Research Paper :

Evaluation of biocontrol potential of *Trichoderma* species against *Sclerotium rolfsii, Aspergillus niger* and *Aspergillus flavus*

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SUMMARY

Forty six isolates of *Trichoderma* spp. belonging to *viz., viride, harzianum, hamanatum, ressei* and *koningii* species groups were screened for their modes of biocontrol ability against *Sclerotium rolfsii* Sacc., *Aspergillus niger* van Teighem and *A. flavus* Link ex Fries, the causal agents of stem rot, collar rot and aflaroot of groundnut, respectively. The isolates T005, T043, T095, T49, T126, T144, T166, T191, 250, 390 and T425 gave maximum inhibition of mycelial growth of *S. rolfsii* in dual culture and killed the sclerotia. The isolate numbers T043, T071, T250, T292, and T425 were most effective against *A. niger*. While the isolate numbers T004, T040, T043, T071, T144, T292, T357, T390 and T425 showed maximum inhibition of *A. flavus*. The isolates T043 and T425 were effective against all the three pathogens *i.e. S. rolfsii, A. niger* and *A. flavus*. Isolates T071 and T292 were effective against *A. niger* and *A. flavus*, while isolates T144 and T390 were effective against *S. rolfsii* and *A. flavus*. The results of this study indicate that the two strains (T043 and T425) have the potential for biological control of *S. rolfsii, A. niger* and *A. flavus*. Thus, use of T043 and T425 as seed treatment or furrow application may enable to reduce soil borne diseases of groundnut. However, filed evaluation of these isolates should be under taken to evaluate their efficiency against the soil borne pathogen of groundnut.

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Out of nine oilseed crops grown in India, groundnut accounts for 35% of the total area cropped under oilseed and 40% of the total oilseed production. Though, India is the largest producer of groundnut, its average productivity levels are very low as compared to USA and China. An important factor contributing to low yield are diseases (Subramanyam and McDonald, 1983). Stem rot, collar rot and aflaroot are the major soil-borne diseases of groundnut causing extensive damage to the crop. *S. rolfsii* attacks the crop at all the stages causing seed rot, seedling blight, stem rot and pod rot, the most common being stem rot. On the contrary, both *A. niger* and *A. flavus* primarily attack the seedling stage causing collar rot and aflaroot. Out of the only economical management measure recommended for these diseases is to treat seed with fungicides, but it can not protect the crop for longer period. The chemical method developed control too has its own limitations such as high capital investment, non-remunerative, poor availability, selectivity, temporary effect, efficacy affected by physico-chemicals and biological factors, development of pest resistance, pollution of food and feeds, health hazards, environmental pollution, etc. Considering these limitations, biological control is an important approach in this direction. It refers to "Reduction of amount of inoculum or disease producing activity of a pathogen accomplished by through one or more organisms other than man" (Cook and Baker, 1983). The potential for biological control of plant diseases has been reviewed by (Blackman and Pokkema, 1982; Mukerji, 1983 and Upadhya and Rai, 1983).

S. rolfsii is a facultative parasite and is found in a wide-range of soil. The fungus survives in soil mainly as sclerotia, which represent the main source of inoculum and remain viable in soil for several months. *S. rolfsii* attacks all parts of the plant but stem

infection is the most common and serious. The first symptom is sudden wilting of a branch, which is completely or partially in contact with the soil. The junction of the branch with the stem near the soil level is the most favoured point of attack and a white coating of fungus mycelium appears there. Sclerotia of mustard seed size appear gradually on the infected areas. *A. niger* (collar rot) is found on groundnut in all the major growing areas of the world (Porter *et al.,* 1984). Although *A. niger* attacks groundnut at all stages, seedlings and young plants are particularly susceptible. Sudden loss of leaf turgidity and wilting of plants are characteristics of collar rot. The infected part is profusely covered with black masses of mycelium and conidia. *A. flavus* thrives in the soil as a saprophyte and invades groundnut seeds, seedlings and pods during pre-and post-maturity stage in the field. Seedling infection with *A. flavus* is characterized by the necrotic lesions on the emerging plumule and cotyledons. Lesions become covered with masses of yellow-green spore heads of *A. flavus*. Infected seedlings lack a secondary root, a condition known as 'aflaroot' (Chohan and Gupta, 1968; Aujla *et al.*, 1978).

