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Trichoderma asperellum: A potential biocontrol agents against wilt of pigeonpea caused by *Fusarium udum* Butler

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ABSTRACT

***Fusarium* wilt caused by *Fusarium udum* (FU) is one of the most devastating soil borne pathogens of pigeonpea. Rhizosphere of pulses is very rich in microbial diversity and possesses several kinds of beneficial microorganisms. Among the beneficial microorganisms, *Trichoderma* is the most widely used biofungicide worldwide. It is soil-borne filamentous fungi, capable of managing several plant pathogenic fungi. Twelve isolates of *Trichoderma asperellum* were isolated from pulses rhizosphere and evaluated against *Fusarium udum* under *in vitro* and *in situ* condition for their antagonistic and growth promoting ability. Out of twelve isolates tested, two isolates (IIPRTas-6 and IIPRTas-11) were most promising and inhibited maximum mycelial growth (70.83 and 69.17% respective-ly) of test pathogen under *in vitro* condition. For growth promotion potential, IIPRTas-4 and IIPRTas-12 were identified as promising in respect of root length (cm) and shoot length (cm) under pot conditions. The aim of this study was to identify the indigenous *Trichoderma asperellum* from pulses rhizosphere for management of wilt of pigeonpea.**

Key words: *Trichoderma asperellum*, Pulses rhizosphere, *Fusarium udum*, Biocontrol

Pigeonpea wilt caused by *Fusarium udum* Butler is one of the most economically important diseases in India inflicting heavy yield losses at various stages of crop (Khare *et al.* 1994). Several methods such as chemical seed treatment, soil solarization, inter/mixed cropping or crop rotation, and through host plant resistance have been recommended for management of wilt disease (Vishwadhar and Chaudhary, 1998). However, wilt still continues to be one of the major biotic constraints to pigeonpea production. Biocontrol agents have assumed special significance in the present day strategy for developing environmentally safe methods of plant disease management. Prospects of biological control of soil borne pathogens using *Trichoderma* species have been described (Papavizas, 1985). *Trichoderma* spp have been widely used as commercial biocontrol agents all over the world (Harman *et al.* 2004, Verma, 2007, Mukherjee *et al.* 2013, Mishra *et.al* 2016, 2018). India alone is having more than 250 commercial formulations which are being used against many diseases of different crops (Mukherjee *et al.* 2013, Singh *et al.* 2012). The indigenous strains of *Trichoderma* spp. seem to function as better antagonists and disease management agents as they are well adapted to local conditions. Since meager information on the exploitation of native isolates of *Trichoderma* spp. against wilt of pigeonpea in Central and Southern region of Uttar Pradesh are available, the present study has been under taken to identify the native *Trichoderma* isolates and explore their antagonistic potential against *F. udum* causing wilt disease of pigeonpea.

MATERIALS AND METHODS

Collection of soil samples and isolation of Trichoderma asperellum from pulses rhizosphere : Twenty random rhizosphere soil samples were collected from healthy pigeonpea, chickpea, lentil and fieldpea from Kanpur and nearby districts (Kanpur Dehat, Fatehpur, Auraiya) and four district of Bundelkhand (Hamirpur, Jalaun, Banda, Jhansi) and stored in sterile plastic bags. The soil samples were air dried. Isolation was done by using serial dilution technique on *Trichoderma* Selective Medium (Elad and Chet, 1983,

Table: 1 Details of *Trichoderma asperellum* isolates from pulse rhizosphere in Bundelkhand region

Isolate details	Rhizospheric soil detail	Locations	Geographical locations	Name of the species	Colony color
IIPRTas-1	Chickpea	IIPR Main Research farm	20° 16' 12.00" N 80° 08' 24.00" E	*Trichoderma asperellum*	Dark green
IIPRTas-2	Fieldpea	Kanpur Dehat	26.5267° N 79.8297° E	*Trichoderma asperellum*	Whitish green
IIPRTas-3	Chickpea	Jalaun	26.1210° N, 79.7484° E	*Trichoderma asperellum*	Whitish green
IIPRTas-4	Lentil	Hamirpur	25.7913° N, 80.0088° E	*Trichoderma asperellum*	Dark green
IIPRTas-5	Lentil	Hamirpur	25.7913° N, 80.0088° E	*Trichoderma asperellum*	Dark green
IIPRTas-6	Chickpea	Fathepur	25.8500° N, 80.8987° E	*Trichoderma asperellum*	Dark green
IIPRTas-7	Pigeonpea	Banda	25.4796° N, 80.3380° E	*Trichoderma asperellum*	Dark green
IIPRTas-8	Fieldpea	Auraiya	26.4626° N, 79.5098° E	*Trichoderma asperellum*	Light Green
IIPRTas-9	Chickpea	Jalaun	26.1271° N, 79.4704° E	*Trichoderma asperellum*	Whitish green
IIRTas-10	Chickpea	Jhansi	25.4484° N 78.5685° E	*Trichoderma asperellum*	Whitish green
IIPRTas-11	Pigeonpea	Kanpur Dehat	26.2017° N 79.8157° E	*Trichoderma asperellum*	Whitish green
IIPRTas12	Pigeonpea	Kanpur Dehat	25.2765° N 83.0335° E	*Trichoderma asperellum*	Dark green

