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Research Article

Effect of Non-Volatile Compounds Produced By *Trichoderma* Spp. On Growth and Sclerotial Viability of *Rhizoctonia Solani*, Incitant Of Sheath Blight of Rice

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ABSTRACT

Nine isolates of *Trichoderma* spp. collected from three different districts were screened for their efficacy against test pathogen, *Rhizoctonia solani* by dual culture technique. The isolate TN3 showed maximum percentage of inhibition (96.25%) followed by TK3 (95.0%) and TC3 (91.25%) against the pathogen, while the least inhibition percentage was observed with the isolate TC2 (77.25%). The effect of culture filtrate of *Trichoderma* spp. on radial growth of *R. solani* indicated that the antagonists produced non-volatile antifungal antibiotics. Maximum inhibition (22.20 %) of the mycelial growth was observed with the culture filtrate of TC3 followed by TK2 and TN2 which inhibited radial growth of *R .solani* (18.80 %). The effect of *Trichoderma* spp. on germination or viability of sclerotia of *R. solani* indicated that the antagonists can reduce the viability of sclerotia. TN3 was able to inhibit the viability of sclerotia upto 62.04% followed by TK3 and TC3 equally (55.53%).

Key Words: *Rhizoctonia solani*, variability, antibiosis, biological control, non-volatile Compounds

Abbreviations:

TK - *Trichoderma* from Kadapa

TC - *Trichoderma* from Chittoor

TN - *Trichoderma* from Nellore

INTRODUCTION

Rice is one of the world's most important cereals for human consumption. India ranks first in area (44.6 m ha) and second in production (116 mt) next to China (176 mt). In India, Andhra Pradesh ranks fourth in area (3.946 m ha) and second in production (19.84 mt) next to West Bengal (22.03 mt) in 2007-08 (www.indiastat.com). Several pathogenic diseases have been found to occur on the rice crop resulting in extensive damage to grain and straw yield. The crop is subjected to attack by many diseases caused by fungi, bacteria, viruses, nematodes and several physiological disorders which caused annual loss to the tune of 12-25 per cent of the total production. Rice is subjected to the attack of over 30 fungi in our country. Major fungal diseases are Blast, Brown spot, False smut, Bunt, Sheath rot, Sheath blight, Leaf scald, Stem rot, Sheath net blotch and Seedling blight. Among these diseases the sheath blight disease caused by *Rhizoctonia solani* Kuhn., earlier considered to be minor disease is now regarded as an internationally important one that is second only to and often rivals of the blast disease, because of the introduction of high yielding varieties since 1960. In recent years, the increasing use of pesticides in agriculture has been the subject of growing concern for both environmentalists and public health authorities. Besides their non-target effects and hazardous to nature, they are becoming more expensive and some are losing their effectiveness due to development of resistant strains. Biological control has emerged as an alternative and most promising means of the management of plant pathogens. Bio-control of *Rhizoctonia solani* can be achieved by either promoting the native antagonists to reach a density sufficient to suppress pathogen(s) or by introducing alien antagonists. The possible use of fungal antagonists of rice pathogen has been viewed as an alternative disease management strategy. Among the several antagonists tested by various scientists, species of *Trichoderma*, *Gliocladium* and *Aspergillus* etc., have been found effective in inhibiting the sheath blight (*Rhizoctonia solani*). Though introduction of several antagonists against sheath blight pathogen seems to hold great promise to suppress the disease, no effective and economic management strategies have been derived. Of the several fungal antagonist(s) tested, *Trichoderma* spp. were extensivel

