lindemuthianum is an important problem of beans in Tamil Nadu. Alternative control methods have not been adequately investigated. This study was conducted with the objective of screening of effective isolates of *Trichoderma*, *Pseudomonas* and *Bacillus* in order to manage the bean anthracnose infection caused by *C. lindemuthianum.*

Materials and methods

Colletotrichum lindemuthianum isolates

C. lindemuthianum was isolated from various markets and fields in and around Coimbatore. Totally sixteen isolates were collected and pathogenecity studies were conducted, among the isolates, the isolate cl7 was found to be highly virulent and was used for further studies. The pathogen, *C. lindemuthianum* isolates were grown on potato dextrose agar (PDA) plates.

Antagonistic strains

The *Pseudomonas* (PFSR, PF1, PFHS, PF8 and PFOOTY1), *Bacillus* (BSG3, M2, CBE 4 and G1) and *Trichoderma* (TVMGLT1, TV21GL, TVMNT7, *T. harzianum*, TV15, TVUV10 and TVMG5) strains were isolated by using the serial dilution technique in Kings' B medium, Nutrient agar medium and *Trichoderma* selective medium respectively. The bacterial strains were identified according to the description given in Bergey's manual for Systematic Bacteriology. These isolates were maintained at -80°C with 50% glycerol.

The strains of *Pseudomonas* (Pf1 and PF8), *Bacillus* (CBE 4) and *Trichoderma* (TVMNT7, *T. harzianum* and TV15) were obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and others are native isolates.

Effect of biocontrol agents on the growth of C. *lindemuthianum*

Fungal and bacterial antagonistic strains were tested for their antagonistic activity against mycelial growth of *C. lindemuthianum* by following the dual culture technique. *Pseudomonas* cultures were streaked in a four cm line (1 cm away from the edge of the plate) on the Petri dish containing equal amount of KB and PDA medium. A nine mm mycelial disc of *C. lindemuthianum* was placed to the most distal point of the dish perpendicular to the bacterial streak.

Similarly, for the fungal antagonist a mycelial disc of the pathogen (9mm dia) was placed at one end and the mycelial disc of *Trichoderma* (9mm dia) was placed at the other end. The plates were incubated at room temperature for 5-7 days and mycelial growth of the pathogen, inhibition zone (mm) and per cent reduction over control were measured.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

Table 1. Effect of different *Tichoderma* isolates on the growth of *C. lindemuthianum*

S. No	Isolates	% growth reduction over control	Inhibition zone (mm)	Antagonistic over growth (mm)
1	TVMG5	32.00[a]	10.00	60.00
2	TVMGLT1	75.00[e]	18.00	65.00
3	TV21GL	62.50[d]	8.00	63.00
4	TVMNT7	50.00[c]	8.00	56.00
5	*T. harzianum*	50.00[c]	15.00	61.00
6	TV15	37.50[b]	10.00	58.00
7	TVUV10	37.50[b]	14.00	50.00
8	Control	-	0.00	0.00

Values are the mean of four replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Table 2. Effect of different isolates of *Pseudomonas* on the growth of *C. lindemuthianum*

S. No	Isolates	% growth reduction over control	Inhibition zone (mm)
1	PFSR	33.33[e]	20.00
2	PF1	26.19[d]	15.00
3	PFHS	4.70[a]	15.00
4	PF8	7.10[b]	8.50
5	PFOOTY1	9.00[c]	10.00
6	Control	-	0.00

Values are the mean of four replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Table 3. Effect of different isolates of *Bacillus* on the growth of *C. lindemuthianaum*

S. No	Isolates	% growth reduction over control	Inhibition zone (mm)
1	BSG3	14.28[d]	5.00
2	M2	6.66[b]	3.50
3	CBE4	4.70[a]	2.00
4	G1	9.50[c]	2.00
5	Control	-	0.00

Values are the mean of four replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

