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Isolation and screening of some rhizosphere fungi for their antagonistic potential against *Fusarium oxysporum* f. sp. *lycopersici* in tomato cv Pusa Ruby

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Abstract

Studies were made to isolate and screen the associated rhizosphere mycoflora of *Ziziphus jujuba* Mill.for their antagonistic effect. Fungi were isolated from rhizosphere and rhizoplane of *Ziziphus* in the month of March. Three fungal genera *Penicillium cyclopium, Trichoderma harzianum* and *Trichoderma pseudokoeningii,* known to have antagonistic potential, were screened against *Fusarium oxysporum f.* sp. *lycopersici* to evaluate their biocontrol potential on tomato cv *Pusa Ruby*. Germination percentage of plant was studied after 5, 10, 15 and 20 days of sowing. All three fungi were found to mitigate the adverse effect of the pathogen. Moreover, fungi have improved germination percentage over uninoculated check.

Soil borne Fusaria wilt diseases causes significant losses in a wide variety of crop plants. Tomato occupies a unique position among vegetables with its large number of uses. It is grown in almost all parts in the country occupying approximately 2 lakh hectares. The yield is 25 tonnes per hectare[9]. The yield is affected by losses to crop by various pests and diseases[15]. In India, tomato productivity loss has been estimated to range from 10 to 80% [14], Large number of diseases due to fungi have been reported[10]. Most important soil pathogen of tomato is wilt fungus, *Fusarium oxysporum f.* sp. *lycopersici* causing great damage to the crop[5].

Management of soil borne diseases is a complex process as the soil borne pathogens can survive in soil for longer period or on alternate hosts. Chemical control is too expensive, and development of resistant varieties is rather difficult. Rhizosphere saprophytic microflora play a key role in managing the soil borne diseases. The flora includes both deleterious and beneficial elements that have the potential to influence plant growth significantly. Beneficial micro-organisms affect plant growth positively[3] and suppress harmful microorganisms including pathogens thorough various mechanisms like competition, antibiosis, hyper parasitism and secretion of cell wall degrading enzymes.[2,6]

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Fusarium oxysporum is widespread pathogen causing wilt in many crop plants including tomato. Many fungal antagonists of *Fusarium oxysporum,* Schlechtendahl, *Trichoderma* and *Penicillium* are known to act by hyper parasitism and antibiosis[4]. In present studies an attempt has been made to determine the effect of three well known antagonists of *Fusarium oxysporum* namely *Penicillium cyclopium,* Westling, *Trichoderma harzianum*, Persoon,and *Trichoderma pseudokonigii* Rifai on the germination of seeds of tomato cv. Pusa Ruby. Their antagonistic potential was measured in dual plate culture method in a previous study. Present study aims at the screening of antagonists in field condition by finding out the germination percentage of host crop.

Isolation of Rhizosphere Mycoflora: The three fungi, *Penicillium cyclopium, Trichoderma harzianum and Trichoderma pseudokoningii* were isolated from the rhizosphere and rhizoplane of wild *Ziziphus* plant from Sasani, Aligarh.

Warcup's soil plate method was used for isolating rhizosphere fungi and serial root washing technique[7] for rhizoplane fungi. The Petri plates were incubated at 28°C for a week. There were ten Petri plates in each replicate. The fungi were isolated and identified. Frequency of fungi in soil (non-rhizosphere) served as control. The frequency of occurrence was determined as follows:

$$\text{Frequency of occurrence} = \frac{\text{No. of plates containing a fungus}}{\text{Total number of plates}} \times 100$$

Identification of isolated antagonists, ***Trichoderma harzianum, Trichoderma pseudokoningii*** and ***Penicillium cyclopium*** and strain of locally isolated ***Fusarium oxysporum f.* sp. *lycopersici*** was done from diagnostic lab in department of Mycology and Plant Pathology, IARI, New Delhi.

Germination Trial :

Pure culture of fungi was raised in 250 ml Erlenmeyer flasks containing potato dextrose broth. The flasks were sterilized at 15 lbs pressure for 15 minutes in an autoclave. After cooling these were inoculated with test fungi under aseptic conditions. The flasks were incubated at 28°C. After 15 days, the mycelial mat was filtered through Whatman filter paper no. 1 and washed with sterilized distilled water. It was later blended in blender for obtaining homogenous suspension in sterilized distilled water in such a way so as to obtain 1g of mycelium/10 ml. Seeds of tomato cv usa Ruby after having sterilized with 0.1% sodium hypochlorite and rinsed three times with sterilized distilled water were sown in 10 cm. pots. Later these were inoculated with 5g /kg soil *of Fusarium oxysporum lycopersici,, Penicillium cyclopium*, *Trichoderma harzianum and Trichoderma pseudokoningii* separately. Soil was also inoculated with *F. oxysporum lycopersici* (5g) together with different inoculum levels of all the three antagonistic fungi. Un- inoculated seeds served as check. Observations were made after 5, 10, 15, 20 and 25 days. Germination percentage and the change in this percentage in relation to the check were calculated.

