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Evaluation of *Penicillium* sp. Eu0013 for management of root rot disease of okra

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Abstract

In this study the interactive effect of a plant growth promoting fungus (PGPF) *Penicillium* sp. EU0013 was used as biocontrol agent using seed priming and soil drench method against *Fusarium solani* which is causal agent of root rot of Okra. Different conidial concentrations of EU0013 (1×10^5, 1×10^6 and 1×10^7 conidia mL^{-1}) were prepared and applied under field condition by using the above mentioned methods. Under field condition data was recorded on disease severity (%) and seedling mortality (%),of fungal species in the okra rhizosphere. Results showed that *Penicillium* sp. EU0013 applied at 1×10^7 conidia mL^{-1} as seed priming significantly reduced disease severity 22.17% and seedling mortality by 13.8%, in okra rhizosphere. It can be concluded that root rot in okra caused by *F. solani* was efficiently controlled by the application of plant growth promoting fungus (PGPF) *Penicillium* sp. EU0013 as biocontrol agent using seed priming method as compared to soil drench method.

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Introduction

Okra or lady's finger (*Abelmoschus esculentus* L. Moench) locally known as Bhindi, is one of the most important summer vegetables of Pakistan. It belongs to Malvaceae family (Tindall, 1983). Okra is grown in warmer areas of the temperate regions and all parts of the tropics in Pakistan (Baloch *et al.* 1994). It is a good source of vitamins like A, B, C, and is rich in minerals, iodine and protein. In paper industries, the stem of okra plants is used for fiber purposes (Qayyum 1990; Mithal 2006). Okra is attacked by many soil and seed-borne diseases. Root or collar rot and damping-off disease caused by *Macrophomina phaseolina (Rhizoctonia bataticola), Rhizoctonia, solani, Fursarium solani* and *Pythium butleri* or root and stem rot caused by *Phytophthora palmivora*. Seed borne diseases in okra are caused by five major fungi *i.e.* foot and root rot, anthracnose and die-back, *Cercospora* leaf spot, *Corynespora* leaf spot and leaf blight, they are caused by *Fusarium oxysporum, Colletotrichum dematium, Cercospora abelmoschi, Corynespora cassiicola* and *Macrophonina phaseolina,* respectively. Among these diseases *Fusarium solani* (Root rot) is causing serious damages to okra and rapidly spread under favorable conditions (Rahim *et al.* 1992). Root rot is more serious problem in okra which is grown on small scale like home gardening have a reported disease incidence of 55-80%. However, incidence is low *i.e.* 10-45% in crops that are grown on large scale under field conditions (Mithal, 2006). Several control and management strategies *i.e.* chemical, cultural and biological controls have been made for the control and management of okra root rot. Various biocontrol agents such as *Trichoderma harzianum* and *T. viride* alone or in combination with certain chemicals *i.e.* Benlate, Ridomil and Dithane M-45 were found significantly effective against root rot caused by *F. solani* in okra (Ahmad *et al.* 2012). The fungus genus *Penicillium* has a lot of bioactive compounds that can simply be used as antifungal agents. *Penicillium* isolates show high antifungal effect on mycelial growth of *F. oxysporum, F. solani, M. phaseolina, Aspergillus japonicus* and *Cladosporium cladosporioides*. *Penicillium italicum* inhibits the fungal growth from 45 to 68% as compared to *P. simplissimum* 25–68% (Khokhar *et al.* 2011). In the present study The *Penicillium* sp. EU0013 was used as bicontrol agent against root rot of okra with objective to determine the effect of different application techniques of *Penicillium* sp. EU0013 on root rot disease of okra.

Materials and methods

Source of Genotype and Pathogen

Locally available okra cultivar "MALIKA" was purchased from the market for the research purpose. Seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 15-30 seconds followed by triple rinsing in sterilized distilled water before sowing. Old culture of *Fusarium* root rot of okra was available in Plant Pathology laboratory. This fungus was sub-cultured on Potato Dextrose Agar (Potato 250g; Dextrose 20g; Agar 20g) medium. *F. solani* was applied in a single conidial concentration 1×10^6 mL^{-1} in field as soil drench at specific seedbeds (8 cm deep) at the time of sowing (Rajput *et al.* 2008).

Biocontrol agent

In this study *Penicillium* sp. EU0013 was used as biocontrol agent and was sub-cultured on PDA medium. Using haemocytometer three conidial concentrations (0, 1×10^5, 1×10^6 and 1×10^7 conidia mL^{-1}) were prepared and applied to seeds as seed priming and to soil as soil drench at 8 cm depth. Gum arabic (2%) was applied as a sticky material to the conidial suspension for seed priming (Dawar *et al.* 2008).

Field experiment

To study the effect of *Penicillium* sp. EU0013 as biocontrol and its different application methods on okra root rot pathogen was demonstrated in Malakandher Research Farm, The University of Agriculture, Peshawar. Three factorial randomized complete block design (RCBD) with 3 replications was used *i.e.*

Factor A: Pathogen

P_0: No pathogen treatment

P_1: Pathogen treatment

Factor B: Application methods of biocontrol
M_1: Seed priming
M_2: Soil drench.

Factor C: *Penicillium* sp. EU0013 concentrations
B_0: Control (No biocontrol)
B_1: 1 x 10^5 conidia mL^{-1}
B_2: 1 x 10^6 conidia mL^{-1}
B_3: 1 x 10^7 conidia mL^{-1}.

Plots for each treatment were kept 4 meter long with 3 rows. Plant to plant and row to row distance was kept 25cm. Data were recorded on okra plants in each treatment on the following parameters.

