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Inhibition of Mycelial Growth of Seed Borne Pathogenic Fungi of Pea (*Pisum sativum* L.)

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Abstract: ***The percent decrease in radial mycelial growth of different seed borne fungi of pea was recorded by dual culture plate assay and the results revealed the significant effect on the radial mycelial growth, inhibition of all pathogenic fungi (at p=0.05) by Trichoderma species as compared to control. The three antagonists used against the seed borne fungi shows significant effect. The most effective antagonists inhibiting the mycelial growth of pathogenic fungi was T. longibrachiatum followed by T. harzianum and T. viride. In the different pathogenic fungi percent inhibition of mycelial growth on average was observed maximum in case of R.solani (47.11) and F. oxysporum (47.55%) followed by Fusarium moniliforme (41.31%) and A.niger (41.94%) and least inhibition of growth was observed in case of F. roseum (25.97%). Significant interaction showed that T. harzianum inhibited the maximum growth of F. roseum among all the pathogenic fungi.***

Keywords: Pea (*Pisum sativum* L.), pathogenic fungi, Seeds, Mycelial growth

1. Introduction

Seeds are important carrier of plant pathogens so either in field or during their ill storage develops various types of damages due to the association of seed-borne microorganisms. Such seeds showed several types of abnormalities with a great loss in their quality and are considered to be poor quality seeds both for industries and consumers. Different pathogens affect seeds by causing disease. Seed germination, mycelial growth, radicle; plumule length gets affected by these fungi by becoming barriers for the seed development. Efforts were made in present work to examine these pathogens from pea seeds so, as to continue the healthy pea production for future generation.

Seed treatment weather physical, chemical and biological may keep down the inoculum to negligible level. Seed treatment becomes more economical and effective when it is carried out with respect to nature of the pathogen and level of infection percentage (Neergard, 1974). Seeds as a basic unit of propagation carry a wide range of microorganisms externally or internally, become active under favorable conditions resulting extensive damage to seeds and diseases on crops raised from them. Most of the food crops grown are propagated by seed and these crops are infected by many harmful seed borne diseases (Neergard 1977).

2. Material and Methods

Isolation of biocontrol agent (BCA) from soil

The isolation of biocontrol agent was done by obtaining the soil from the rhizosphere of the host to be protected rather than from soil mass. The samples were collected where antagonistic are more common. The collection was made in root zone at 5-15cm (Baker *et al* 1979). The rhizosphere soil of healthy pea plant (*Pisum sativum* L.) was collected from the agricultural fields of district Pulwama of Jammu and Kashmir. An aliquot(4ml) of 10^{-4} ml dilution were spread on sterilized PDA Petriplates added with chloremphenicol at 1g per liter after autoclaving the medium and were incubated at 25^0C under 12 hours of light and darkness. The growing mycoflora were observed under compound microscope, identified with available literature Rifai and Webster (1996), Barnett (1980) and Bissett (1991). The identified *Trichoderma viride, Trichoderma harzianum and Trichoderma longibrachiatum* spp. were purified by hyphal tip culture technique and preserved in refrigerator at 5^0C for further use (Tuti 1969).

Dual culture and antagonistic inhibition on mycelial growth of pathogenic fungi

The *In-vitro* screening was done to obtain the suitable antagonist. The different antagonists were tested against pathogenic fungi following dual culture method (Dennis and Webster, 1971). The inhibition of mycelial growth of pathogenic fungi with the effect of antagonitics were studied by positioning 5mm discs of pathogen fungi diametrically opposite to 5mm disc inoculated in each petriplates or medium. These inoculated discs were harvested from margins of actually growing seven day old cultivar of antagonistic. The pathogenic fungi and the discs were placed at 6cm distance and were incubated at 25^0C. The experiment was conducted in quadruplicate CRD arrangements. The growth of the pathogen was recorded every day .The measurement was taken on the day before contact or after 28 days if no contact occurred between colonies. Percentage inhibition of growth was calculated following the formula suggested by Sunder *et al* (1995). The data were analyzed subjected to analysis of variance (ANOVA) and the Duncan's Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1980).

% inhibition = dc-dt /c x100
dc = average increase in mycelial growth (control)
dt= average increase in mycelial growth in treatment

3. Results

The percent decrease in radial mycelial growth of different seed borne fungi was recorded by dual culture plate assay and the results revealed the significant effect on the radial mycelial growth ,inhibition of all pathogenic fungi (at

p=0.05) by *Trichoderma* species as compared to control (Table 1).

The three antagonists used against the seed borne fungi shows significant effect. The most effective antagonists inhibiting the mycelial growth of pathogenic fungi was *T. longibrachiatum* followed by *T. harzianum* and *T. viride.* In the different pathogenic fungi percent inhibition of mycelial growth on average was observed maximum in case of *R.solani* (47.11%) and *F. oxysporum* (47.55%) followed by *Fusarium moniliforme* (41.31%) and *A.niger* (41.94%) and least inhibition of growth was observed in case of *F. roseum* (25.97%). Significant interaction showed that *T. harzianum* inhibited the maximum growth of *F. roseum* among all the pathogenic fungi.

Table 1: Percent inhibition of mycelial growth of pathogenic fungi by different BCA *in vitro*

Treatment	% inhibition of mycelial growth						
	F. oxysporum	***F. moniliforme***	***F. roseum***	***F. solani***	***R. solani***	***A. niger***	**Means**
T. viride	50.04± 1.32 fg	35.72± 1.64 ij	17.35± 1.42 l	26.62± 3.15ij	45.83± 4.69g	50.75±1.48fg	37.72± 2.75B
T. longibrachiatum	20.40± 1.10 jk	26.81± 0.93 h	12.09± 1.12 kl	16.16± 1.26 kl	19.28± 3.21 kl	28.92± 0.70 hi	20.61± 1.33 D
T. harzianum	26.71± 1.35 ij	34.77± 1.32 b	18.23 ± 0.67cd	29.18± 3.65 hi	33.3±3 4.72 hi	17.63± 0.57kl	26.64± 1.79 C
Control	90.00 ±1.65 b	67.94 1.04±h	56.20± ±0.39 kl	62.29± 1.76 de±	90.0±0± 3.57a	70.43±1.22c	71.9±7 8.29A
Means	47. 55 ±5.35A	41.31±5.98B	25.97± 4.71D	33.57± 4.56C	47.11± 4.10A	41.94± 5.33	
DMRT (P = 0.05)	BCA=2±.748		Fungi=3.365				BCA=6.71

Data are means of four replications
*Means followed by the same letter within a column are not significantly different at (P>0.05) according to Duncan's Multiple range (DMR) Test.
Mean± S.E.

Appendix 1: Analysis of variance for inhibition of mycelial growth of pathogenic fungi of *Pisum sativum* L. by different biocontrol agents

Source	D.F.	S.S.	M.S	F. Value
BCA	5	16123.32	3224.66	141.43**
Media	3	65507.38	21835.79	957.954**
BCA ×Media	15	27213.59	1814.24	79.5756**
Error	12	1641.52	22.79	
Total	95	110485.833		

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