Trichoderma species are asexual, soil-inhabiting filamentous fungi and have the ability of antagonizing a series of plant pathogenic fungi (Papavizas, 1985). Proposed mechanisms of antagonism include mycoparasitism by the action of cell-wall degrading enzymes, antibiosis by the production of antibiotics, competition for space and nutrients through rhizosphere competence, facilitation of seed germination and growth of the plants via releasing important minerals and trace elements from soil and induction of the defense responses in plants (Herrera and Chet, 2003; Howell, 2003; Benýtez *et al.*, 2004). Biological control of soil borne disease by microbial antagonists has been widely reported (Deacon, 1988, Hornby, 1990). *Trichoderma* species are considered as promising biological control agents against numerous phytopathogenic fungi (Sarhan *et al.*, 1999). In the present study, attempts have been made to investigate the potential of these biological resources in controlling growth and sporulation of *S. rolfsii*, *A. niger* and *A. flavus* under *in vitro* conditions.

MATERIALS AND METHODS

Study site:

The present study was carried out in Department of Plant Pathology, Directorate of Groundnut Research (formerly National Research Centre of Groundnut), Junagadh, Gujarat, during 2009.

Isolation and maintenance of fungal plant pathogens:

Economically important three fungal plant pathogens *viz.*, *S. rolfsii, A. flavus,* and *A. niger* causing soil borne diseases of groundnut were isolated from infected plants. The infected groundnut plants samples were collected from Latur, Nanded, and Solapur districts of Maharashtra state. Infected plants were collected during *Kharif* 2008 and 2009 and brought to the laboratory in separate polythene bags. As soon as the infected plants were brought to the laboratory, small bits of infected plant parts were removed carefully and surface sterilized with 0.1% $HgCl_2$ and were transferred to PDA Petridis. These plates were incubated for 5 days at 28^0C in BOD. After three days of incubation, pure culture of individual pathogen was obtained by sub-culturing the culture. Pure cultures of these pathogens were maintained on Potato dextrose agar (PDA) slants and stored at 4^0 C for further studies. Twenty seven isolates of *S. rolfsii*, 19 isolates of *A. flavus and* 11 isolates of *A. niger* were isolated from infected plants and rhizospheric soil samples collected from groundnut growing fields. Thereafter, virulence of these pathogens was studied and based on this study, the most virulent isolates of *S. rolfsii* (SR-17), *A. flavus* (AF-5) and *A. niger* (AN-9) were selected for the present study.

Trichoderma species:

Five spp. of *Trichoderma viz., T. viride, T. harzianum, T.hamanatum, T.ressei and T. koningii,* were used in the present investigations. All the biocontrol agents were obtained from culture bank of Plant Pathology Department, Directorate of Groundnut Research, Junagadh. For the laboratory experiments, the pathogens as well as antagonists were grown on Maltose peptone agar medium (maltose-20g, peptone-2g, agar-12g and 1000 ml water).

Test of antagonistic potential:

Five spp. of *Trichoderma viz. T. viride, T. harzianum, T. hamanatum, T. ressei and T. koningii* were used for antagonistic test by dual culture technique using 20 ml of Potato dextrose agar medium in 90 mm culture plates. Potato dextrose agar medium in the culture plates was seeded with the *Trichoderma* species and test pathogen (5 mm culture discs of three days old culture) opposite each other near the periphery of Petriplates. The medium inoculated with the pathogen alone served as control. These plates were incubated in BOD at 28°C and 70% relative humidity. Five replications were maintained for each isolate. After 72 hrs of inoculation,

diameter of the mycelial growth of both the antagonists and test pathogens was measured. The data from the replicated plates were averaged and the result was expressed as per cent inhibition of fungal plant pathogens growth over the control. The percentage growth inhibition of pathogens was obtained by using the formula:

$$\text{Percentage growth inhibition} = \frac{A - B}{A} \times 100$$

where A = Area covered by test pathogen in control (mm)

B= Area covered by test pathogen confronted with *Trichoderma* (mm)

RESULTS AND DISCUSSION

The percentage growth inhibition of three fungal plant pathogens by tested *Trichoderma* species are shown in Table 1 Antagonism between the potential antagonists and test pathogen indicated that, the test pathogen stops growing upon contact with the antagonist which continues its growth over the test fungus colony. The maximum growth inhibition of *S. rolfsii* was exerted by isolate numbers T005, T043, T095, T49, T126, T166, T144, T191, T250, T390 and T425. The isolate numbers T042, T071, T250, T292, and T425 were most effective against *A. niger*. While the isolate numbers T004, T040, T043, T071, T144, T292, T537, T390 and T425 showed maximum inhibition of *A. flavus*. Only two isolates T043 and T425 were effective against all the three pathogens *i.e. S. rolfsii, A. niger* and *A. flavus* (Plate 1).

