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Effect of Volatile and Non Volatile Compounds of *Trichoderma* spp. against Soil Borne Diseases of Chickpea

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A B S T R A C T

Biological control represents an important approach of agricultural biotechnology for controlling many fungal plant pathogens. *Trichoderma* spp. are the most promising and effective bioagents against many plant pathogenic fungi. In present paper, twenty *Trichoderma* isolates were isolated from chickpea rhizosphere soil and screened for their efficacy against soil borne plant pathogens namely *R. bataticola, F. oxysporum ciceri* and *S. rolfsii*. In chickpea the isolates ATPU 1, ATPP 6, KNN 4, KNO 2, KNPG 3 and KNP 1 were most efficient in the production of volatile and non-volatile compounds.

Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume, grown mainly for human consumption. Low yield of chickpea is primarily attributed to its susceptibility to several fungal, bacterial and viral diseases (Rehman *et al.*, 2013). Among the economically important diseases, wilt (*Fusarium oxysporum* f. sp. *ciceris*), dry root rot (*Rhizoctonia bataticola*) and collar rot (*Sclerotium rolfsii*) are the major and widespread diseases affecting chickpea cultivation (Nene and Sheila 1999). Biological control represents a viable alternative to the use of chemical fungicides and it is considered to be a safe, effective and eco-friendly method for plant disease management (Benitez *et al.*, 2004).

Trichoderma is known to be one of the best candidates of biocontrol agents. Modes of action of this fungus include mycoparasitism, antibiosis, competition for nutrients and space, tolerance to stress through enhanced root and plant development. A range of antibiotics are produced by species of *Trichoderma*, which are active against pathogens in *in-vitro* and consequently antibiotic production has been suggested as a mode of action of these fungi against plant pathogens. It has also been claimed that the antagonistic possessing both the phenomena of antibiosis and mycoparasitism would be able to effectively combat plant pathogens such as *R. bataticola, F. oxysporum ciceri* and *S. rolfsii.*

Materials and Methods

Isolation of *Trichoderma* from rhizosphere soil sample

The soil samples were collected from the different locations of Kurnool, Kadapa and Anantapur districts of Andhra Pradesh at a depth ranging 5-6 cm, by removing top 2 cm surface soil. Isolation was done by serial dilution technique. The probable colonies were observed closely and picked up from Petri plates and transferred to PDA slants and finally pure culture was obtained by repeated subculture. They were identified up to species level on the basis of their morphological and molecular characters (Rifai, 1969; Bhagat and Pan, 2010).

Evaluation of antagonistic activity through production of antifungal volatile metabolites

Productions of volatile metabolites by *Trichoderma* spp were assayed as described by (Dennis and Webster, 1971b) with slight modifications. The *Trichoderma* isolates were centrally inoculated by placing 3mm disc taken from three days old cultures on the PDA plates and incubated at 28 ± 2° C for three days. The top of each Petri dish was replaced with bottom of PDA plate inoculated centrally with the pathogen. Petri dish with PDA medium without *Trichoderma* spp at the lower lid and the upper lid with pathogens was maintained as control. The pair of each Petri dish were sealed together with paraffin tape and incubated for 4-6 days. After incubation the inhibition of mycelia growth was calculated according to (Vincent, 1947).

Evaluation of antagonistic activity through production of antifungal non-volatile metabolites

The ability of *Trichoderma* isolates to produce the non-volatile substances was studied following methods described by (Dennis and Webster 1971 a). All test isolates of *Trichoderma* were grown in 100 ml sterilized potato dextrose broth (PDB) for 10 days in 250 ml Erlenmeyer flasks with periodical shaking. Culture filtrate of *Trichoderma* was harvested by filtering it through Whatmann filter no. 42 filter paper into sterilized flasks. The culture filtrate was centrifuged at 6000 rpm for 10 min, sterilized by passing it through Cellulose Millipore membrane filter paper (0.4 µm pore size). The required volume of culture filtrate was added with known volume of melted PDA to obtain final concentration of 10%, 15% and 20% (v/v) culture filtrate. The amended media was poured into Petri dishes and inoculated with mycelial plug (6 mm dia) picked up from young culture of test pathogen and incubated at 28 ± 1°C for 4 days. The PDA medium without addition of culture filtrate of antagonist was served as control. The radial mycelial growth of test pathogen was measured and per cent inhibition of mycelial growth of pathogen was calculated according to (Vincent, 1947) as mentioned earlier.