Morton and Stroube 1955). Cultures were purified by single spore culture technique on PDA plates (point inoculation) and incubated at 27°C for 24-48 hr. They were identified on the basis of their cultural and morphological characters (Rifai, 1969). Morphological and cultural identification was done based on colony colour, nature of sporulation and their growth, conidia and conidiophores characteristics through microscopic observation.

***Collection and maintenance of Fusarium udum* pathogen:** *Fusarium udum* was isolated from wilted plants of pigeonpea (cv. Bahar) grown in pigeonpea wilt sick field using standard method. Pure culture was maintained using single spore culture on Potato Dextrose Agar (PDA) at 27°C. The pathogenicity of the isolates was confirmed in pigeonpea cv. Bahar under greenhouse conditions as described by Kannaiyan and Nene, 1981.

Antagonistic potential Trichoderma asperellum against F. udum

Dual culture technique: Petridishes (90mm) containing PDA were inoculated with 5mm diameter mycelial disc of 7 days old culture of *F. udum* and *Trichoderma* spp. at equal distance from the periphery. Inoculated petridishes were incubated at 27°C in BOD incubator and the radial growth of FU was measured 2, 4, 5, 6 and 7 days after incubation. Control without *Trichoderma* was maintained and each treatment replicated thrice. Percent inhibition of FU radial growth was calculated with following formula (*Vincent* (1927) :

$$\text{Percent (\%) inhibition (I)} = (C - T/C) \times 100$$

I = % inhibition

C = radial growth of test pathogen with absence of antagonist (mm)

T = radial growth of test pathogen with antagonist (mm)

In situ efficacy of T. asperellum for growth promotion activity : Twelve isolates of *Trichoderma asperellum* were evaluated for their growth promotion potential. *T. asperellum* were inoculated in 100ml Potato Dextrose Broth (PDB) medium in 250 ml conical flasks and incubated for 7 days at 27°C. 7 days old fungal mycelia were harvested, crushed and pigeonoea seeds (var: Bahar) were treated for a 10 minutes. Treated seeds were sown in earthen pots (13×13×5 cm) containing 1.0 kg of sterilized field soil. Each treatment was replicated thrice in Completely Randomized Block Design (CRD). The growth parameters *viz*. shoot length and root length were recorded at 60 day after sowing (DAS) and germination percentage was observed after 15 DAS. Length of shoot was measured from the base of stem to its tip, while root length was measured from its point of attachment on stem base to the tip of the tap root.

RESULTS AND DISCUSSION

Identification of Trichoderma asperellum : Total 12 *T. asperellum* isolated from pulses rhizosphere. White to dark greenish colony was observed on the basis of their cultural characteristics (Fig.1). Variability was also observed in size and shape of conidia, conidiophores and phialides. In most of the isolates, conidia were globose to subglobose in shape, conidiophores are generally branched, paired and chlamydospores was typically present. Phialides are straight or hooked and almost cylindrical and swollen in the middle (Fig. 2).

Fig. 1: Cultural variability among the *Trichoderma asperellum* isolates

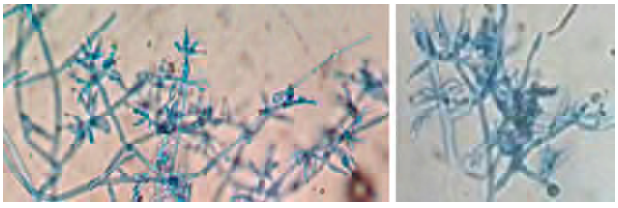

Fig. 2: Morphological characteristics of *T. asperellum*

Screening of the antagonistic activity of T. asperellum isolates : All the 12 isolates of *T. asperellum* tested caused significant reduction in the mycelial growth of the fungus. The percent inhibition ranged between 65.21 to 70.83% was recorded in the isolates of IIPRTas-6, IIPRTas-11, IIPRTas-10, IIPRTas-1, IIPRTas-8, IIPRTas-7 and IIPRTas-5 (Table 2). The highest inhibition was caused by isolates IIPRTas-6 and IIPRTas-11 (70.83 and 69.17% respective-ly). However, minimum percent inhibition was recorded in IIPRTas-2