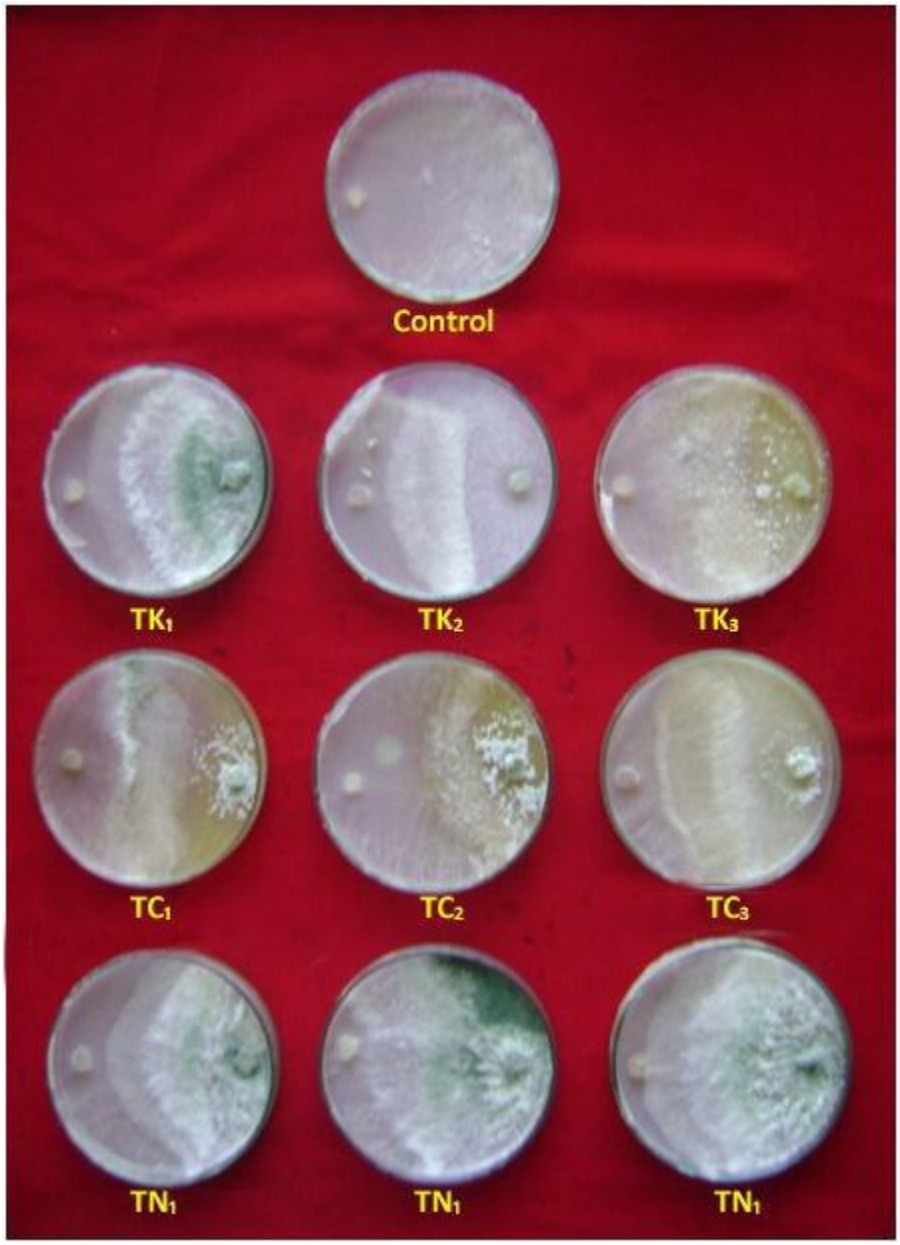

Figure 1. *In vitro* inhibition of *R. solani* by *Trichoderma* isolates by dual culture technique

Abbreviations:

TK - *Trichoderma* from Kadapa
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exposed for the control of soil-borne pathogen. They have been found effective in inhibiting the growth of *R. solani* under *in vitro* conditions. Keeping in view the importance of disease, the present investigations were therefore undertaken.

MATERIALS AND METHODS

Rice plants showing characteristic symptoms of sheath blight were collected from Agricultural Research Station (ARS), Nellore. The infected leaf sheath samples were thoroughly washed in running tap water and cut into small pieces of 3 mm size along with the lesion having half healthy and half diseased tissue. The pieces were surface sterilized with 0.1% mercuric chloride solution for 30 sec. The tissue pieces were subsequently washed in three changes of sterile distilled water to eliminate excess mercuric chloride and then the pieces were transferred onto PDA medium in Petri dishes. Plates were incubated at 28 ± 2°C and observed periodically for growth of the fungus. Axenic culture of the pathogen was obtained by single hyphal tip method and maintained on PDA slants throughout the investigation. Sheath blight pathogen was identified on the basis of cultural and morphological characteristics. Slides were prepared in cotton blue.

Soil samples were collected from Kadapa, Chittoor and Nellore districts of Andhra Pradesh where the disease incidence was high. Serial dilution plate technique (Johnson and Curl, 1959) was used to isolate native antagonistic *Trichoderma spp*. on PDA. *Trichoderma* species were isolated using *Trichoderma* specific medium (Elad and Chet, 1983). The cultures were purified by single spore isolation technique and maintained on PDA slants.