Results and Discussion

Antagonistic effect volatile substances against soil borne pathogens

Twenty *Trichoderma* spp. isolated from different region of Kurnool, Kadapa and Anantapuram district of Andhra Pradesh has different levels of antagonism against three pathogens. All the *Trichoderma* isolates produced toxic volatile metabolites having significant effect in reducing the radial growth of test pathogens. In case of *R. bataticola*, *T. asperelum* (KNO 2) inhibited the mycelial growth of test pathogen by 82.5% per cent followed by *T. asperellum* (ATPU 1and KNPG 3) with 80.6 per cent inhibition over control and least recorded in *T. viride* (KJ 12) with 64.7% whereas in case of *S. rolfsii*, *T. asperellum* (KNPG 3) was

found most efficient in reducing the mycelial growth of test pathogen by 86.7 % and least recorded in *T. viride* (KJ 12) with 73.0%. Volatile metabolites produced by *T. asperellum* (ATPU 1) was found most efficacious in reducing the mycelial growth of *F. o.* f. sp. *ciceri* by 86.7 per cent followed by *T. asperellum* (KNPG 3) and *T. harzianum* (ATPP 6) recording 83.9 and 83.5 per cent inhibition over control, respectively (Fig. 1).

Species of *Trichoderma* have been demonstrated *in vitro* to act against fungal plant pathogens by producing diffusible volatile antibiotics. Vey *et al.*, (2001) reported that there are large varieties of volatile secondary metabolites produced by *Trichoderma* such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogens (Bhagat *et al.*, 2014). Similarly, Amin *et al.*, (2010) reported volatile activity of six isolates of *Trichoderma* spp. against seven different fungal plant pathogens. Among the six *Trichoderma* isolates studied, *T. viride* (Tv-1) was found to most effective in reducing the mycelial growth of *F. oxysporum* (41.8%). *S. rolfsii* mycelium growth and sclerotial production were inhibited by 40.68 and 48.1 per cent, respectively.

Fig.1 Antagonistic potential of *Trichoderma* isolates against three pathogens by Production of volatile substances

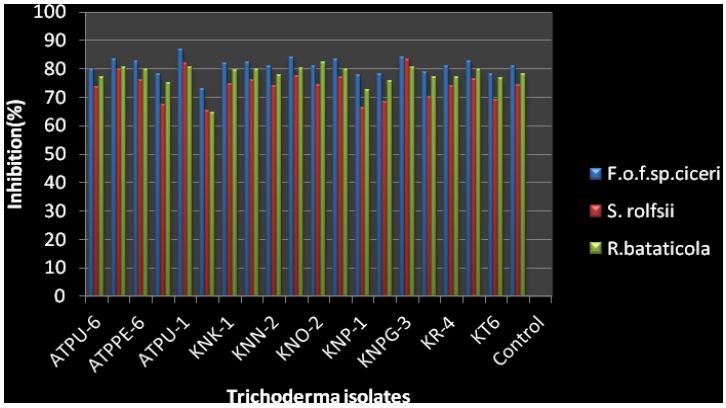


Fig.2 Antagonistic potential of *Trichoderma* spp against *R.bataticola* through non volatile compounds

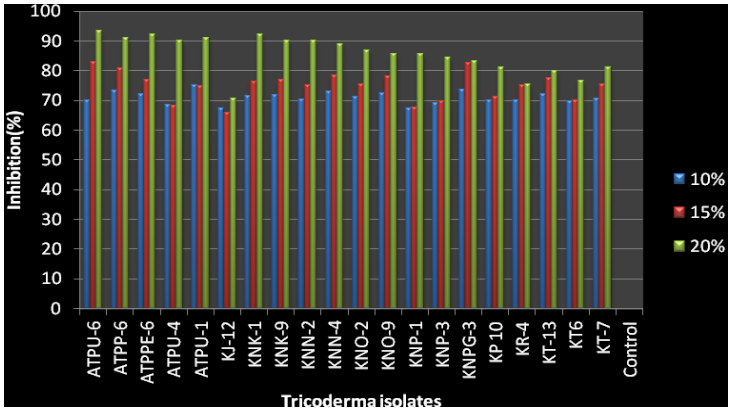


Fig.3 Antagonistic potential of *Trichoderma* spp against *S. rolfsii* through non-volatile compounds

