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IN VITRO EVALUATION OF BIOCONTROL POTENTIAL OF *PAECILOMYCES* SPECIES AGAINST *SCLEROTIUM ROLFSII AND PYTHIUM APHANIDERMATUM*

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ABSTRACT

Paecilomyces species viz., four strains of *Paecilomyces variotii*, *Paecilomyces lilacinus* and *Paecilomyces fumosoroseus* were evaluated *in vitro* for biocontrol potential against *Sclerotium rolfsii and Pythium aphanidermatum* by dual culture plate method. Colonies of *Paecilomyces* species and *Sclerotium rolfsii* met each other but *Sclerotium rolfsii* later overgrew the colonies of tested fungi. whereas, growth of *Pythium aphanidermatum* inhibited by the *Paecilomyces* species.

Key words: *Paecilomyces*, Biocontrol potential, *Sclerotium rolfsii, Pythium aphanidermatum.*

INTRODUCTION

The presence of *Sclerotium rolfsii* (Sacc.), first time reported in Pakistan by Ahmed *et al.* (1984) on maize (*Zea mays* L.), is an economically important pathogen in warm, moist climate worldwide, causing diseases on more than 500 species of plants (Aycock, 1966). The pathogen causes stem and root rots on a wide variety of fruit and vegetable crops (Domsh *et al.*, 1980). This pathogen propagates by sclerotia under favourable conditions. After germination, sclerotia may cause chlorosis and wilting of entire plants (Yaqub and Shahzad, 2005). It causes serious damages to rice, apple, sugar beet, sunflower, and mash and mung beens in Pakistan (Ruqia, 2001; Shahzad and Ghaffar, 1995; Yaqub and Shahzad, 2005). Considering the health risks of living beings farmers prefer to use biocontrol agents against pathogens over fungicides.

Pythium aphanidermatum is a cosmopolitan pathogen with a wide host range (Plaats-Niterink, 1981; Domsh *et al.*, 1980). It is an aggressive pathogen and produces damping-off, root and stem rots, and blights of grasses, fruits, vegetables and crop plants. It is of economic concern on most annuals, cucurbits, and grasses. This fungus was associated with all the agronomic and horticultural crops in Pakistan (Shahzad and Ghaffar, 1995; Abdul-Haq and Shahzad, 1998; Lodhi *et al*., 2013).

Of the various biocontrol agents, species of *Paecilomyces*, especially *P. lilacinus* and *P. variotii* have shown biocontrol potential against root infecting fungi like *Macrophomina phaseolina* (Shahzad and Ghaffar, 1987, 1989; Abbas *et al*., 2011; Qureshi *et al*., 2012). *Rhizoctonia solani* (Shahzad and Ghaffar, 1989; Mansoor *et al*., 2007)), *Fusarium solani* (Shahzad and Ghaffar, 1989; Siddiqui *et al*., 2000; Mansoor *et al*., 2007), *a*nd *Pythium aphanidermatum* . (Hashem-al-Sheikh and Abdelzaher 2010) No literature on the use of *Paecilomyces* species against *S. rolfsii*, and *P. aphanidermatum* in Pakistan is available. In our previous studies (Perveen, 2015), species of *Paecilomyces* showed biocontrol potential against root-knot nematodes. In view of the importance of the root rot and root-knot disease complexes caused by the interaction of the root infecting fungi and root knot nematodes (Shahzad and Ghaffar, 1992; Perveen and Ghaffar, 1998; Siddiqui *et al*., 2000), biocontrol potential of *Paecilomyces* species was evaluated against *S. rolfsii* and *P. aphanidermatum in vitro.*

MATERIAL AND METHODS

Microorganisms used: *Paecilomyces variotii* and *Paecilomyces fumosoroseus* were isolated from soi**l** using serial dilution technique (Waksman and Fred, 1922). Culture of *Paecilomyces lilacinus* was acquired from ARS collection of entomopathogenic fungal cultures, USDA-ARSRW Centre for Agriculture & Health, USA. *Sclerotium rolfsii* used in the present studies was isolated from sugar beet seed (Ruqia, 2003). Isolation of *Pythium aphanidermatum* was by baiting techniques (Harvey, 1925):

***In vitro* interaction of *Sclerotium rolfsii and Pythium aphanidermatum* with *Paecilomyces* species:**
Dual culture plate method: In dual culture plate essay, a 5mm diameter inoculum disc of four strains of *P. variotii*, *P. lilacinus* and *P. fumosoroseus* placed near the edge of a Petri plate containing PDA medium; a similar inoculums

disc of a root infecting fungus placed at the opposite end of Petri plate. There were three replicates for each treatment and the plates were incubated at 28 ± 2°C. The colony diameters of pathogen and test microorganism recorded daily type of interaction observed using the following key:

A= Colonies of the test microorganism and the pathogen met each other. No further growth of either the microorganism or the pathogen observed.
B= Colonies of test organism and pathogen met each other; the pathogen later overgrew the colony of test microorganism.
C= Test microorganism produced a zone of inhibition.