The effect of culture filtrates of the nine isolates of *Trichoderma* spp. on the growth of *R. solani* was studied as per method given by Dennis and Webster (1971). Fifty ml of sterilized potato dextrose broth taken in 250 ml flask was inoculated with a 5mm mycelial disc of the biocontrol agent(s) cut from the edge of four day old culture. Inoculated flasks were incubated at 28 ± 2°C for 5 days with constant shaking in water bath. The culture filtrate was filtered through Whatman no.1 filter paper and the filtrate was collected in a flask. The culture filtrate of bioagent and molten double strength PDA were mixed together in equal proportion (1:1). The medium was then sterilized and poured into the Petriplate @ 20 ml/plate. After solidification the Petriplates were carefully inoculated with 5 mm discs of the test pathogen cut from the four day old culture. PDA plates inoculated with the test pathogen but not amended with culture filtrate was maintained as control. Plates were then incubated in an incubator at 28±2°C. Three replications were maintained for each treatment. Periodic observations on radial growth of mycelium were recorded (Khan 2007). Inhibition percentage of mycelial growth of test pathogen was calculated by the formula: I = (C- T)/C X100 Where, I = Per cent inhibition in growth of test pathogen , C = Radial growth of pathogen (mm) in control , T = Radial growth of pathogen (mm) in treatment. To assess the efficacy on sclerotial viability, sclerotia of *R. solani* were placed on the surface of PDA plate which was overgrown with the mycelium and spores of a 4 day-old culture of *Trichoderma* spp. Ten sclerotia were kept in each treatment and three replications were maintained. The cultures were incubated at 26° C in the darkness for upto 30 days. Untreated sclerotia served as the control. The viability of *R. solani* sclerotia was estimated by placing them on water- agar for 24 hrs at 26°C and the germination was detected with a stereomicroscope.

RESULTS AND DISCUSSION

In the present investigation, susceptible rice cultivar NLR-34449 was collected from Agricultural Research Station (ARS), Nellore. The pathogen was isolated from the infected leaf sheath by tissue segment method on potato dextrose agar medium (Rangaswami and Mahadevan, 1999).Initially the fungus was producing pale to dark brown colonies on potato dextrose agar (PDA) medium. Mycelium was hyaline, irregular with right angle branches and formation of septum in the branched hyphae, which are characteristic features of the fungus. It produced sclerotia that were pale brown to blackish at maturity. The size and shape of sclerotia was varying and some times many sclerotia were joined together. Dugger (1915) described in detail about the morphology and growth habit of the fungus and stated that the young hyphal branches inclined in the direction of growth were invariably some what constricted at the point of union with the main hyphae. A septum was formed in the branch near the constriction. Akino and Ogoshi, 1995 described the characteristics of the genus *Rhizoctonia* like branching near the distal septum of cells in young, vegetative hyphae. Formation of septum in the branch near the point of origin, constriction of the branch, dolipore septum, no clamp connections, no conidium except moniloid cells, sclerotia not differentiated into rind and medulla, no rhizomorph. In the present studies, the antagonistic *Trichoderma* spp. were screened against the test pathogen, *R. solani*. All 9 isolates of *Trichoderma* spp. collected from three different districts were screened for their efficacy against test pathogen.

Table 1. *In vitro* screening and comparative effect of non-volatile compounds of *Trichoderma* isolates on growth and sclerotium viability of *R. solani*

S.No.	Isolates	Dual culture technique		Non-volatile compounds		Sclerotium viability	
		Radial growth of *R.solani* (mm)*	Per cent inhibition over control	Radial growth of *R.solani* (mm)*	Per cent inhibition over control	Number of sclerotia germinated*	% inhibition on viability of sclerotia
1	TK1	10.0	87.50 (69.28)	80.0	11.10 (19.46)	6.33	34.85 (36.18)
2	TK2	5.0	93.75 (75.52)	73.0	18.80 (25.70)	5.33	45.19 (42.24)
3	TK3	**4.0**	**95.00 (77.08)**	75.0	16.67 (24.09)	4.33	55.53 (48.17)
4	TC1	15.0	81.25 (64.34)	80.0	11.10 (19.46)	7.33	24.50 (29.67)
5	TC2	18.0	77.50 (61.68)	90.0	00.00 (00.00)	7.67	20.68 (27.05)
6	TC3	7.0	91.25 (72.80)	**70.0**	**22.20 (28.12)**	4.33	55.53 (48.17)
7	TN1	8.0	90.00 (71.57)	80.0	11.10 (19.46)	5.67	41.36 (40.02)
8	TN2	7.0	91.25 (72.80)	73.0	18.80 (25.70)	4.67	51.70 (45.97)
9	TN3	**3.0**	**96.25 (78.84)**	76.0	15.50 (23.18)	**3.67**	**62.04 (51.97)**
10	Control	80.00	00.00 (00.00)	90.00	00.00 (00.00)	9.67	00.00 (00.00)

