Journal of Stored Products and Postharvest Research Vol. 3(4), pp. 49 – 51, 16 February, 2012
Available online http://www.academicjournals.org/JSPPR
DOI: 10.5897/JSPPR11.049
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Short Communication

Effect of *Pseudomonas fluorescens* on *Fusarium oxysporum* f.sp. *gladioli* causing corm rot disease of gladiolus

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Accepted 12 January, 2012

A detailed study was conducted to find out the efficacy of *Pseudomonas fluorescens* on fusarial corm rot pathogen *Fusarium oxysporum* f. sp. *gladioli* in Department of Plant Protection, Allahabad Agricultural Institute Deemed-University, Allahabad. The strains of *P. fluorescens* isolates from gladiolus rhizosphere had significantly inhibited the growth of *F. oxysporum* f. sp. *gladioli*. Among the strains, PsL-4 of *P. fluorescens* has higher inhibitory action in comparison with other strains. The strains of *P. fluorescens* also produced hydrogen cyanide (HCN) which limited the growth of pathogen. The study also indicated the involvement of Siderophore-mediated antagonism in *P. fluorescens.*

Key words: *Fusarium oxysporum*, gladiolus*, Pseudomonas fluorescens,* siderophore antagonism.

INTRODUCTION

Flowers symbolize purity, beauty, peace, love and patience apart from providing fresh air and fragrance. Gladiolus is a “queen of bulbous flowers” owing to its beauty and its economic value. Gladiolus is considered to be a profitable floricultural crop. A few bulbous flowers have found a place among the top 10 cut flowers in the international floricultural trade. Gladiolus took the first place and accounted for 60% of the total turnover of cut flower. Though, India has suitable agro climatic condition for Gladiolus cultivation but it is being grown barely over an area of 289 ha, producing 459 lakh spikes annually. Evidently, gladiolus occupies a premier position among the vast number of crop available for exportation on a commercial scale. However the crop is prone to attacks by a large number of diseases. The most destructive disease of gladiolus throughout the world is *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *gladioli*; Gladiolus suffers annually serious losses due to wilt and storage rot caused by *F. oxysporum* f. sp. *gladioli*. The importance of the disease can be judged from the losses caused by it in different parts of the world, which have been estimated as 30 to 80% (Maurhofer et al., 1995) Corm rot disease caused by *F. oxysporum f.* sp. *gladioli* is a devastating disease of gladiolus and is the serious limiting factor in the commercial cultivation of Gladiolus.

The disease occurs both in storage, as well as in the field causing huge losses up to 60 to 80% in higher contaminated areas. In Himachal Pradesh the disease incidence ranged from 6.1 to 64.2%, comparatively more in sub-mountain regions than temperate zones (Tomar, 1997), though chemical control is a regular practice in managing the diseases continuous use of fungicide leads to a pollution problem, residual effects, toxicity, resistance in pathogen, imbalance in soil microbial associations (Nandakumar et al., 2002). Therefore, alternative means of disease control are advisable. The use of biocontrol agents are good fungal antagonists of many soil borne pathogens like *Fusarium* spp. Mukhopadhy and Mukherjee, (1996). The present study was undertaken to study the effect of different strains of *Pseudomonas fluorescence* on the development of *F. oxysporum* f. sp.*gladioli* causing corm rot disease of gladiolus.

MATERIALS AND METHODS

The present study was conducted in the Research Laboratory Department of Plant Protection College of Agriculture, Allahabad

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Table 1. *In vitro* and inhibition of *Fusarium oxysporum* f. sp. *gladioli* by different strains of *Pseudomonas fluorescens* at different time intervals in dual culture.

Treatment	Growth (mm) 24 h	Growth (mm) 48 h	Growth (mm) 72 h	I.O.C. (in %)
PsL-1	22.3	40.5	78.0	29.25
PsL-2	20.8	34.8	53.1	43.17
PsL-3	21.5	32.1	44.5	47.25
PsL-4	13.8	16.8	23.6	69.97
PsL-5	28.6	41.0	63.0	29.21
PsL-6	23.6	32.5	55.5	40.86
PsL-7	27.3	35.0	62.0	33.76
PsL-8	31.6	38.5	45.1	34.74
PsL-9	14.8	20.3	32.5	63.86
PsL-10	24.1	44.0	57.8	33.80
PsL-11	27.0	34.0	53.8	37.58
PsL-12	22.0	30.1	58.	42.26
PsL-13	15.1	25.6	27.3	62.95
Control	37.0	67.0	83.0	-
SE±	1.75	1.74	1.1	1.84
CD =5%	3.55	3.57	3.12	3.815

Agricultural Institute-Deemed University, Allahabad. U.P. in 2008 to 2009.

