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BIOEFFICACY EVALUATION OF *SERRATIA MARCESCENS* AGAINST ANTHRACNOSE (*COLLETOTRICHUM LINDEMUTHIANUM* (SACC. & MAGNUS) BRIOSI & CAVARA) DISEASE IN DOLICHOS BEAN

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Abstract

Anthracnose is one of the most destructive diseases in dolichos bean plant. In the present study, we investigated the *in vitro* biological control of *Colletotrichum lindemuthianum* by using biocontrol agent such as *Serratia marcescens.* The antifungal activity was tested against the *C. lindemuthianum* by poisoned food technique. Among the different concentration of *S. marcescens* culture filtrate at 40 and 50 % conc. completely inhibited the mycelial growth, mycelial dry weight and conidial germination of *C. lindemuthianum* and also showed maximum germination, shoot, root length and vigour index of dolichos bean.

***Keywords*:** Dolichos bean, *Colletotrichum lindemuthianum, Serratia marcescens,* Mycelial growth, Mycelial dry weight, Conidial germination

Introduction

Dolichos bean (*Lablab purpureus* L. Sweet) is an important pulse-cum vegetable crop. It is widely cultivated throughout the tropics and subtropics as a vegetable crop because of its high nutritive value (up to 40% protein), ability to fix nitrogen, improving soil fertility and its drought tolerance. In India the young pods are consumed as fresh vegetables and mature dry seeds (18–36% protein content) are important in the diet of the vegetarian population. It is an important food source to people of all income categories, especially to the poor farmers as a source of dietary protein (Wortmann *et al.*, 1998).

Dolichos bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara is one of the most important seed borne diseases of dolichos bean in the world (Amin *et al.*, 2014). The disease is serious in areas that had cool and wet weather conditions, causing up to 100% yield loss (Pastor-Corrales and Tu, 1989). It is difficult to control *C. lindemuthianum* due to its wide host range, seed borne, air borne and prolonged survival in the seed and plant debris. Despite wide spread use of synthetic chemical for the management of plant diseases, recent advances about their environmental hazards warrants eco-friendly alternative method for disease control. Application of biocontrol agents is emerging as an acceptable method. In the present investigation, the biocontrol agent was taken up and their efficacy was studied against *C. lindemuthianum* under *in vitro* conditions.

Materials and Methods

Preparations of culture filtrate of *S. marcescens*

S. marcescens was inoculated into Erlenmeyer flasks containing 50 ml of sterile chitin luria broth and kept on a rotary shaker at 100 rpm for 48 h. Then the culture was filtered through bacteriological filter under vacuum and the filtrate thus obtained was used for testing their antagonistic ability over the pathogen.

Effect of culture filtrate of *S. marcescens* on the mycelial growth of *C. lindemuthianum*

The culture filtrate of *S. marcescens* was separately incorporated into sterilized PDA medium at 10, 20, 30, 40 and 50 per cent by adding the calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as control. The amended media were transferred to sterile Petri dishes separately @15 ml and allowed to solidify. Each plate was inoculated at the centre with seven days old (9 mm) PDA culture disc of *C. lindemuthianum*. The diameter of the mycelial growth (in mm) of *C. lindemuthianum* was measured when the mycelial growth fully covered the control plates.

Effect of culture filtrate of *S. marcescens* on the mycelial dry weight of *C. lindemuthianum*

Potato dextrose broth was poured 250 ml Erlenmeyer flasks and sterilized. The culture filtrates of *S. marcescens* were incorporated into sterilized PDA broth at 10, 20, 30, 40 and 50 per cent by adding the calculated quantity of the culture filtrate to the broth by means of a sterile pipette. Carbendazim at 0.1 per cent was added to the broth for comparison. Broth without any extracts served as control and three replications were maintained for each treatment. After the incubation period, the mycelial mat was harvested on a previously weighed filter paper and dried at 105°C in hot air oven till constant weight is reached, cooled in a desiccators and the mycelial weight was recorded and expressed as mg / 50 ml broth.

Effect of culture filtrate of *S. marcescens* on the conidial germination of *C. lindemuthianum* (Cavity slide method)

The culture filtrate of *S. marcescens* at 0.5 ml and spore suspension of the test fungus at 0.5ml (100 spores/ml) were mixed in a cavity slide and incubated for 48 h in Petri dish glass bridge moist chamber at $28+2^0$ C. Carbendazim 50% WP 0.1 per cent conc. was used for comparison. Cavity slides with sterile distilled water having spore suspension alone were kept as control. Observations were taken from 20 microscopic fields from each slide. The total number of conidia germinated under each microscopic field was recorded and per cent germination was calculated.