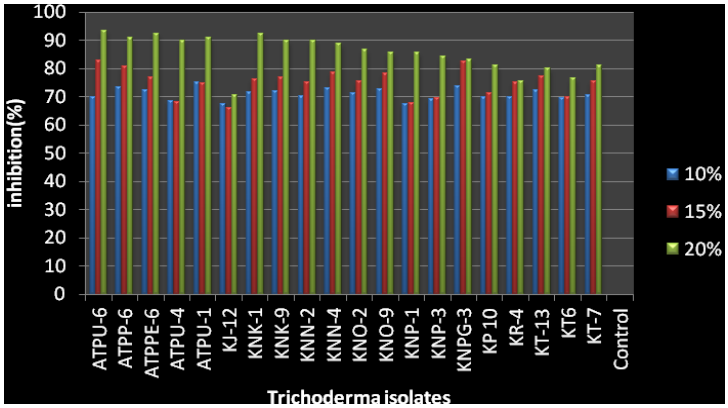
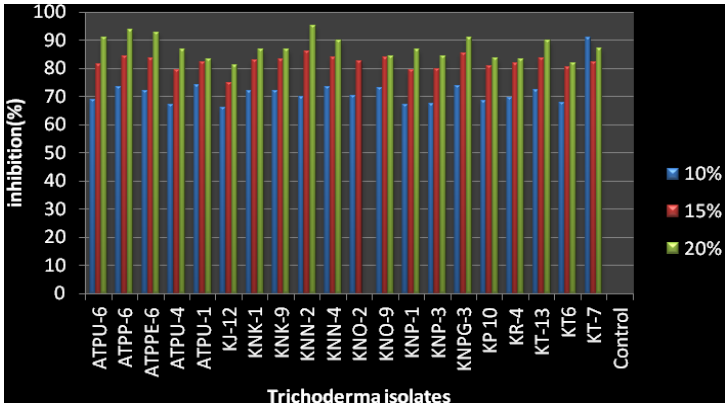


Fig.4 Antagonistic potential of *Trichoderma* spp against *F. oxysporium. f.sp. ciceri through* non-volatile compounds



In case of *R. solani*, *T. viride* (Tv-2) accounted for maximum reduction in mycelial growth (30.58%) and sclerotial parasitization (65.6%). Several workers like Pan and Bhagat (2008), Stoppacher *et al.,* (2010) and Pan *et al.,* (2013) have also reported the effectiveness of diffusible volatile compounds by *T. viride* and *T. harzianum* under in vitro. Antifungal volatile compounds produced by strain SQR-T037 highly effective to suppress the growth of *F. oxysporum* up to 9 days causing watermelon wilt (Waseem *et al.,* 2013).

Antagonistic effect non -volatile substances against soil borne pathogens

All the *Trichoderma* isolates significantly inhibited the test pathogens by production of non-volatile inhibitors at 10%, 15% and 20%. The pathogen, *R. bataticola* was significantly inhibited by *Trichoderma* spp. and ranged from 82.2% - 91.1% inhibition at 20% concentration. The maximum zone of inhibition for non-volatile metabolites of *T. longibrachiatum* (ATPPE 6) and *T. harzianum* (KNO 9) were found significantly over other isolates with 91.1% inhibition (Fig. 2). While, in case of *S. rolfsii, T. asperellum* (ATPU 6) was found most efficacious in reducing the mycelial growth of test pathogen by 93.3% and least recorded in *T. longibrachiatum* (KR 4) with 75.5 % (Fig. 3). The highest inhibition was recorded with *T. viride* (KNN 2) against *F. o. f.* sp. *ciceri* with 95.0% and *T. harzianum* (ATPP 6) with 93.8 % inhibition and least inhibition was recorded by *T. longibrachiatum* (KR 4) with

83.3 % of mycelial growth inhibition at 20% of the non-volatile compounds (Fig. 4).

Antagonisim of *Trichoderma* species against several pathogens were reported by Reddy *et al.,* (2013), Sundaramoorthy and Balabaskar (2013), Hanan and Mohamed (2014). The degree of inhibition varied from one strain to another. In the present investigation, some of the *Trichoderma* isolates like ATPU 1, ATPP 6, KNO 2, KNPG 3 and KNN 4 were highly efficient whereas some isolates have exhibited relatively less inhibition of mycelial growth of test fungus. The possible reason may be due to their inherent potentiality to adapt well in introduced conditions and aggressiveness of the *Trichoderma* isolates towards certain plant pathogens (Bae and Knudsen, 2005; Pan and Jash, 2009).

Several workers studied on the production of volatile and non-volatile antibiotics revealed that *T. harzianum* and *T. viride* were highly effective in reducing the radial growth of *S. rolfsii* by the production of these substances (Rao and Kulkarni, 2003). Dubey and Suresh (2006) found that non-volatile substances produced by *T. harzianum* are inhibitory to *F. o.* f. sp. *ciceri* causing chickpea wilt. Similarly *T. viride* isolate, followed by *T. harzianum* inhibited maximum mycelial growth of the *F. o.* f. sp. *ciceri* through production of volatile and non-volatile inhibitors in dual culture (Dubey *et al.,* 2007). Waseem *et al.,* (2013) reported that non-volatile antifungal compounds extracted from the liquid culture *Trichoderma* strain SQR-T037, significantly inhibited the growth of *F. oxysporum. f. sp. niveum* incitant watermelon of wilt.