Table: 2 ***In vitro* evaluation of potential native *T. asperellum* against *F. udum***

Isolate details	Name of the species	Mycelial growth (mm) of *F. udum* after 7 days of incubation*	Percent inhibition over control (%)
IIPRTas-1	*Trichoderma asperellum*	27.67	68.95
IIPRTas-2	*Trichoderma asperellum*	37.93	57.45
IIPRTas-3	*Trichoderma asperellum*	31.67	64.47
IIPRTas-4	*Trichoderma asperellum*	32.00	64.09
IIPRTas-5	*Trichoderma asperellum*	31.00	65.21
IIPRTas-6	*Trichoderma asperellum*	26.00	70.83
IIPRTas-7	*Trichoderma asperellum*	30.50	65.78
IIPRTas-8	*Trichoderma asperellum*	29.70	66.78
IIPRTas-9	*Trichoderma asperellum*	32.17	63.91
IIRTas-10	*Trichoderma asperellum*	27.60	69.03
IIPRTas-11	*Trichoderma asperellum*	27.47	69.17
IIPRTas12	*Trichoderma asperellum*	31.30	64.89
CONTROL	*Fusarium udum*	90.0	-

*Average three replication

Table 3. Characterization of *T. asperellum* isolates for growth promotion potential

Isolate details	Name of the species	Germination percent (%)	Method of application	Root length* (cm)	Shoot length* (cm)
IIPRTas-1	*Trichoderma asperellum*	86.76	ST	7.90	24.62
IIPRTas-2	*Trichoderma asperellum*	85.39	ST	9.00	24.50
IIPRTas-3	*Trichoderma asperellum*	88.02	ST	9.70	25.90
IIPRTas-4	*Trichoderma asperellum*	89.15	ST	11.80	25.90
IIPRTas-5	*Trichoderma asperellum*	88.36	ST	8.50	22.50
IIPRTas-6	*Trichoderma asperellum*	80.49	ST	8.75	24.52
IIPRTas-7	*Trichoderma asperellum*	79.49	ST	8.25	27.65
IIPRTas-8	*Trichoderma asperellum*	84.49	ST	8.66	25.33
IIPRTas-9	*Trichoderma asperellum*	88.89	ST	7.20	24.81
IIRTas-10	*Trichoderma asperellum*	79.49	ST	9.65	31.15
IIPRTas-11	*Trichoderma asperellum*	80.56	ST	7.52	25.87
IIPRTas12	*Trichoderma asperellum*	90.49	ST	11.65	26.50
CONTROL		79.86	-	6.25	17.50

*Average three replication; **ST:** Seed Treatment

isolate i.e. 57.45 percent followed by isolate IIPRTas-9 i.e. 63.91 percent (Table-2).

In situ evaluation of T. asperellum for plant growth promotion activity: Results revealed that, the significant differences were observed in all the isolates of *T. asperellum*. The maximum root and shoot length were recorded higher in isolates, IIPRTas-12 (11.65cm and 26.50cm) and IIPRTas-4 (11.80cm and 25.90cm). Whereas in control 6.25cm root length and 17.50cm shoot length were recorded. The maximum seed germination was recorded in IIPRTas-12 (90.49%) and the lowest germination was recorded in IIPRTas-7 (70.49%) and IIPRTas-10 (70.49%) (Table.3). Several research findings that have appeared in the literature revealed the fact that various species of *Trichoderma* suppress mycelial growth of soilborne pathogens, increase plant growth and induce resistance in various crops (Mukherjee, 1993; Tian *et al.* 2001; Dutta and Das, 2002; Palomar *et al.* 2002, Mishra and Gupta, 2012, Kumar *et al.* 2012, 2016). The present study clearly indicates that, the high potential of *Trichoderma asperellum* isolates differ in its biocontrol ability against *Fusarium udum* and plant growth promotion ability in pigeonpea. Therefore, the identified potential isolates of *T. asperellum* can be used for making formulations as well as consortia for their further exploitation.

