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Suppression of *Fusarium* wilt disease in gladiolus by using rhizobacterial strains

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ABSTRACT

Gladiolus is an important commercial flower crop fetching high returns in national and international markets. Fusarium wilt caused by Fusarium oxysporum f. sp. gladioli is a serious disease which has become a limiting factor in its production. With an objective to have a biological control over this biotic stress, an experiment was conducted during 2012 and 2013 at Department of Microbiology, CCSHAU, Hisar to evaluate certain rhizobacterial strains for suppression of Fusarium wilt disease in gladiolus. Twenty five rhizobacterial strains were tested under dual technique for antifungal activity against fungal pathogen Fusarium oxysporum f. sp. gladioli. Two Pseudomonas strains HWM13and HCS2 as well as two Bacillus strains RSD8 and NNY19 caused maximum inhibition of the pathogen. Under pot house conditions, single inoculation of HCS2 showed increased plant height (66.7 cm) and superior floral attributes over control. Coinoculation of HCS2 with pathogen resulted in complete suppression of disease with no symptoms of wilting. Thus, Pseudomonas strain HCS2 could be further tested under field conditions for the suppression of Fusarium wilt in gladiolus.

***Keywords*:** *Bacillus*, disease incidence, *Fusarium*, Gladiolus, *Pseudomonas*

Gladiolus (*Gladiolus grandiflorus* Ness*)* is considered to be the "queen of bulbous flowers". The genus *Gladiolus* belongs to family Iridaceae. It has gained popularity owing to its magnificent, unsurpassed beauty, attractive colours, various sizes and shapes of flowers with long lasting spikes. *Fusarium* corms rot and wilt of gladiolus caused by *Fusarium oxysporum* f. sp. *gladioli* are considered to be the most destructive and widely distributed disease in most gladiolus growing countries of the world. The pathogen infects the corm and root system leading to deterioration in quality and quantity of spikes and planting material owing to heavy yield losses (Rana *et al*., 2004). Attempts have been made to control the disease by steeping corms or cormels and drenching the soil with fungicides. The continuous use of fungicides proved to be hazardous; polluting the environment and leading to residual toxicity, creating resistance in pathogens and reducing soil fertility (Nazir and Riazuddin, 2008; Riaz *et al*., 2008).

Application of PGPR represents a potentially attractive alternative disease management approach. Since PGPR are known for growth promotion and disease reduction in crops (Jetiyanon and Kloepper, 2002). Among bacterial antagonists, fluorescent pseudomonades are reported to be effective against broad spectrum of plant pathogens in many plant species like tomato, carnations, tobacco etc (Van loon *et al.,* 1998). The sporulating Gram-positive bacteria like *Bacillus* sp. have also been used successfully for plant disease control (Kloepper *et al.,* 2004). Therefore, biological control alone or integrated with other control methods is an alternative method for the management of this disease. Therefore, this study was carried out to study to detect the effective rhizobacterial strains for combating fusarium wilt disease in gladiolus.

MATERIALS AND METHODS

The study was carried out in Bio-control laboratory, Department of Microbiology, CCS Haryana Agricultural University, Hisar during August 2012 to March 2013.

The pathogen causing *Fusarium* wilt in gladiolus (*Fusarium oxysporum* f. sp. *gladioli*) was procured from Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi. The culture was multiplied using potato dextrose agar (PDA) media. The gladiolus corms of variety Advanced Red was obtained from Department of Horticulture, CCS Haryana Agricultural University, Hisar.

Twenty five PGPR strains were procured from the Department of Microbiology, CCS Haryana Agricultural University, Hisar. The rhizobacterial strains were maintained by periodic transfer on Luria Bertani (LB) slants (Sambrook *et al*., 1989). These bacterial cultures were stored at 4°C in refrigerator for further use. *Fusarium oxysporum* culture was maintained on PDA medium slants.

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The interaction of rhizobacterial strains with *Fusarium oxysporum* was studied by the spot test method (Sindhu *et al.*, 1999) on PDA medium plates. Spore suspension of fungi was prepared in 3 ml sterilized water. About 0.2 ml of fungal spore suspension was spread over PDA medium plates. A loopful of 48-hour old growth of the rhizobacterial strains was spotted on pre-seeded plates. Five cultures were spotted on each plate. Plates were incubated for 48 hours at 28±2°C and growth inhibition of fungi was recorded after 2-3 days. Detection of antagonistic activity of rhizobcterial strains was based upon the ability of rhizobacterial strains to inhibit fungal growth on PDA containing petri plates. The best four antagonistic strains showing zone of inhibition were selected and evaluated for further testing under pot conditions.