Seed Germination Bioassay

Roll Towel Method (ISTA, 1976)

The germination paper was soaked in water for 2 to 4 h to moist it evenly and to remove water soluble toxic substances present in it. Seeds of dolichos bean were surface sterilized with one per cent sodium hypochlorite for 30 sec., rinsed in sterile distilled water and dried overnight. Ten ml of the culture filtrate of *S. marcescens* with different concentration was taken in a Petri dish. To this, 100 mg of Carboxy methyl cellulose (CMC) was added as an adhesive material. One gram of seeds was soaked in 10 ml of culture filtrate suspension for 2 h and air dried overnight in a sterile Petri dish. The treated seeds were equidistantly placed between the two sheets of paper towel (27x20 cm), rolled carefully ensuring to pressure on seeds, wrapped with a polythene sheet to reduce surface evaporation and kept in germination chambers in an upright position. Each treatment was replicated thrice. They were incubated at room temperature (28 ± 2°C) for seven days and the following observations were recorded.

Seedling growth and vigour

The normal seedlings were selected at random from each replication and the shoot and root length from the collar at the tip of the primary root was measured and the respective mean values were recorded. The vigour index (VI) was calculated by using the formula suggested by Abdul Baki and Anderson, (1973).

Vigour Index = (Root length + Shoot length) x Germination percentage

The germination percentage was calculated by using the following formula.

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds sown}} \times 100$$

Results and Discussion

Effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *C. lindemuthianum*

The results of the *in vitro* studies conducted to find out the effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *C. lindemuthianum* are summarized in (Table 1).

The mycelial growth of *C. lindemuthianum* was found to be reduced with an increase in the conc. of culture filtrates of *S. marcescens* and the reduction was significantly the maximum in the case of *S. marcescens* with 37.54, 28.44, 9.25, 0.00 and 0.00 mm at 10, 20, 30, 40 and 50 per cent conc. of the culture filtrate respectively as against the maximum growth of 90.00 mm in the control. The same trend was maintained in the case of liquid medium assay. The flasks inoculated with pathogen and amended with culture filtrate of *S. marcescens* recorded significant reduction in the mycelial dry weight whereas, the flasks inoculated with *C. lindemuthianum* alone (control) recorded the maximum mycelial dry weight (341.65 mg). The minimum mycelial dry weight (1.20 mg) of *C. lindemuthianum* was recorded in 50 per cent conc. of the culture filtrate of *S. marcescens* which was at par with *S. marcescens* @ 40% and Carbendazim @ 0.1 per cent (1.23 and 2.30 mg respectively).

The antifungal metabolites produced by *S. marcescens* might be attributed as the reason for the reduction in the growth of the test pathogen. *S. marcescens* inhibited of mycelial growth of several pathogens including *Colletotrichum* spp. (Stefan Kurze *et al.,* 2001). The cell free extracts of *S. marcescens* effectively inhibited the growth of *R. Solani* (Strit *et al*., 1993). Someya *et al.* (2000) reported that *S. marcescens* completely inhibited the radial growth of *R. Solani* and *F. oxysporum* f.sp. *cyclaminis*. The culture filtrates of *S. marcescens* @ 40% completely inhibited on the mycelial growth and mycelial dry weight of *M. phaseolina* (Ezhilarasi, 2006). These earlier reports corroborates with the present findings.

In liquid media, the biomass production of *C. lindemuthianum* was strongly inhibited by *S. marcescens* at 40 per cent conc. (Table 1). Perusal of literature revealed that the inhibitory effect of *S. marcescens* against various fungal pathogens (Bruton *et al.,* 2003; Jaiganesh *et al.,* 2007). Sanjeevkumar, (2008) reported that the biomass production of *F. oxysporum* f.sp. *cubense* was strongly inhibited by *S. marcescens*. Several antifungal compounds from *S. marcescens* have been purified and characterized, such as chitinase, prodigiosin, and protease. These compounds attack the cell walls of phytopathogenic fungi to cause cell lysis and subsequent death. Interestingly, each strain of *S. marcescens* may have their own set of fungicides that allow them to fight against different spectra of fungi. The two chitinolytic enzymes and prodigiosin from *S. marcescens* strain B2 inhibited spore germination of grey mold pathogen *Botrytis cinerea* (Someya *et al*., 2001; Giri *et al*., 2004) while the chitinase from *S. marcescens* MO-1 inhibited the development of a number of phytopathogens such as *Alternaria citri, Fusarium oxysporum, Trichoderma harzianum, Aspergillus niger* and *Rhizopus oryzae* (Okay *et al.,* 2013). These reports are in line and add support to the present findings.

Effect of culture filtrates of *S. marcescens* on the conidial germination of *C. lindemuthianum*

The effect of culture filtrates on the conidial germination of *C. lindemuthianum* was studied and the results are summarized (Table 2). Among the various conc. of *S. marcescens* the culture filtrate tested, the conidial germination of *C. lindemuthianum* was completely inhibited by 40 and 50 per cent conc. of the culture filtrate and it was found to be on par with carbendazim 50% WP @ 0.1 per cent. The culture filtrates of *S. marcescens* @ 30 per cent conc. ranked next by significantly reducing the conidial germination to 10.15 per cent at 48 h observations. The culture filtrate of *S. marcescens* @ 10 per cent was found to be the least effective (48.43%) at 48h.

