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Effect of *Rhizobium* sp., on Growth of Pathogenic Fungi under *in vitro* Conditions

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Abstract: In a laboratory study chickpea root nodulating bacterium *Rhizobium* sp., strain Thal-8 significantly inhibited the growth of pathogenic fungi (*Alternaria alternata*, *Fusarium* sp. *Ascochyta rabiei, Drechslera* sp. and *Curvularia* sp.) in potato dextrose agar media. The inhibition rate displayed differences in accordance with different fungi cultures. However, the inhibitory effect of *Rhizobium* sp., strain Thal-8 occurred most on pure cultures growth of *Alternaria alternata* and *Drechslera* sp. (54 and 45%, respectively) and least on *Curvularia* sp. (3%).

Key words: Rhizobium, pathogenic fungi, biological control

INTRODUCTION

Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents since, the rhizosphere provides front line defence for roots against attack by pathogens (Weller, 1998). Of these, the rhizobia are reported as effective biocontrol agents for the inhibition of certain soil-borne plant pathogens (Chakrabotry and Purkayastha, 1984; Ehteshamul-Haque and Ghaffar, 1992). Many species of rhizobia promote plant growth and also inhibit the growth of certain pathogenic fungi. There are reports where *Paecilomyces lilacinus*, *Trichoderma harzianum* (Ehteshamul-Haque *et al*., 1992) *Stachybotrys atra* (Butt and Ghaffar, 1972) and *Memnoniella ehinata* (Dawar *et al*., 1993) have shown promising results for the control of root infecting fungi. Rhizobia are also reported to significantly inhibit the growth of pathogenic fungi i.e *Macrophomina phaseolina* (Tassi) Gold, *Rhizoctonia solani* Kuhn and *Fusarium* sp., in both leguminous and non-leguminous plants (Ehteshamul-Haque and Ghaffar, 1993). An experiment was therefore carried out to study the effect of *Rhizobium* sp., strain Thal-8 on growth of leguminous, non-leguminous and anaerobic pathogens. Thal-8 is a local isolate that is being used by NARC for producing bioinoculant for chickpea. This inoculum is being widely used by chickpea farmers in Pakistan. It was personally communicated by some farmers that Thal-8 inoculated plants not only gave better yield but they were also more resistance to the disease (Ascochyta blight). The reported study was conducted with the objective to find out the additional benefits of *Rhizobium* sp. strain Thal-8 so that it may be used as biocontrol agent both for leguminous and non-leguminous plants.

MATERIALS AND METHODS

Three leguminous pathogens (*Ascochyta rabiei, Alternaria alternata* and *Fusarium* sp.) were isolated from infected plants of chickpea. A non-leguminous pathogen (*Drechslera* sp.) was isolated from infected wheat plant while an anaerobic pathogen (*Curvularia* sp.) was isolated from rice. *Rhizobium* sp., strain Thal-8 was obtained from Soil Biology and Biochemistry Lab., National Agricultural Research Centre, Islamabad. Potato dextrose agar media was used for culture growth. All the cultures were inoculated against *Rhizobium* sp. on solidified media in Petri dishes, which were divided into two halves by marking with a permanent marker on under sides. *Rhizobium* sp. was inoculated in the form of streak while fungi cultures were inoculated in the form of 1mm disc cut from fifteen days previously grown cultures. Both *Rhizobium* sp. and fungi cultures were inoculated at the same day. After inoculation cultures were incubated at 25°C (±3°C) in an incubator. The colony radii of all fungi cultures were measured after five days of incubation while colony radius of *Ascochyta rabiei* was measured after 10 days. Percent decrease in colony radii of all fungi cultures due to *Rhizobium* sp., strain Thal-8 was calculated by using following formula.

Percent decrease in colony radius = B-A/A x 100

where

B = Colony radius away from *Rhizobium* sp.
A = Colony radius facing *Rhizobium* sp.

RESULTS AND DISCUSSION

In this study, *Rhizobium* sp., strain Thal-8 significantly inhibited the growth of leguminous as well as non-leguminous pathogens. But the growth of anaerobic pathogen was not much affected. The inhibition rate displayed differences in accordance with different fungal cultures (Table 1). This is due to influence of antifungal compounds of rhizobia on the fungal colonies. Thal-8 is also reported to produce acid which have antifungal effect (Khokhar *et al*., 2001). Difference in the efficacy of

Table 1: Percent decrease in colony radii of pathogenic fungi due to Rhizobium (Thal-8)

Fungi sp.	A: Colony radii facing *Rhizobium* (cm)	B: Colony radii away from *Rhizobium* (cm)	Decrease in Colony Radii (%)	Mean (%)
Alternaria alternata	1.5	2.5	66	
	2.0	2.8	40	54
	1.7	2.7	58	
Fusarium sp.	1.9	2.5	31	
	1.7	2.3	35	36
	1.8	2.6	44	
Ascochyta rabiei	1.5	2.0	33	
	1.2	1.7	41	36
	1.4	1.9	35	
Drechslera sp.	1.5	2.2	46	
	1.4	2.0	42	45
	1.7	2.5	47	
Curvularia sp.	2.5	2.5	0	
	2.5	2.7	8	3
	2.6	2.7	3	

rhizobia on different hosts may also be due to the quality/quantity of root exudates which influence the bacterial colonization (Parke, 1991). Rhizobia are also reported to produce toxic metabolites (Chakrabotry and Purkayastha, 1984) which have inhibitory effect on soil-borne plant pathogens.

The rhizobia present in the rhizosphere of plants presumably prevent the contact of pathogenic fungi by covering hyphal tip of the fungus and by parasitizing it (Tu, 1978). Besides parasitizing the hyphae rhizobia also produce antibiotics (Malajezuk, 1983) which resulted in the lysis of the fungal hyphae (Malajezuk *et al*., 1984). Due to aerobic nature of *Rhizobium* the growth of anaerobic pathogen was not much affected. Since, the rhizosphere provide front line defense for roots against attack by pathogens, the rhizobia present in the rhizosphere are ideal for use as biocontrol agents. The growth of *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* was significantly inhibited due to rhizobia (Omar and Abd-Alla, 1998). Beside the fixation of nitrogen rhizobia are reported to produce plant growth regulators such as auxins, cytokinins and gibberallins like substsances that stimulate and enhance plant growth (Triplett *et al*., 1981; Sheng, 1993). There is therefore need to select potential *Rhizobium* (biocontrol agents) that could be effective against more than one pathogens on more than one host plants.

Integration of two or more *Rhizobium* strains could also provide better results than their separate use. *Bradyrhizobium* sp. and *Rhizobium trifolii* significantly controlled *M. phaseolina, Rhizoctonia solani* and *Fusarium solani* infection on sunflower and chickpea (Siddiqui *et al.,* 1998). There is also need to establish a correlation between the population of pathogens in the soil and inoculum dose of biocontrol agents required for effective suppression of the disease as suggested by Dawar *et al.* (1993). It is concluded from this study that *Rhizobium* sp. (Thal-8) could be used as a biocontrol agent both for leguminous and non-leguminous plants.

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