A pot house experiment was conducted by inoculation of four efficient antagonistic bacteria selected on the basis of growth inhibition zone under plate conditions. Their effect on growth of gladiolus and suppression of *Fusarium* wilt was studied under pot house conditions during the month of October 2012 to March 2013.

Well prepared garden soil was collected from Horticulture farm, CCS Haryana Agricultural University, Hisar. The earthen pots of 25 cm diameter and 10kg capacity were filled with mixture of garden soil and sand in ratio of 1:1. The cultures of selected bacterial strains were grown in LB broth and fungus was grown in PDA broth for 3 days. Corms were inoculated with 50 ml growth suspension of selected bacterial strains and fungus according to the treatment schedule and grown in the respective pots. About 20 ml growth suspension was added after one week in respective pots according to treatment schedule. Observations were taken for plant height (cm), days taken for spike initiation, florets per spike, floret diameter (cm), % disease incidence and quantification of *Pseudomonas* and *Bacillus* at sixth leaf stage and at harvest stage. The experiment consists of ten treatments *viz.,* T_1: control, T_2: Pathogen inoculated, T_3: PS_1(selected *Pseudomonas* strain 1), T_4: PS_2(selected *Pseudomonas* strain 2), T_5: Pathogen + PS_1 (selected *Pseudomonas* strain 1), T_6: Pathogen + PS_2 (selected *Pseudomonas* strain 2), T_7: BS_1 (selected *Bacillus* strain 1), T_8: BS_2 (selected *Bacillus* strain 2), T_9: Pathogen + BS_1 (selected *Bacillus* strain 1), T_{10}: Pathogen + BS_2 (selected *Bacillus* strain 2).

On the basis of symptoms observed, percent disease incidence was calculated by following formula.

$$\text{\% Disease incidence (PDI)} = \frac{\text{Total no. of disease plants}}{\text{Total no. of plants}} \times 100$$

Soil samples were collected from rhizosphere zone of each treatment**.** The serial dilutions of soil samples (up to 10^{-6}) were made in 9.0 ml sterilized water blank and 0.1 ml of diluted soil suspension was plated on King's B medium plates (Sindhu *et al.,* 1999). *Pseudomonas* and *Bacillus* colonies were counted based on morphological and pigment production characteristics after 3 days of incubation at 28±2°C.

RESULTS AND DISCUSSION

Twenty five rhizobacterial strains were screened for their antagonistic interaction against the fungal pathogen *Fusarium oxysporum* f. sp. *gladioli* on PDA medium plates using spot test method (Sindhu *et al.,* 1999). Detection of antifungal activity of bacterial strains depended on their ability to inhibit fungal growth under cultural conditions. Out of twenty five strains, four rhizobacterial strains were selected i.e., two from *Pseudomonas* sp. HWM13 and HCS2 and two from *Bacillus* sp. RSD8 and NNY19. Several rhizobacterial strains have been reported with the potential to control various root and foliage diseases in agricultural crops (Goel *et al*., 2000; Weller *et al*., 2007; Sindhu *et al*., 2012). Siddiqui *et al*. (2001) showed that *Pseudomonas aeruginosa* and *Bacillus subtilis* strains produced inihibition zone by inhibiting radial growth of *Macrophomina phaseolina*, *Fusarium oxysporum* and *Rhizoctonia solani*. Similarly, Karuppiah and Rajaram (2011) showed that out of 63 different *Bacillus* isolates, six species inhibited the growth of *Pencillium* sp., *Cercospora* sp. and *Fusarium oxysporum*. The inhibition of fungal pathogen was found due to the production of some specific siderophores, antibiotics, secondary metabolites or hydrolytic enzymes (Buysens *et al*., 1996; Kinner *et al*., 1998).

A total of 10 treatments including control, single inoculation of selected strains and coinoculation of strains with fungal pathogen were evaluated under pot house conditions.

