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Bioefficacy of *Aspergillus niger* isolates against Soil Borne Pathogens of Field Pea

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Abstract

Three pathogens *viz. Fusarium oxysporum*f sp. *pisi*, *Rhizoctonia solani* and *Pythium ultimum* were in vitro tested for their sensitivity towards the isolates of antagonists *Aspergillus niger*. Results showed that *Aspergillusniger* proved potential *in vitro* test through inhibiting the mycelial growth. The antagonists grew on the mycelial of all the test pathogens and reduced the mycelial growth of *Fusarium oxysporum*f sp. *pisi*, *Rhizoctonia solani* and *Pythium ultimum*. Among all isolates of *Aspergillus niger*, isolates An1 exhibites significantly the highest antagonistic potential against *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* there by resulting in 68.00, 65.85 and 65.94 percent growth inhibition, respectively over control.The inhibition of the soil borne pathogens is due to the antagonistic effects of locally isolated *A. niger.*

Key words: Pea, Biological Control, Soil Borne Pathogens, wilt, root rot

Introduction

Pea (*Pisum sativum*) is a herbaceous annual plant belongs to family fabaceae, grown for its edible seeds. Wilt and root rot are an important and widespread disease of pea that often causes significant reduction in the yield and quality. Soil borne pathogens like *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium ultimum* were an important cosmopolitan fungal pathogen that infectspea plant species worldwide (Prokinova and Markova, 1997; Maheshwari *et al*., 1982; Poehlman, 1991). In recent years, the occurrence of wilt and root rot disease in leguminous plant has affected yield tremendously. Hence, the management of plant diseases is an imperative need in the present scenario to meet the increasing demand for the continuous and healthy food supply for an ever-increasing human population. Several methods, like cultural, chemical, biological and genetic manipulations are being employed to minimize the losses caused by the plant pathogens.

Among all the methods, Biological control using beneficial microbes, like *spergillus niger* offers an environmental friendly approach to the management of plant diseases and may be incorporated into the cultural, physical controls and limited chemical usage for an effective integrated disease management (IDM) programme. Therefore the present investigation was undertaken to screen the efficacy of rhizospheric fungal isolates against wilt and root rot pathogens of pea.

Materials and Methods

The present investigation was carried out under laboratory conditions at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U. P. (India)

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Source of culture

(a) Causal pathogens

Isolation of Pathogens (*Fusarium oxysporum, Rhizoctonia solani and Pythium ultimum)* done in culture media from diseased plant part collected from field. The infected plants of pea exhibiting characteristic symptoms of wilt and root rot were collected and brought to the laboratory for isolation. Such plants were washed thoroughly under the running tap water to remove the adhering soil. The roots of infected plants were cut into small pieces and then sterilized with sodium hypochlorite or mercuric chloride for 2 minutes followed by 2-3 washing with distilled water. Two to three surface sterilized pieces were placed on solidified potato dextrose agar medium poured in previously sterilized 90 mm diameter Petri plates, aseptically in laminar flow. The inoculated Petri plates were incubated in the BOD incubator at 27± 2°C. These plates were observed daily for fungal growth, if any, was repeatedly sub-cultured on PDA slants for obtaining pure culture. Thereafter, isolated fungus was identified and confirmed on the basis of their cultural and morphological characteristics, respectively.

(b) Isolation and identification of *Aspergillus niger* isolates

Total 3 isolates of *Aspergillus niger* were isolated by using fungal specific media from soils collected from different rhizospheric soils of pea. Soil samples were serially diluted and plated on Potato Dextrose Agar (PDA) media containing 30µg each of Chloramphenicol and Streptomycin sulphate to inhibit any bacterial growth. Pure cultures of *Aspergillus niger* were obtained by subculturing on Potato Dextrose Agar medium. The inoculated cultures were incubated for 4-5 days at 28^{0}C in order to achieve full growth. Initial identification of isolates was done morphologically by observing the color and growth pattern of mycelium in PDA plates. Fungus were analyzedby the microscopically and confirmed by looking on appearance of their hyphal and conidial characteristics

The antagonistic potential of fiverhizospheric fungal isolates of *Trichoderma harzianum* against wilt and root rot pathogens of pea*viz., Fusarium oxysporum* f.*sp. pisi, Rhizoctonia solani* and *Pythium ultimum* was evaluated *in vitro* by dual culture technique.

