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RESEARCH ARTICLE

Biological control of collar rot of sunflower using rhizobacteria

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ABSTRACT

A study was conducted to screen potential biocontrol agents, *Pseudomonas fluorescens, Bacillus subtilis, Pseudomonas* sp.-I, *Bacillus* sp.-I and *Bacillus* sp.-II for the management of *Sclerotium rofisii* the causal agent of collar rot of sunflower in dual culture test and they overgrew the pathogen upto 76.2, 88.8, 80.01, 35.9 and 77.8 per cent *in vitro,* respectively. Among these biocontrol agents, *P. fluorescens, B. subtilis, Pseudomonas* sp.-I and *Bacillus* sp.-II were tested against collar rot of sunflower when delivered as seed dressing or soil application in pot trials in greenhouse conditions. *B. subtilis* as seed treatment was more effective in disease control by producing minimum disease incidence (10-17 %) followed by other biocontrol agents. Significant increase in seedling emergence, plant stand and biomass were recorded in all biocontrol agents treatments compared with the untreated control. Treatment with the *Bacillus* sp.-II as seed treatment showed maximum plant height (90.80 cm), maximum number of leaves per plant (20.37) and biomass (10.87g/plant) followed by other biocontrol agents treated as seed treatment as well as soil drenching under greenhouse conditions. *Bacillus subtilis* was more effective in disease suppression compared to other strains and *Bacillus* sp.-II was more effective in plant growth promoting activity followed by other biocontrol agents.

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INTRODUCTION

Sunflower is one of the major oilseed crops grown in India. It is a common observation that the reduction in yields is mainly due to the diseases rather than pests in the crop. Collar rot is one of the major diseases of sunflower caused by *Sclerotium rolfsii* and has become a big threat to crop production. The pathogen causes pre and post emergence damping-off, root/collar rot and wilt of sunflower and is considered as one of the economically important diseases in India (Hebbar *et al.,* 1991). Controlling this disease by chemical means is very much temporary and deteriorating the soil health. Under these circumstances, biological control is the best alternative to safeguard crop yields. Biological control of plant pathogens has been considered as a potential control strategy in recent years (Weller, 1988). A large number of soil bacteria like *Bacillus* spp. and *Pseudomonas* spp. have earlier been reported to posses inhibitory properties against a number of plant pathogens (Hegde *et al.,* 1980; Fiddaman and Rossell, 1993; Hebbar *et al.,* 1991). An attempt has been made in this experiment to compare the efficacy of soil bacteria among selected strains *viz.*, *Pseudomonas fluorescens, Bacillus subtilis* and strains isolated from rhizosphere of infected plant, applied through seed treatment and soil drenching for controlling collar/root rot of sunflower caused by *Sclerotium rolfsii* and to know their effect on growth of sunflower under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

The pathogen (*Sclerotium rolfsii*) was isolated from infected collar rot of sunflower plant. Two soil bacteria

(*Pseudomonas fluorescens* and *Bacillus subtilis*) were collected from the Project Directorate of Biological Control (PDBC), Bangalore and three soil bacteria, *viz*., *Pseudomonas* sp.-I, *Bacillus* sp.-I and *Bacillus* sp.-II were isolated from pathogen infected rhizosphere soil of sunflower used in the present study.

In vitro antagonistic potential of rhizobacteria were tested by dual culture techniques (Dennis and Webster, 1971) by observing the inhibition of mycelial growth of pathogen (*Sclerotium rolfsii*). Greenhouse effect of soil bacteria against collar rot of sunflower was conducted in pot experiment pathogen inoculum was prepared by growing pathogen in 200 g mixture of autoclaved sand and sorghum (95:5) mixture in 500 ml conical flask for 25 days. Then 25 g of pathogen inoculum mixture was mixed with 5 kg sieved non-sterile soil and later filled in washed plastic pots.

Bacterial antagonists were grown in 100 ml nutrient broth in 250 ml conical flask as a broth culture for 10 days for seed treatment. The cultures were blended in mixer carboxyl methyl cellulose was added as an adhesive. Surface sterilized sunflower seeds (KBSH-1) were soaked in blended broth culture of biocontrol agents suspension for 20 minutes and then air dried and sown in pots. For soil drenching, 50 ml of blended broth culture of biocontrol agents suspension was drenched in each pot immediately after sowing seeds. Observations were recorded on per cent germination, per cent disease incidence, plant height and biomass of plants on 45th days after sowing.