Under pot house conditions, observations were taken for plant height (cm), days taken for spike initiation, spike length (cm), number of florets and floret diameter (cm) (Table 1). The results revealed that use of Pseudomonas and bacillus strains increased vegetative and floral attributes of gladiolus over control. This might be due the positive effect of rhizobacterial strains on soil nutrition and overall growth of the crop. *Pseudomonas* and *Bacillus* are

predominantly found in the rhizosphere of cereal and legume crops. Some rhizobacteria have immense utility for use as biofertilizer, biocontrol agent and in bioremediation due to their plant growth-promoting ability and antagonistic activity (Vessey *et al*., 2003; Weyens *et al*., 2009). Maximum plant height (66.7 cm) was recorded with single coinoculation of *Pseudomonas* strain HCS2 followed by *Bacillus* strain NNY19. Among coinoculation treatments, *Pseudomonas* strain HCS2 with fungal pathogen recorded maximum plant height (61.0 cm). Among single inoculation treatments, HCS2 recorded maximum plant height (66.7 cm) followed by NNY19 (64.2 cm) and whereas control recorded the least plant height (55.9 cm). Early spike initiation was observed in treatment with single inoculation of HCS2 after 113 days which was 3 days earlier than uninoculated control. Whereas, HWM13 and RSD8 coinoculated with pathogen and pathogen inoculated control showed no flowering. Single inoculation of *Pseudomonas* strain HCS2 recorded maximum spike length (55.4 cm), florets per spike (13.0) and floret diameter (12.1 cm) which was followed by single inoculation of *Bacillus* strain NNY19 (53.4 cm, 11.5, 11.3cm, respectively). Among coinoculation with pathogen treatments, HCS2 recorded maximum spike length, florets per spike and floret diameter of 52.7 cm, 11.0, 11.0 cm, respectively. The present findings are supported by Dua and Sindhu (2012) who reported that *Pseudomonas* isolate WPS3 and WPS90 resulted in 131% and 47% increase in plant dry weight as compared to uninoculated control and also coinoculation with *Rhizoctonia solani* showed 115% and 98% increase in plant dry weight with both the strains, respectively. Fluorescent pseudomonads in the plant rhizosphere have been found to improve plant growth through multitudinous factors viz. production of plant growth promoting substances (Ahmad *et al*., 2008; Sindhu *et al*., 2010), early colonization of root surfaces (Benizri *et al*., 2001), secretions of vitamins (Derylo and Skorupsca, 1993) and through suppression of plant diseases by production of antibiotics, siderophores, hydrolytic enzymes and HCN (Stockwell and Stack, 2007; Sindhu *et al*., 2009; Ahemad and Khan, 2011). Similar findings were also reported by Nagorska *et al*. (2007) in gladiolus that besides disease suppression, the bacterial treatments greatly enhanced corm and cormel production and promoted flowering in gladiolus. This could be due to the ability of microbes to activate the host defense system so that the plant is poised to resist potential pathogens and making certain nutrients like nitrogen and phosphorus more readily available to the plants. These results are in agreement with the findings of Koley and Pal (2011) in tuberose cv. Prajwal who reported that use of biofertilizers like *Bacillus, Azotobacter, Pseudomonas* etc. have resulted in increased plant growth and yield. Similar results were also reported by Singh *et al.* (2011) in lentil, Das *et al.* (2014) in mungbean.

Table 1: Effect of coinoculation of *Pseudomonas* and *Bacillus* strains with *Fusarium oxysporum* f. sp. *gladioli* on growth parameters of gladiolus

Treatments	Plant height (cm)	Days to spike initiation	Spike length (cm)	Florets per spike	Floret diameter(cm)
Control	55.9	116	50.5	10	9.6
Pathogen inoculated	43.5	-	-	-	-
HWM13	62.8	117	52.4	9.5	10.3
HSC2	66.7	113	55.4	13.0	12.1
Pathogen + HWM13	49.7	-	-	-	-
Pathogen + HSC2	61.0	119	52.7	11.0	11.0
RSD8	59.1	115	51.2	10.5	9.8
NNY19	64.2	115	53.4	11.5	11.3
Pathogen + RSD8	46.5	-	-	-	-
Pathogen+ NNY19	51.3	121	48.4	9.0	9.4