Several workers have reported about the inhibitory effect of *S. marcescens* on the conidial germination of *Fusarium* sp. (Jones *et al*., 1986; Brurberg *et al*., 1994). The cell free suspensions of *S. marcescens* drastically reduced the sclerotial germination and viability of *S. rolfsii* (Ordentlich *et al*., 1988). When sclerotia of *R. solani* were treated with the low and high molecular-weight fraction culture filtrate of *S. marcescens*, more than 70 per cent of the sclerotia failed to germinate (Someya *et al.,* 2000). *S. marcescens* effectively inhibited the conidial germination of *Phaeoisariopsis personata* causing late leaf spot in groundnut (Krishna

Kishore *et al.,* 2005). These earlier reports are analogous to the results of present findings.

Effect of *S. marcescens* on the dolichos bean seed germination and plant growth promotion (Roll towel method)

The data on the effect of antagonists on dolichos bean seed germination and growth promotion are presented in (Table 3). The culture filtrate of *S. marcescens* at various conc. showed no inhibitory effect on the germination of dolichos bean seeds and in general all the treatments induced the plant growth promotion *viz.,* shoot and root length significantly over control. Among the different conc. tested, *S. marcescens* @ 50% recorded numerically superior values with 91.23 per cent germination, 12.3 and 13.7 cm shoot and root length, respectively and a vigour index of 2327.93, which was statistically on par with *S. marcescens* @ 40% conc. (90.25%, 12.1 cm, 13.5cm and 2310.40 respectively). This was followed by Carbendazim 50% WP (0.1%) treatment (87.49%, 11.2cm, 11.6cm and 1994.77 respectively). The minimum germination percentage root and shoot length and vigour index was noticed with control (75.80%, 4.5cm, 7.8cm and 932.34 respectively). Among all concentrations tested, *S. marcescens* @ 10 per cent conc. was found to be the least effective.

The antagonist *S. marcescens* not only suppressed the growth of pathogen and control the disease, but also has got its growth promoting effect on plants. Ezhilarasi, (2006) reported that *S. marcescens* reduced root rot disease and increased root and shoot length of blackgram seedlings. The stimulatory effects of plant growth promotion by PGPR strains have not been completely elucidated, but they may result from synthesis of antibiotics (Ge *et al.*, 2006) or siderophores (Kloepper *et al.*, 1980), synthesis of phytohormones (Bashan *et al.*, 2004), reduction of membrane potential of the roots (Bashan *et al.*, 2004), synthesis of some enzymes (such as ACC deaminase) that modulate the level of plant hormones (Shaharoona *et al.*, 2006), as well as the enhancement of availability of some minerals (Roesti *et al.*, 2006). Subharathinam, (2018) reported that *S. marcescens* had the ability to significantly increase the vigour index of brinjal seedlings. These earlier reports lend support to the present findings. Our study clearly demonstrated that the *S. marcescens* was highly inhibitory to the test pathogen and increased the plant growth parameters.

Table 1: Effect of culture filtrate of *S. marcescens* on the mycelial growth and mycelial dry weight of *C. lindemuthianum*

Tr. No	Conc. of culture filtrate(%)	Mycelial growth (mm)	Per cent inhibition over control	Mycelial dry weight (mg)	Per cent inhibition over control
1	10	37.54	58.28	156.43	54.21
2	20	28.44	68.40	112.25	67.14
3	30	9.25	89.72	45.61	86.65
4	40	NG	100.00	1.23	99.63
5	50	NG	100.00	1.20	99.65
6	Carbendazim 50% WP @0.1%	NG	100.00	2.30	99.33
7	Control	90.00	-	341.65	-
	S.Ed CD (p=0.05)	0.79 1.92	--	0.61 1.31	--

NG- Nil growth

Table 2: Effect of culture filtrate of *S. marcescens* on the conidial germination of *C. lindemuthianum*

Tr. No	Conc. of culture filtrate (%)	Conidial germination (%) @ 48 h
1	10	48.43
2	20	30.50
3	30	10.15
4	40	0.00
5	50	0.00
6	Carbendazim 50 % WP @ 0.1%	0.00
7	Control	92.25
	S.Ed CD (p=0.05)	1.01 2.34

Table 3: Effect of *S. marcescens* on the seed germination and plant growth promotion of dolichos bean (Roll towel method)

Tr. No	Conc. of culture filtrate (%)	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour Index
1	10	82.33 (65.14)	6.5	10.4	1391.37
2	20	84.64 (66.92)	7.5	11.2	1582.76
3	30	86.51 (68.45)	9.0	12.0	1816.71
4	40	90.25 (71.80)	12.1	13.5	2310.40
5	50	91.23 (72.77)	12.3	13.7	2327.93
6	Carbendazim 50 % WP @ 0.1%	87.49 (69.28)	11.2	11.6	1994.77
7	Control	75.80 (60.53)	4.5	7.8	932.34
	S.Ed CD (p=0.05)	0.42 0.99	0.10 0.42	0.11 0.39	-

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