Isolation of *F. oxysporum* f. sp *gladioli*

The infected tissues of corms were removed, surface sterilized with 0.1% mercuric chloride and washed 3 times with sterilized water aseptically and transferred to the plated potato dextrose agar (PDA) media and incubated at 24 ± 1°C for three days. The fungus was seen in the plated media and subsequently sub-culturing was done to maintain the pure culture.

Isolation of *P. fluorescens*

Strains of *P. fluorescens* were isolated from the different location of Gladiolus fields at random. The collected soil samples were dried in shade and grind to powder using motor and pestle. 10 g of soil was mixed with 90 ml of sterilized distilled water (SDW) to a preparation of 10^{-1}, this suspension was used for serial dilution up to 10^{-5}. The dilution 10^{-1} of the suspension was used for plating in King's B media and incubated at 27±1°C, after incubation the colonies of *P. fluorescens* were identified (Johnsan et al., 1958).

Characterization of isolates

The *P. fluorescens* isolates were characterized as follows:

Cultural characterization: The bacteria were examined under ultra violet (UV) light and colonies with yellow green colour action were marked and recorded.

Morphological characterization: Morphological characterization was done by cell shape and arrangement, gram staining and colony morphology.

Biochemical characterization: The characterization was done by gelatin hydrolysis, urea test, ammonification, Levan production from sucrose, iodole production test, starch hydrolysis, oxidase test and HCN production.

Inhibition zone recorded: The growth of *F. oxysporum* f. sp. *gladioli* against *P. fluorescens* (in mm) was recorded at 24, 48, and 72 h, in dual culture medium. Plates were incubated at 28°C and the inhibition zone was recorded.

I.O.C. in percent = [(Growth in C. P – Growth in T. P)/(Growth in C. P)] ×100

The data was statistically analyzed by randomized block design (RBD).

RESULTS

P. fluorescens isolated from rhizosphere region of Gladiolus used for assessing their antifungal efficacy against *F. oxysporum* f. sp. *gladioli*. The results demonstrated that all strains isolated from the rhizosphere of Gladiolus had significant inhibitory effect on the growth of *F. oxysporum* f. sp. *gladioli*. In the strains, PsL-4 strain had higher inhibition effect on the growth of the pathogen. It is revealed from Table 1 that during investigation different strains of *P. fluorescens* reduction in the growth of *F. oxysporum* f.sp.*gladioli*. The growth of test fungus was reported highest (83.00 mm) in control (T_0) at 72 h.

The highest percentage of inhibition was found in PsL-4 (13.8), PsL-9 (14.8) and PsL-13 (15.1) at 24 h. 16.80% at 48 h and 23.60% at 72 h, respectively; this was followed by PsL-9 and PsL-13 in solid medium during investigation significantly inhibited the growth of *F. oxysporum* f.sp *gladioli.* The morphological, biochemical characteristics of isolated strains of *P. fluorescens* are summarized in

Table 1. The strain PsL-4 (13.8), PsL-9 (14.8) and PsL-13 (15.1) were found to be significantly superior to the other isolates. The inhibition zones were recorded at 24, 48 and 72 h.

DISCUSSION

In vitro antibiosis was the criterion used in this study to select efficient strains of *P. fluorescens* for use in assessing their metabolite production and antifungal effect. Efficient strains of *P. fluorescens* were selected for suppression of pathogen. The metabolite production by the strain PsL-4 of *P. fluorescens* had shown the antifungal activity against *F. oxysporum* f. sp*. gladioli*. The antibiotics namely phenazine, phocyanine, pyreolnitrin and phoroglucinol were produced by some strains *of P. fluorescens* against various diseases. Earlier a number of strains of *P. fluorescens* have been shown to produce phlorogluicinol. The severity of tobacco black root rot was reduced when soil was added with pholoroglucinol. Phlorogluicinol metabolites are phenolic and are produced by bacteria with broad spectrum anti bacterial, antifungal and phytotoxic properties. Maurphofer et al. (2002), reported that phlorogluicinol producing strains of *P. fluorescens* have been shown to be effective against root pathogens.

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