The idea of a sustainable agricultural practice and environmental protection enhances the importance of biocontrol. The adoption of a sustainable agricultural practice, using strategies that are environmentally

Table 1 : *In vitro* efficacy of *Trichoderma* species against *S. rolfsii, A. flavus* and *A. niger*

Trichoderma species	Growth inhibition (%) by *Trichoderma* species			*Trichoderma* species	Growth inhibition (%) by *Trichoderma* species		
	S. rolfsii	*A. flavus*	*A. niger*		*S. rolfsii*	*A. flavus*	*A. niger*
T003	82.8	79.5	83.8	T49	88.8	81.3	82.1
T040	79.2	92.4	90.5	T144	88.4	91.9	90.3
T004	74.2	96.9	94.5	T145	70.1	69.7	71.6
T005	96.6	92.8	91.6	Tv267	66.1	70.0	72.4
T017	76.4	77.8	76.4	T071	77.4	81.1	95.6
T043	98.7	97.4	98.0	T115	56.7	59.4	63.0
T082	70.3	72.2	73.1	T117	68.3	70.4	71.6
T093	66.2	67.6	69.5	T134	69.9	71.2	72.1
T095	97.6	87.2	89.8	T166	87.8	84.8	85.1
T107	69.3	71.7	75.4	T191	86.9	84.9	85.6
T116	67.8	66.6	68.6	T218	50.8	54.3	60.9
T119	75.5	73.7	76.1	T221	58.5	62.8	68.7
T126	92.1	89.4	90.6	T226	74.0	77.4	79.4
T127	61.0	63.9	70.3	T257	51.0	57.3	68.0
T146	69.1	70.0	72.4	T268	75.7	77.5	82.3
T171	79.3	94.2	92.5	T29	75.1	79.4	82.7
T190	79.9	78.2	76.5	T356	66.0	69.5	75.3
T215	63.4	58.7	62.4	T357	66.2	92.7	90.8
T219	67.8	71.4	74.9	T359	79.9	79.5	80.5
T250	91.4	86.3	95.3	T361	78.1	75.5	77.1
T253	55.6	55.6	60.2	T362	69.1	73.4	76.5
T257	80.3	77.6	79.3	T390	84.2	93.7	87.1
T292	75.4	89.5	93.4	T425	91.9	93.1	95.1

C.D. (P=0.05)	0.33	*S. rolfsii*
C.V. %	2.37	
C.D. (P=0.05)	0.50	*A. flavus*
C.V. %	3.49	
C.D. (P=0.05)	0.51	*A. niger*
C.V. %	3.51	

S. rolfsii -VS-T48

S. rolfsii -VS-T005

S. rolfsii -VS-T126

A. niger -VS-T043

A. niger -VS-T071

A. niger -VS-T250

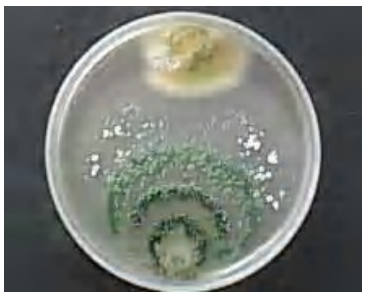
A. niger -VS-T292

A. niger -VS-T425

A. niger -VS-T191

A. niger -VS-T126

A. niger -VS-T043



A. niger -VS-T040

A. flavus-VS-T004

A. flavus-VS-T071

A. niger -VS-T390

Plate 1: Antagonistic interactions between *Trichoderma* species and fungal plant pathogens (*S. rolfsii*, *A. niger* and *A. flavus*)

friendly, less dependent on agricultural chemicals is gaining world wide recognition. One of the key elements of such sustainable agriculture is the application of biocontrol agents. *Trichoderma* species are antagonistic by nature with rich resource and a broad action scope. Appreciably, the results of the present study, confirmed clearly that *Trichoderma* species inhibited the growth of pathogens remarkably well. The competence shown by *Trichoderma* species to inhibit the growth of the tested pathogens *in vitro* suggests that the phenomenon of hyphal interaction described by Dennis and Webster (1971) for *T. viride* and some other species of *Trichoderma* is implicated in the biocontrol mechanism.

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