Dual culture technique. Efficacy of antagonistic fungal isolates of *Trichoderma harzianum* on radial growth inhibition of test pathogens *i.e*. *Fusarium oxysporumf.sp. pisi, Rhizoctonia solani* and *Pythium ultimum* was studied *in vitro* by dual culture technique. Twenty ml sterlized melted PDA was poured in 90 mm diameter Petri plate. After solidification mycelial discs having diameter of 5mm diameter were cut from the young culture of fungal bioagents and test fungus with the help of sterilized cork borer. These discs were placed in the Petri plate containing PDA, maintaining the distance of 4cm between the discs of the test fungus. All the Petri plates were incubated for five days at 28 ±1°C. Each treatment replicated three times. Radial growth inhibition of test pathogens i.e. *Fusarium oxysporum, Rhizoctoniasolani* and *Pythium ultimum* was measured at an interval of 24h for five days to record different stages of antagonism. The observations were recorded after 24, 48, 72, 96 and 120 hrs in the same way as described earlier. The percent inhibition over check, noted after 5 days of incubation was calculated by the formula given by Vincet, 1947.

I = 100 X C T-C

Where, I = Percent Inhibition C = Colony diameter in check T =Colony diameter in treated petriplate.

Results and Discussion

Bio-efficacy of fungal isolates namely *isolates of Aspergillus niger* (An1, An2, An3) were evaluated *in vitro* against *Fusarium oxysporum*f sp. *Pisi Rhizoctonia solani* using dual culture technique on Potato Dextrose Agar. The observations, thus, recorded on radial growth of antagonists and test fungus is represented in Table 1, fig. 1

It is evident from Table 1 that all the 3 isolates of *Aspergillus niger* (An1, An2, An3) significantly inhibited the radial growth of *Fusarium oxysporum*f sp. *pisi Rhizoctonia solani* and *Pythium ultimum* in comparison to control. A persual of the data presented in Table 1 reveal the significants effect of all isolates of *A. niger* (An1, An2 and An3) on radial growth inhibition of wilt and root rot pathogens over control. Of all isolates, An1 exhibites

Table 1. Efficacy of *Aspergillus niger* isolates against wilt and root rot pathogens of pea

Fungal Antagonists	Isolate no.	*Fusarium oxysporum* f.sp. *pisi*		*Rhizoctonia solani*		*Pythium ultimum*	
		Radial growth* (cm)	inhibition (%)	Radial growth* (cm)	Inhibition (%)	Radial growth* (cm)	Inhibition (%)
Aspergillusniger	An1	2.1	68.0	2.3	65.8	2.1	56.9
	An2	2.4	64.0	3.4	50.2	2.5	59.4
	An3	3.2	51.0	3.1	54.1	3.1	49.7
Control	C	6.6	00.0	6.8	00.0	6.1	00.0
CD at 5%		0.39		0.50		0.65	