RESULTS AND DISCUSSION

All the five bacterial biocontrol agents showed antagonism against *Sclerotium rolfsii in vitro* test. Pattern of inhibition of mycelial growth by them was uniform and differed significantly between the isolates. The isolate, *Bacillus subtilis* showed highest inhibition (88.80 %) followed by *Pseudomonas* sp. –I (80.01 %). *Bacillus* sp.-II (77.8 %) and *Pseudomonas fluorescens* (76.2 %). They were selected for further study. *Bacillus* sp.-I showed minimum inhibition (35.90 %) of mycelial growth (Table 1). A large number of reports of rhizobacteria being inhibitory towards plant pathogens including *Sclerotium rolfsii* are available. Hegde *et al*. (1980), Keyser and Ferreira (1988) and Rangeshwaran and Prasad (2000) and others have studied different aspects of inhibition of *Sclerotium rolfsii* by soil bacteria under *in vitro* condition.

Table 1: Inhibition of *Sclerotium rolfsii* by antagonistic bacterial isolates in dual culture test

Sr.No.	Antagonists	Per cent inhibition over control
1.	*Pseudomonas fluorescens*	76.20
2.	*Bacillus subtilis*	88.80
3.	*Pseudomonas* sp.	80.10
4.	*Bacillus* sp.	35.09
5.	*Bacillus* sp.	77.80

Effects of soil bacteria against collar rot of sunflower (Table 2) when applied as seed treatment as well as soil drenching was studied under green house conditions. The per cent germination of sunflower seeds ranged from 76.67 per cent to 100 per cent. Highest per cent germination (100 %) was observed in biocontrol agents treated as seed treatment followed by soil bacterial biocontrol agents treated as soil drenching and significantly superior over lowest per cent germination (76.67 %) in fungicide treated seeds.

Table 2: Effect of bacterial biocontrol agents against collar rot of sunflower under greenhouse condition

Sr. No.	Treatments	Germination	Disease incidence (%)	Plant height (cm)	No. of leaves/ plant	Biomass (g/pl)
1.	Untreated control	94.40	3.70	80.80	18.44	9.16
2.	S. *rolfsii*	83.33	53.60	77.18	18.05	8.61
3.	*P. fluorescens* (ST)	100.00	19.50	86.68	19.82	10.62
4.	*P. fluorescens* (SD)	88.89	13.42	85.25	19.89	10.83
5.	*Bacillus subtilis* (ST)	100.00	10.17	85.85	19.04	10.33
6.	*Bacillus subtilis* (ST)	88.89	15.00	85.73	19.33	10.67
7.	*Pseudomonas* sp-(ST)	100.00	13.92	85.73	19.97	10.56
8.	*Pseudomonas* sp-(SD)	94.40	15.92	86.84	20.00	10.15
9.	*Bacillus* sp-II (ST)	100.00	11.25	85.05	20.37	10.87
10.	*Bacillus* sp-II (SD)	88.89	13.25	90.80	19.93	10.75
11.	Bavistin (0.2%)	77.67	14.67	75.80	17.11	8.18
	C.D.	5.86	2.81	2.41	0.48	0.32

ST - Seed Treatment, SD - Soil Drenching

At 45 days after sowing, disease incidence ranged from lowest (3.70 %) in untreated control and highest (53.60 %) in *Sclerotium rolfsii* treated alone. Similarly, *Bacillus* sp. strains S12 and S17 showed high antagonistic properties against *Sclerotium rolfsii* causing foot rot of groundnut and sugarbeet (Ray and Mukherjee, 1997). Seed bacterization with one of the endophytes (Endo PR8) of *Pseudomonas fluorescens* isolated from root and stem of health cotton seedlings, reducing damping off disease incidence caused by *Sclerotium rolfsii* by 76 per cent (Bowmik *et al.,* 2002).

Growth factors of sunflower at 45 days after sowing observed maximum plant height (90.05 cm), maximum number of leaves per plant (20.37) and biomass (10.87g / plant) in Bacillus sp.-II treated as seed treatment followed by other biocontrol agents treated as seed treatment as well as soil drenching were more effective than fungicide treatment. Minimum plant height (75.8 cm), lowest number of leaves per plant (17.11) and lowest biomass (8.18 g / plant) produced in fungicide treated plants. *Bacillus* strains previously reported to colonize tomato roots and showed an increased in plant growth and biomass production (Bochow, 1992). Similarly seed bacterization of groundnut with *Pseudomonas fluorescens* significantly improved the groundnut seed germination, nodulation and dry matter production besides protection from *Sclerotium rolfsii* (Ray and Mukherjee, 1997).

In general *Bacillus* strains are highly efficient in disease suppression and plant growth promotion of sunflower *in vitro* and greenhouse conditions.

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