At sixth leaf stage, disease incidence ranged between 0 - 33.3%. Based on wilting symptoms coinoculation treatments HWM13 and RSD8 with pathogen recorded 22.2% and 33.3% disease incidence, respectively and single inoculation of *Bacillus* strain RSD8 recorded 11.1% disease incidence (Table 2). At harvest stage disease incidence increased considerably. Maximum % disease incidence was observed in treatment RSD8 coinoculated with pathogen (83.3%) and followed by coinoculation treatments with HWM13 and NNY19 (66.6% in both), whereas in single inoculation treatments only RSD8 strain recorded 33.3% disease incidence. *Pseudomonas* strain HCS2 on coinoculation with pathogen did not show any disease symptoms. So this strain was found to be best in combating the wilt disease in gladiolus (Table 2). Sahu and Sindhu (2011) also reported that on coinoculation of Bradyrhizobium strain with *Pseudomonas* strain CP56, no disease symptoms were observed on green gram plants indicating that this bacterial strain completely controlled the root rot disease under pot house conditions. *Bacillus* and *Pseudomonas* count were higher at sixth leaf stage and then reduced at harvest stage. *Bacillus* count was maximum in single inoculation of *Bacillus* strain NNY19 at sixth leaf and harvest stages (87.5 x 10^4 and 78.5 x 10^4 Colony Forming Unit/ml, (CFU/ml), respectively and its coinoculation with pathogen also recorded maximum count (80.1 x10^4 and 74 x 10^4 CFU/ml) at both the stages among all other coinoculation treatments. *Bacillus* count was higher as compared to *Pseudomonas* count in all the treatments. This might be due to the persistence of *Bacillus* sp. in soil for longer duration and their survival even under adverse conditions. *Pseudomonas* strain HCS2 recorded maximum *Pseudomonas* count at sixth leaf and harvest stages among single inoculation treatments (29.0 x 10^4 and 30 x 10^3 CFU/ml, respectively). Besides its coinoculation with pathogen also recorded maximum *Pseudomonas* count (19 x 10^4 and 20 x 10^3 CFU/ml) among coinoculation treatments. Dua and Sindhu (2012) reported that coinoculation of *Pseudomonas* isolate WPS3 with *R. solani* caused 88.9% disease control in wheat. Similarly, antagonistic effect of *Pseudomonas* sp. on different pathogenic fungi and enhancement of plant growth in green gram and other plants has been reported (Hultberg *et al*., 2000; Sindhu and Dadarwal, 2001). Shanmugam *et al.* (2011) reported that application of talc-based formulation of strain mixture, S2BC-2 (*B. subtilis*) + TEPF-Sungal (*B. cepacia*) as corm dressing and soil application significantly reduced vascular wilt and corm rot of gladiolus as good as chemical treatment under greenhouse on challenge inoculation with the pathogen. Similarly, *Pseudomonas* strain PsL-4 showed higher inhibition effect and antifungal activity against *Fusarium oxysporum* f. sp. *gladioli* due to the metabolite production by this strain as reported by Nazir *et al*. (2011).

Table 2: Percent disease incidence and bacterial count on inoculation with *F. oxysporum* f. sp. *gladioli*

Treatments	% Disease incidence at		*Bacillus* countat (CFU/ml)		*Pseudomonas* count at (CFU/ml)	
	6th leaf	Harvest	6th leaf	Harvest	6th leaf	Harvest
Control	-	33.3	66.5 x 10^4	55.5 x 10^4	4.0 x 10^4	-
Pathogen inoculated	33.3	100	20.5 x 10^4	33.5 x 10^4		-
PS_1 (HWM13)	-	-	45.0 x 10^4	42.0 x 10^4	17.0 x 10^4	10 x 10^3
PS_2 (HSC2)	-	-	26.2 x 10^4	23.0 x 10^4	29.0 x 10^4	30 x 10^3
Pathogen + HWM13	22.2	66.6	53.0 x 10^4	47.5 x 10^4	10.5 x 10^4	
Pathogen + HCS2	-	-	74.5 x 10^4	57.2 x 10^4	19.0 x 10^4	20 x 10^3
BS_1 (SCD8)	11.1	33.3	77.5 x 10^4	63.5 x 10^4	6.8 x 10^4	-
BS_2 (NNY19)	-	-	87.5 x 10^4	78.5 x 10^4	7.6 x10^4	10 x 10^3
Pathogen + RSD8	33.3	83.3	72.5 x 10^4	65 x 10^4	4.2 x 10^4	-
Pathogen + NNY19	-	66.6	80.1 x 10^4	74.5 x 10^4	5.5 x 10^4	-

The results from the present investigation suggest that inoculation with rhizobacterial strains enhance the growth and quality of gladiolus over control besides suppression of *Fusarium* wilt disease. Thus, further evaluation of these bacterial strains under field conditions is needed before commercialization in order to reduce the pesticide cost and for an eco-friendly disease management approach.

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