*Mean of three replicate

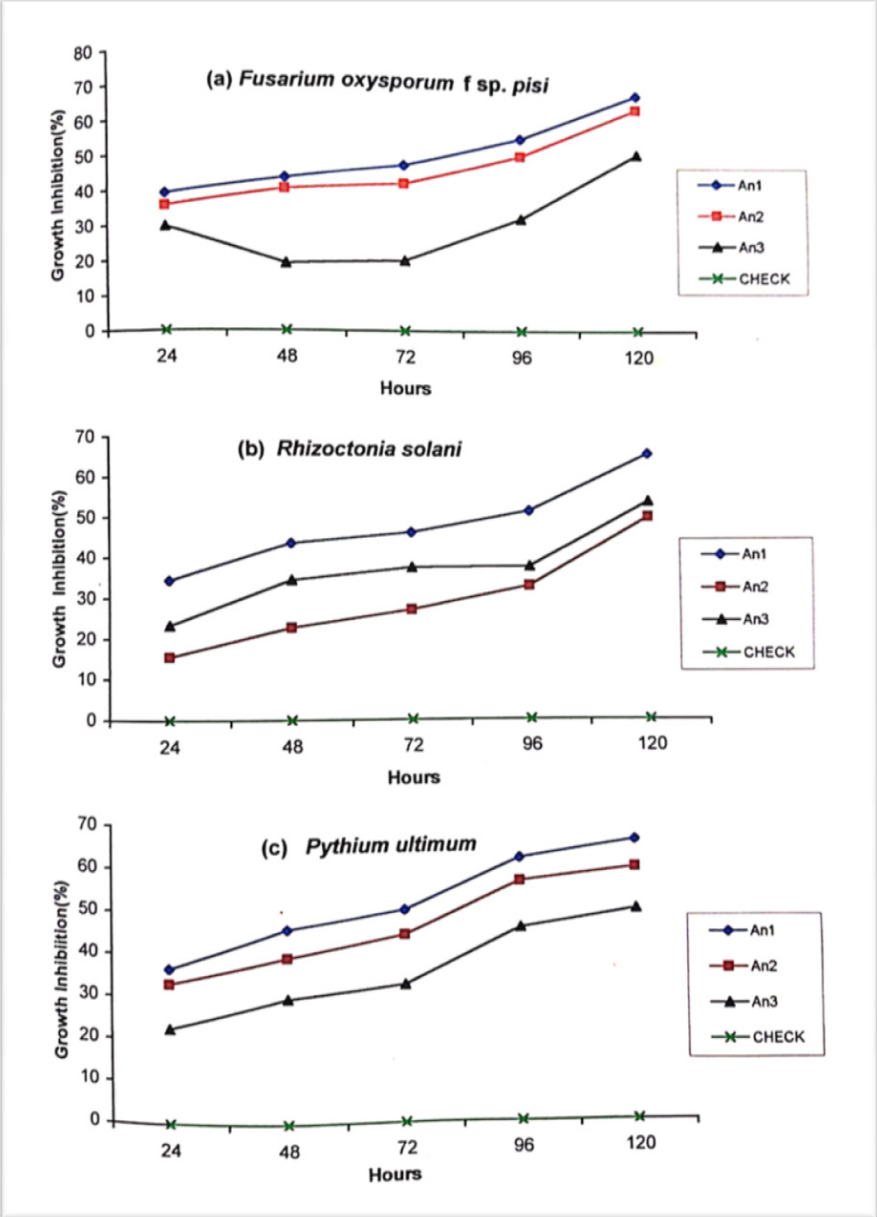

Fig. 1. Efficacy of *Aspergillus niger* isolates against wilt and root rot pathogens of peain subsequent hours.

significantly the highest antagonistic potential against *F. oxysporum* (2.1), *R. solani* (2.3) and *P. ultimum* (2.1) thereby resulting in 68.00, 65.85 and 65.94 percent growth inhibition, respectively over control. However, the efficacy of isolates, An1 and An2 did not visualize any statistical difference against *F. oxysporum* and *P. ultimum* but it was not true in case and statistically at par in their efficacy (Table 1).

The percent growth inhibition resulted due to isolates of *A. niger* at subsequent intervals is depicted in Fig. 2. A close look of the same indicate that all the isolates showed a successive trend in growth inhibition of the pathogens at different hours after inoculation. However, isolate An1 proved to be more effective followed by An2 in inhibiting the growth of *F. oxysporum* f. sp. *pisi* and *P. ultimum*. *Aspergillus niger* can be readily isolated from soil, growth on a wide variety of media with the production of abundant conidia and is known to be a good soil saprophyte. It attacks plant pathogens in culture and treated soils and produces metabolites against the pathogens. The versatility and broad spectrum of activity paved the way to exploit *A. niger* AN 27 as a potential biocontrol for plant disease management. *A. niger* plays a significant role in phosphate solubilization (Vassilev *et al*., 1996).

Buchi *et al*., (1983) explained that *Aspergillus* spp. are well known for producing various kinds of active compounds including antifungal and antibacterial agents. The inhibitory effect exhibited by AN 27 in dual culture and in medium amended with its culture filtrate, persuaded to search for the substance(s) responsible for the action. GC-Mass, IR and NMR spectroscopy analyses of the isolated compound revealed a gamma lactone compound, viz., trans and cis-4 (3 acetoxy-6-methoxy-2-hydroxyphenyl)-2-methoxy butanolide. Minimum inhibitory concentration (MIC) bioassay of the compound against *F. oxysporum* melonis established that it inhibits the mycelial growth of the pathogen above 10 ppm concentration and no growth was observed at 150 ppm (Angappan *et al*., 1996). Strong antagonism by *Aspergillus* sp. Against *Fusarium oxysporum, Fusariun oxysporum* f. sp. *radicislycopercisi*, *Fusarium oxysporum* f. sp. *Vasinfectum* and *F. solani* have been reported by Bora (1977) and Maoris *et al.* (1981).

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