All twenty-four species of fungi were isolated (Table-1) with many genera common

Table-1 Study of Rhizosphere and Rhizoplane Mycoflora of *Ziziyphus jujuba* in the month of March in at Aligarh

S.No.	Name of Fungi	Frequency of occurrence in		
		S	R.S.	R.P.
1	*Alternaria alternata* (Fr.) Keissler	40	00	10
2	*Alternaria humicola* Qudemans	00	30	00
3	*Aspergillus clavatus* Desmazieres	00	10	10
4	*Aspergillus flaviceps*	30	20	10
5	*Aspergillus flavus* Link	10	10	00
6	*Aspergillus fumigatus*	20	30	10
7	*Aspergillus niger* van Tiegham	40	30	10
8	*Aspergillus terreus* Thom	00	20	00
9	*Aspergillus ochraceous* Wilhelm	00	20	00
10	*Aureobasidium* sp.	40	30	00
11	*Chaetomium* sp.	00	30	20
12	*Cladosporium cladosporioides* Fres. (deVries)	60	50	40
13	*Curvularia* sp.	30	00	00
14	*Epicoccum* sp.	00	30	00
15	*Fusarium oxysporum* Schlechtendahl	60	40	40
16	*Penicillium citrinum* Thom	30	40	30
17	*Penicillium cyclopium* Westling	00	40	50
18	*Penicillium glaucaum* Stoll	00	30	20
19	*Rhizopus nigricans* Ehrenb	90	60	50
20	*Sclerotium* sp.	30	00	30
21	*Trichoderma harzianum* Persoon	00	40	40
22	*Trichoderma pseudokonengii* Rifai	00	40	00
23	*White sterile mycelium*	60	00	30
24	*Yellow sterile mycelium*	30	40	40
		12	19	13

Each figure is a mean of ten replicates R= Rhizosphere ; Rp= Rhizoplane; S= Soil

in soil, rhizosphere, and rhizoplane. However, frequency of antagonist fungi was significantly higher in rhizosphere. Out of twenty four, four genera, *Aspergillus niger, Penicillium cyclopium, Trichoderma harzianum and Trichoderma pseudokonigii* were screen for their antagonistic properties in dual culture method in a separate study[1]. Since *A.niger* have not shown good transition zone remaining three were tested for germination trial.

Table-2. Effect of antagonistic rhizosphere fungi on germination percentage of tomato variety *Pusa Ruby* separately and together in soil

Treatment	5 Days		10 Days		15 Days		20 Days	
	% Emergence	% Change	% Emergence	% Change	% Emergence	% Change	% Emergence	% Change
Uninoculated	20	–	32	–	40	–	42	–
F.o.l	8	-60.00	26.6	-16.88	39.3	-1.75	34.7	-17.38
Penicillum								
cyclopium	22.7	+13.50	33.3	+4.06	41.3	+3.25	64	+52.38
T. harzianum	8.0	-60.00	20.6	-35.63	36	-10.00	44	+4.76
T.pk.	18.7	-6.50	34.7	+8.44	44	+10.00	67	+59.52
F.o.l.+ P.c	20	0.00	34.7	+8.44	43.3	+8.25	58	+38.10
F.o.l +T.h.	12.7	-36.50	34.7	+8.44	41.3	+3.25	46.7	+11.19
F.o.l.+ T.pk	29.3	+46.50	53.3	66.56	58.3	+45.75	66.7	+58.81

F.o.l. = Fusarium oxysporum f. sp. *lycopersici* — *T. h. = Trichoderma harzianum*
P.c.= Penicillium cyclopium — *T. pk = Trichoderma pseudokoningii*

Results presented in table-2 and summarizes the effect of various treatments on percentage of germination of tomato seeds. *Fusarium oxysporum* f. sp. *lycopersici* proved deleterious when inoculated separately. In general, all three fungi when inoculated separately, shown improvement in percentage of germination compared to uninoculated check in final come out.

The improvement in germination percentage varied with three antagonists. Initially at 5 days' time interval the germination percentage has shown reduction or marginal increase over check. The percentage improvement was increased with progression of number of days. Most effective in improving the germination percentage was *T. pseudokonigii* (+59%) followed by *P. cyclopium* (+52%. The results agreed with Poliquit,[13] and Hend *et al,*[8]. However, strain of *T. harzianum* has been less effective in improving germination percentage. In combined inoculations also the trend was same. Strain of *T. pseudokoningii* was most effective of all three genera screened.

The genus *Trichoderma* spp are most commonly used biocontrol agent which acts as a symbiotic fungus that inhibits the *Fusarium* wilt through the production of various lytic enzymes, and other antagonistic secondary metabolites. It is an enhancement in the growth of the host plant by the production of plant growth hormones. *T. pseudokonigii* is coming as potential producer of commercial fermenting enzymes. *Penicillium* though less exploited than *Trichoderma* has come out as a promising biocontrol agent for *F. oxysporum lycopersici* in tomato[12]. The mechanism of control action is predominantly production of antimicrobial substances , cell wall degrading

enzymes and growth promoting hormones.[11]

It could be concluded from above studies that these antagonists can be exploited in agro ecosystems by influencing them in host rhizosphere and the intensity of *Fusarial* wilt can be regulated.

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