Percent disease severity

The disease severity was calculated as a percentage by visually observing the roots and stem near soil surface with symptoms (rotting or browning) using 0-3 scale where 0 = no disease, 1= less than 50% rottning, 2 = more than 50% and less than 75% rotting while 3 = completely damaged plants (Ahmed *et al.* 2012).

Percent seedling mortality

The percent seedling mortality was calculated according to the following formula;

$$\text{Percent Seedling Mortality} = \frac{\text{Total number of dead seedlings}}{\text{Total number of seedlings}} \times 100$$

Statistical analysis

The data obtained for each parameter was subjected to analysis of variance techniques appropriate for randomized complete block design using statistical software MstatC (Steel and Torrie, 1980).

Results and discussion

Disease severity (%)

The analysis of variance revealed that disease severity was significantly ($P<0.05$) affected by biocontrol, pathogen and its method of application. The data presented in Table 1 showed disease severity in okra cultivar malika as affected by pathogen, biocontrol and its application methods. Disease severity (39.97%) was found in plots treated with biocontrol at the rate of 1×10^5 which is statistically similar to 39.87% from biocontrol level 1×10^6 conidia mL^{-1}, further increase in biocontrol level to 1×10^7 conidia mL^{-1} significantly reduced disease severity to 29.58% in comparison with 47.08% of the control. Minimum disease severity was recorded when biocontrol was applied at the rate of 1×10^7 using seed priming in plots without treated with pathogen (24.70%), which is statistically at par with soil drench method (26.70%). Similarly, biocontrol significantly reduced disease severity (31.40%), applied at rate of 1×10^7 in plots treated with pathogen using seed priming as with soil drench method (35.50%) in comparison with control (47.08%). In addition, disease severity was significantly reduced using *Penicilium* sp. EU0013 as seed priming at the rate of 1×10^7 conidia mL^{-1} (Table 1).

Table 1. Disease severity (%) in okra as affected by pathogen and biocontrol with different methods of application.

		Biocontrol			
Pathogen	Methods	B_0	B_1	B_2	B_3
P_0	M_1	39.33DE	26.70G	28.47H	24.70G
	M_2	40.00C	37.67CD	35.50DE	26.70GE
P_1	M_1	53.57A	48.90B	46.60B	31.40EF
	M_2	55.40A	46.60B	48.90B	35.50DE
Mean		47.08 A	39.97 B	39.87 B	29.58 C
LSD value for biocontrol		= 1.13			
LSD value for pathogen × method × biocontrol = 2.26					

Means of the same category followed by the different letters are significantly different from one another at $P\leq0.05$ using LSD test.

Note: P_0= no Pathogen, P_1=Pathogen, M_1=Seed priming, M_2= Soil drench, B_0= no biocontrol, B_1=10^5, B_2=10^6, B_3=10^7 conidia mL^{-1}.

Mansoor *et al.* 2007 reported that *Pseudomonas aeruginosa* and *Paecilomyces lilacinus* affected and restricted the growth of *F. solani* and other root rot causing fungi. These Bicontrol agents are known to produce antifungal compounds that inhibit the growth of other plant pathogenic fungi (Fatma *et al.* 2010; Ahmad *et al.* 2012). *Penicillium* sp produces certain chemicals like grise of ulvin, dechlorogrise of ulvin, curvulinic acid and many others that inhibits the growth of pathogenic fungi and does not allow them to grow well (Nicolitte *et al.* 2007). Similar results were also recorded with Alam *et al.* (2011). *T. harzianum* and *Bacillus subtilis* significantly decreased root rot disease severity by restricting the growth of *F. solani* (El-Mohamedy, 2009).

Table 2. Seedling mortality (%) of okra as affected by pathogen and biocontrol with different methods of application.

		Biocontrol			
Pathogen	Methods	B_0	B_1	B_2	B_3
P_0	M_1	33.27GH	29.57I	26.40J	20.27L
	M_2	32.67H	29.67I	27.30J	22.17K
P_1	M_1	47.97B	42.90C	38.57E	34.17G
	M_2	52.67A	47.17B	41.00D	36.47F
Mean		41.64 A	37.40 B	33.32 C	28.27 D
LSD value for biocontrol		= 0.88			
LSD value for pathogen × method × biocontrol = 1.55					

Means of the same category followed by the different letters are significantly different from one another at P≤0.05 using LSD test.

Note: P_0= no Pathogen, P_1=Pathogen, M_1=Seed priming, M_2= Soil drench, B_0= no biocontrol, $B_1=10^5$, $B_2=10^6$, $B_3=10^7$ conidia mL^{-1}.

Seedling mortality (%)

Data in Table 2 revealed that percent seedling mortality was affected by pathogen, biocontrol and its application techniques. The minimum seedling mortality (28.27%) was recorded in plots treated with the biocontrol applied at the rate of 1×10^7 followed by 33.32% where biocontrol was applied at the rate of 1×10^6 conidia mL^{-1}, when compared with (41.64%) the control. Minimum seedling mortality 20.27% was recorded in plots where biocontrol was applied as seed priming at the rate of 1×10^7 conidia mL^{-1} in absence of the pathogen as with soil drench method (22.17%). Similarly, minimum values for seedling mortality (35.32%) were observed in plots using seed priming for biocontrol application at level 1×10^7 conidia mL^{-1} with pathogen as with soil drench method (36.47%) in comparison with control (41.64%). In addition, application of the biocontrol as seed priming at level 1×10^7 conidia mL^{-1} was found effective in reducing seedling mortality (Table 2). Saman Abeysinghe, (2007) also reported the same reduction in seedling mortality (caused by *F. solani*) by using certain microbial antagonists such as *Trichoderma herzianum* and *Bacillus subtilis*.

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