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REFERENCES

Datta P and BC Das. 2002. Management of collar rot of tomato by Trichoderma spp. and chemicals. Indian Phytopathol. **55**: 235-237.

Elad Y and Chet I. 1983. Improved selective media for isolation of *Trichoderma* spp. and *Fusarium* spp. Phytoparasitica. **11:** 55–58.

Harman GE, Howell CR, A Viterbo, I Chet and M Lorito. 2004. Trichoderma species–opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. **2:** 43–56.

Singh HB, BN Singh, SP Singh and BK Sarma. 2012. Exploring different avenues of *Trichoderma* as a potent bio-fungicidal and plant growth promoting candidate- an overview, Rev Plant Pathol, **5:** 315–426.

Mukherjee K, AK Sampath, S Kranthi, PK Mukherjee. 2013. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation, 3 Biotech. DOI 10.1007/s13205-013-0150-4.

Kannaiyan J. and Nene YL 1981. Influence of wilt at different growth stages on yield loss in pigeonpea, Trop. Pest Manage. **27** (1): 141.

Khare D, Satputi, RG and Tiwari, AS 1994. Present status of the wilt and sterility mosaic of pigeonpea. Indian Journal of Genetics and Plant Breeding, **54**: 337–346.

Kumar K, N Amaresan, S Bhagat, K Madhuri, RC Srivastava. 2012. Isolation and characterization of Trichoderma spp. for antagonistic activity against root rot and foliar pathogens. Indian Journal of Microbiology **52**(2): 137-144

Kumar K, Manigundan K, Amaresan N, 2016. Influence of salt tolerant Trichoderma spp. on growth of maize (*Zea mays*) under different salinity conditions. J. Basic Microbiol. **57**(2): 141-150.

Mishra RK, Naimuddin, Monika Mishra and Sandeep Sharma. 2016. *Trichoderma*: Potential biocontrol agents for pulses. Dalhan Alok **14**: 80-86.

Mishra RK, Naumuddin, Mohd. Akram, DK Sachan, Monika Mishra and Krishna Kumar. 2016. Characterization of Indigenous *Trichoderma* spp through ITS based sequences. Pulses News Letter. **27**(2): 5.

Mishra RK, A Bohra, Naimuddin, Krishna Kumar, Sujayanand GK, PR Saabale, Satheesh Naik SJ, BK Sarma, D. Kumar, Monika Mishra, DK Srivastava and NP Singh. 2018. Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects. Egyptian Jr. Biological Pest Control. DOI 10.1186/s41938-017-0004-1.

Mishra RK and RP Gupta. 2012. *In vitro* evaluation of plant extracts, bio-agents and fungicides against Purple blotch and Stemphylium blight of onion. Journal of Medicinal Plants Research. **6(48):** 5840-5843.

Morton DJ and WH Stroube. 1955. Antagonistic and stimulating effects of soil micro-organism of *Sclerotium*. Phytopathol., **45**: 417-420.

Mukherjee PK. 1993. Biological control of collar rot, dry root rot, wilt and gray mold of chickpea. In: Legumes pathology progress report no. 20 of ICRISAT, Patencheru, India p. 36.

Palomar MK, YC Mangaoang, VG Palermo, GE Escuadra and MB Posas. 2002. Biocontrol of root crop diseases through microbial antagonism. In: Microbial Biotechnology in the Asia- Pacific Region: Prospectus and Challenges for the 21st Century": Proceedings of the 4th Asia Pacific Biotechnology Congress and 30th Annual Convention of the PSM, Inc. College, Laguna, Philippines. p. 322.

Papavizas GC. 1985. *Trichoderma* and *Gliocladium*: their biology, ecology and potential of biocontrol. Annual review of Phytopathology **23**: 23–47.

Rifai M.1969. A revision of the genus *Trichoderma*. Mycological Paper. 116: 1–56.

Tian LS, WH Wang, WL Shi, SS Li, YM Shi, GW Zhang and LP Zhang. 2001. Studies on mechanisms of antagonism of *Trichoderma viride* to *Fusarium oxysporum* f. sp. *lycopersici* and its effect of biocontrol. Plant Protect. **27:** 47-48.

Vincent JM (1927). Distortion of fungal hyphae in the presence of certain inhibitors. Nature **59:** 850.

Vishwadhar and Chaudhary, RG. 1998. Diseases of Pigeonpea and field pea and their management, pp. 217–238. In: Thind, T. S. (Ed.). *Diseases of field crops and their management*. National Agricultural Technology Centre, Ludhiana, India.