RESULTS AND DISCUSSION

Colonies of four strains of *Paecilomyces variotii* met with *Sclerotium rolfsii* then pathogen overgrew on the tested fungi (Table 1; Fig. 1B, C, D, E). Colonies of four strains of *Paecilomyces variotii* and *Pythium aphanidermatum* met each other. No further growth of either the microorganism or the pathogen observed (Table 2; Fig. 1b, c, d, e). Colonies of *Paecilomyces lilacinus* produced zone of inhibition against *sclerotium rolfsii* then pathogen later overgrew the colony of test microorganism (Table 3; Fig. 1A). *Paecilomyces fumosoroseus* failed to inhibit the growth of *sclerotium rolfsii* but pathogen overgrew the tested fungi (Table 4; Fig. 1F). *Paecilomyces lilacinus* and *Paecilomyces fumosoroseus* produced a zone of inhibition against *Pythium aphanidermatum* (Table 3 and 4; Fig. 1a, f).

Table 1. Effect *of Paecilomyces variotii* on *in vitro* growth of *Sclerotium rolfsii.*

Biocontrol agents	Days of incubation	Colony Diameter (mm)		Comments
		Pathogen	Test fungi	
Strain 1	5	44	46	B
Strain 2	5	40	50	B
Strain 3	5	41	49	B
Strain 4	5	45	45	B

Table 2. Effect of *Paecilomyces variotii* on *in vitro* growth of *Pythium aphanidermatum.*

Biocontrol agents	Days of incubation	Colony Diameter (mm)		Comments
		Pathogen	Test fungi	
Strain 1	6	30	60	A
Strain 2	6	28	58	A
Strain 3	6	28	62	A
Strain 4	6	25	65	A

Table 3. Effect *of Paecilomyces* lilacinus on *in vitro* growth of *Sclerotium rolfsii and Pythium aphanidermatum.*

Biocontrol agents	Days of incubation	Colony Diameter (mm)		Comments
		Pathogen	Test fungi	
Pythium aphanidermatum	6	30	30	C
Sclorotium rolfsii	5	55	22	B

Table 4. Effect *of Paecilomyces fumosoroseus* on *in vitro* growth of *Sclerotium rolfsii and Pythium aphanidermatum.*

Biocontrol agents	Days of incubation	Colony Diameter (mm)		Comments
		Pathogen	Test fungi	
Pythium aphanidermatum	6	44	46	C
Sclorotium rolfsii	5	44	47	B