RESULTS

In vitro experiments

Evaluation of *Trichoderma viride* isolates against *C. lindemuthianum* growth under *In vitro* condition showed that all the isolates were effective in inhibiting the pathogen growth. Among the seven isolates (TVMGLT1, TV21GL, TVMNT7, *T. harzianum*, TV15, TVUV10 and TVMG5) tested, isolate TVMGLT1 recorded highest mycelial inhibition of 75.00% reduction over control with the highest inhibition zone of 18.00mm. The increased antagonist overgrowth on the *C. lindemuthianum* was observed in TVMGLT1 (65.00mm) and TV21GL (63.00mm) (Table 1). Though all the isolates showed mycelial inhibition, the isolate TVMGLT1 recorded greater antagonistic activity against the growth of *C. lindemuthianum*. The bacterial antagonists like, *Pseudomonas fluorescens* and *Bacillus subtilis* isolates were evaluated against *C. lindemuthianum*. Among five *Pseudomonas fluorescens* isolates (PFSR, PF1, PFHS, PF8 and PFOOTY1) tested, PFSR showed higher inhibitory effect (33.33%) with highest inhibition zone of 20.00mm (Table 2). Similarly, among four *Bacillus subtilis* isolates (BSG3, M2, CBE 4 and G1) tested, BSG3 recorded higher inhibition in mycelial growth (14.28%) with a inhibition zone of 5.00mm (Table 3).

DISCUSSION AND CONCLUSION

The management of post-harvest diseases by the use of chemicals has been the primary method. However, a number of chemicals are withdrawn from the market because of development of resistance by the pathogen or for human health hazards due to residual toxicity. Biological control offers a best alternative for the control of post-harvest diseases of fruits and vegetables. Biocontrol agents were selected based on their antagonistic activity against mycelial growth of the pathogen by dual culture technique.

Plant growth promoting rhizobacteria (PGPR) are potential agents for biological control of plant pathogens [10]. However, the practical value of a biocontrol agent is greater when it is proved to be effective against a variety of pathogens and in various conditions. The mycoparasitic potential of *Pseudomonas* spp. is well documented. This phenomenon has often been used as means for *In vitro* screening of biocontrol agents. This study deals with assessment of the effect of *P. flourescens*, *B. subtilis* and *T. viride* on mycelial growth of *the C. lindemuthianum.* Bardas et al [11] reported that *P. chlororaphis* PCL1391 alone or combined with *P. fluorescens* WCS365 can be a potential factor in integrated control systems against bean anthracnose in Greece.

In the present study, *In vitro* experiments showed that, among the biocontrol agents tested, *T. viride* TVMGLT1 recorded highest mycelial inhibition of 75.00% reduction over control and highest inhibition zone of 18.00mm. The increased antagonist overgrowth on the *C. lindemuthianum* was observed in TVMGLT1 (65.00mm). Among five *P. fluorescens* isolates tested, PFSR showed higher inhibitory effect (33.33%) and highest inhibition zone of 20.00mm. Similarly, among four *B. subtilis* isolates tested, BSG3 recorded higher inhibition in mycelial growth (14.28%) and inhibition zone of 5.00mm. Soytong et al tested the efficacy of crude extracts from *Chaetomium cupreum* CC, *C. globosum* CG, *Trichoderma harzianum* PC01, *T. hamatum* PC02, *Penicillium chrysogenum* KMITL44 and antibiotic substances Rotiorinol, Chaetoglobosin-C and Trichotoxin A50 against the grape anthracnose caused by *Colletotrichum gloeosporioides* WMF01. All extracts and compounds inhibited the growth

of strain WMF01, with average ED50 values between 1 to 50 ppm. The inhibition of mycelial growth may be due to the production of antibiotics by the biocontrol agents. Production of antibiotics *viz.*, HCN, pyrrolnitrin, phenazine and 2, 4-diacetyl phloroglucinol and lytic enzymes by *P. fluorescens* against fungal pathogens were reported by many workers Ramamoorthy and Samiyappan [12], Ramamoorthy et al [13] and Radjacommare et al [14].

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