References

Amin, F., Razdan, V.K., Bhat F.A and Saba B.M.K. 2010. Potential of *Tricoderma* species as biocontrol agents of soil borne fungal propagules. *Journal of Phytology*. 2(10): 38-41

Bae, Y.S and Knudsen, G.R. 2005. Soil microbial biomass on growth and biocontrol efficacy of *Trichoderma harzianum*. *Biological Control.* 32: 236-242.

Benitez, T., Rincon, A.M., Limon, M.C and Codon, A.C. 2004. Biocontrol mechanisms of *Trichoderma* strains. *International Journal of Microbiology*. 7: 249-260.

Bhagat, S and Pan, S. 2010. Cultural and phenotypic characterization of *Trichoderma* species from Andaman and Nicobar Islands. *Journal of Mycology and Plant Pathology*. 40(1): 145-157.

Bhagat, S., Birah, A., Kumar, R., Yadav, M.S and Chattopadhyay, C. 2014. Plant disease management: prospects of pesticides of plant origin. *Advances in Plant Biopesticides.* 119-129.

Dennis, C and Webster, J. 1971a. Antagonistic properties of species groups of *Trichoderma*, I. production of non-volatile antibiotics. *Transactions of the British Mycological Society*. 57: 41-48

Dennis, C and Webster, J. 1971b. Antagonistic properties of species groups of *Trichoderma*, II. Production of volatile antibiotics. *Transactions of the British Mycological Society*. 57: 363-369

Dubey, S.C and Suresh, M. 2006. Randomly amplified polymorphic DNA markers for *Trichoderma* species and antagonism against *Fusarium oxysporum f. sp. ciceris* causing chickpea wilt. *Journal of Phytopathology*. 154: 663-669

Dubey, S.C., Suresh, M and Singh, B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. *Biological Control*. 40: 118-127.

Hanan, I.M and Mohamed, O.I. 2014. The efficacy of *Trichoderma* species and Bacillus isolates in the control of chickpea wilt pathogens. *Agriculture, Forestry and Fisheries*. 3(5): 346-351.

Nene, Y.L and Sheila, V.K. 1999. ICRISAT Newsletter: proceeding on international workshop on chickpea, improvement. Feb-March: 172-180.

Pan, S and Bhagat, S. 2008. Characterization of antagonistic potential of *Trichoderma*

species against some soil borne plant pathogens. *Journal of Biological Control*. 22: 43-49.

Pan S., Riya Mukherji and Someshwar Bhagat. 2013. Evaluation of *Trichoderma* species against soil borne plant pathogens. *Annals of Plant Protection Sciences*. 21(1): 176-223.

Pan, S and Jash, S. 2009. Production and regulation of cell wall degrading hydrolytic enzymes in mycoparasitic *Trichoderma* species. *Journal of Mycology and Plant Pathology*. 39(2): 208-215

Rao, S. N. and Kulkarni, S. 2003. Effect of *Trichoderma* spp. on growth of *Sclerotium rolfsii* Sacc. *Journal of Biological Control*, 17: 181-184.

Reddy N.G., Thahir B.S and. Eswara R.N.P 2013. Evaluation of *Trichoderma* Sp. against *Rhizoctonia bataticola* Causing Dry Root Rot of Chick Pea (*Cicer arietinum* L.). *Progressive Research 8 (Special):* 572-574

Rehman, W. A. Dar, S. A. Ganie, Javid A. Bhat, Gh. Hassan Mir, Rubina Lawrence, Sumati Narayan and Pardeep Kumar Singh.2013. Comparative efficacy of *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum* f sp. c*iceris* causing wilt of chickpea. *African Journal of Microbiology Research*, Vol.7 (50), pp 5731-5736

Rifai, M.A. 1969. A revision of the genus *Trichoderma*. *Mycological Papers*.116: 1-56

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R and Schuhmacher, R. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GCMS. *Journal of Microbiology and Methods*. 81(2): 187-193.

Sundara Moorthy, S and Bala Baskar, P. 2013. Biocontrol efficacy of *Trichoderma* species against wilt of tomato caused by *Fusarium oxysporum f. sp. Lycopersici*. *Journal of Applied Biology and Biotechnology*. 1(3): 36-40.

Vey A., R. E. Hoagland and T.M. Butt. 2001. Toxic metabolites of fungal biocontrol agents. Fungi as biocontrol agents: Progress, Problems and Potential. Butt, T.M., Jackson, C.N. (Eds). 311-346 CAB international, Bristol.

Vincent, J.M. 1947. Distortion of Fungal hyphae in the presence of certain inhibitors. *Nature*. 150: 850.

Waseem R, Muhammad F, Sohail Y, Faheem U R, Muhammad Y 2013. Volatile and non-volatile antifungal compounds produced by *Trichoderma harzianum* SQR-T037 suppressed the growth of *Fusarium oxysporum* f. sp. *niveum*. *Science letters*. 1(1): 21-24.

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