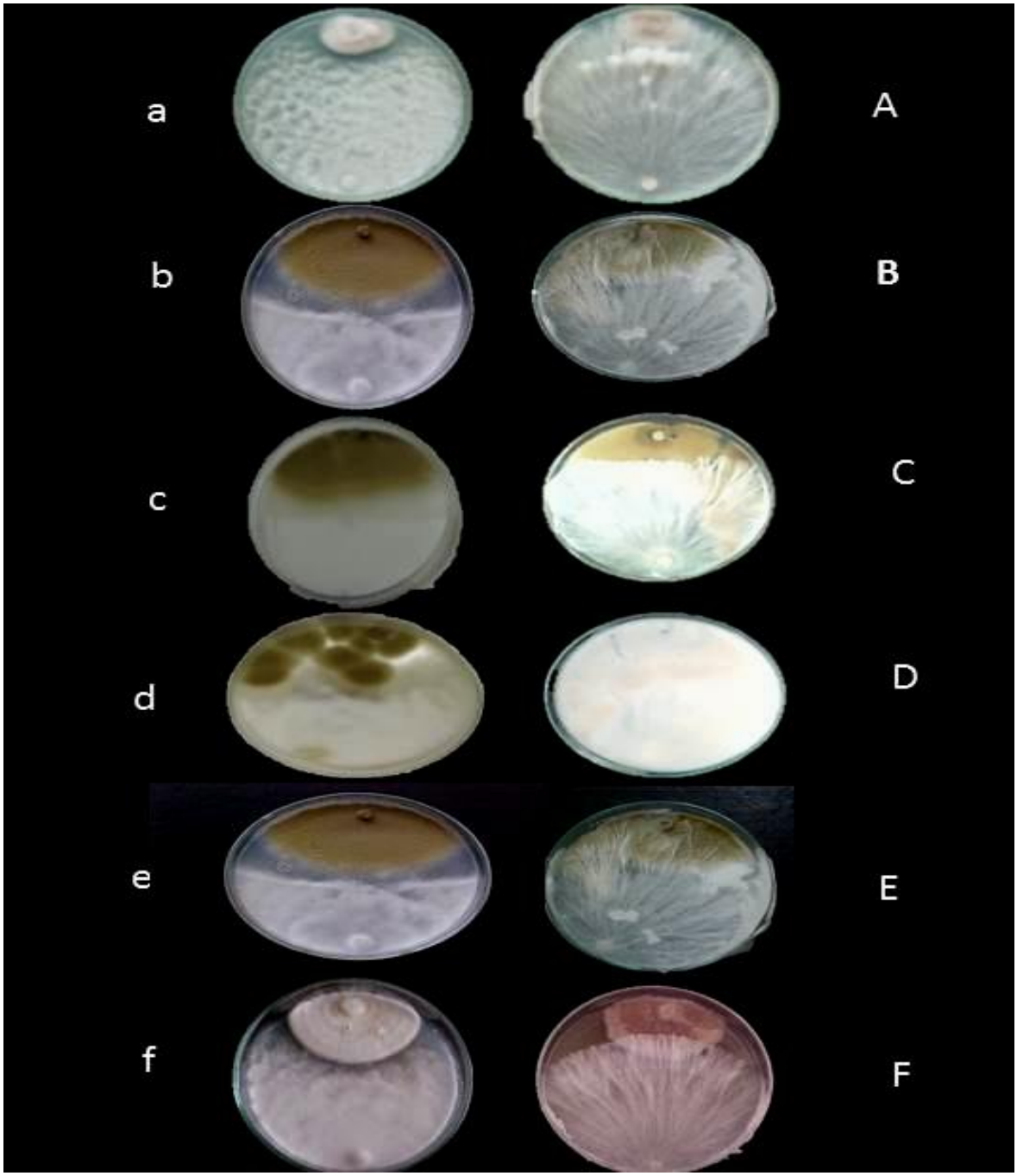

Fig. 1. Interaction of *Sclerotium rolfsii* with A) *Paecilomyces lilacinus*, B) *Paecilomyces variotii* strain 1, C) *Paecilomyces variotii* strain 2, D) *Paecilomyces variotii* strain 3, E) *Paecilomyces variotii* strain 4 and F) *Paecilomyces fumosoroseus.*
Interaction of *Pythium aphanidermatum* with a) *Paecilomyces lilacinus*, b) *Paecilomyces variotii* strain 1, c) *Paecilomyces variotii* strain 2, d) *Paecilomyces variotii* strain 3, e) *Paecilomyces variotii* strain 4 and f) *Paecilomyces fumosoroseus.*

Colonies of all the four strains of *P. variotii* and *S. rolfsii* met each other; the pathogen later overgrew the colony of the test microorganism. Whereas, *P. lilacinus* produced zone of inhibition the pathogen later overgrew the colony of test microorganism. In case of *P. fumosoroseus* first there was inhibition of the growth of pathogen but later on pathogen overgrew the test organism. Shahzad and Ghaffar (1987) has reported that *Paecilomyces lilacinus* produced zone of inhibition against *Sclerotium oryzae* but after 3 days of inhibition, the pathogen over grew the colonies of *P. lilacinus.* Yaqub and Shahzad (2005) have reported that *Trichoderma* species inhibited the growth of

S. rolfsii in dual culture plates. Whereas, four strains of *P. variotii* inhibited the growth of *P. aphanidermatum* Colonies of the test microorganism and the pathogen met each other. No further growth of either the microorganism or the pathogen observed. *Paecilomyces lilacinus* and *P. fumosoroseus* produced zone of inhibition against *P. aphanidermatum.* Hashem-al-Sheikh and Abdelzaher (2010) reported the biological control of *Pythium spinosum* by *P. variotii, Aspergillus sulfureus,* and *Penecillium islandicum. P. fumosoroseus* produced 2-3mm wide zone of inhibition against *F. solani, R. solani* and *P. aphanidermatum,* and inhibited the growth of *M. phaseolina* when colonies met each other. The growth inhibition of pathogens by the biocontrol agents may be attributed to hyperparasitism or antibiosis (We *et al.,* 1986), or production of chitinase and B-1,3-glucanase enzymes that degrade the cell wall of the pathogens (Ahmed and Baker, 1987). Similarly, Muhammad and Amusa (2003) also concluded that the antagonists inhibit the growth of the pathogens either by producing biologically active metabolites, or by colonizing the agar surface much faster as compared to the pathogen.

These results suggest that the chemicals produced by different microorganisms have different effects on pathogens. It could be the reason for inefficacy of *Paecilomyces* species against *Sclerotium rolfsii*

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