** Mean of three replications*

Figures in parenthesis are angular transformed values

S.Ed	*0.4220*	*0.0476*	*0.2505*
C D(0.05)	*0.8806*	*0.0992*	*0.5262*
CV (%)	*0.80*	*0.31*	*0.82*

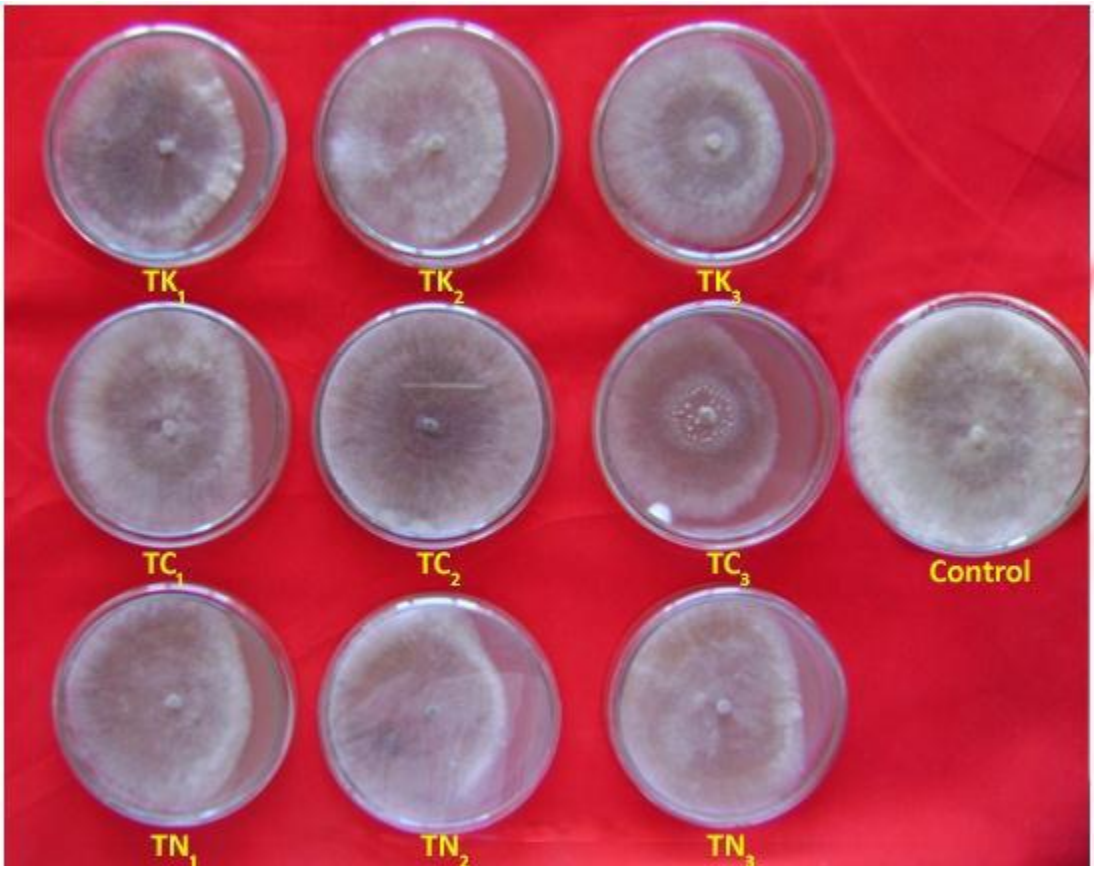

Figure 2. *In vitro* efficacy of non-volatile compounds of *Trichoderma* on growth of *R. solani*

Abbreviations:

TK - Trichoderma from Kadapa
TC - Trichoderma from Chittoor
TN - Trichoderma from Nellore

Among the 9 isolates of *Trichoderma* spp. tested, maximum percentage of inhibition (96.25%) was recorded with TN3 isolate. All the nine isolates of *Trichoderma* inhibited the growth of *R. solani* in dual culture. The isolate TN3 showed maximum percentage of inhibition (96.25%) (Table 1) followed by TK3 (95.0%) and TC3 (91.25%). However, both were found to be on a par with each other in inhibiting the pathogen. The inhibition percentage of other isolates were TK1 (87.5%), TK2 (93.75%), TC1 (81.25%), TC2 (77.25%), TN2 (90.0%) and TN2 (91.25%). Least percentage inhibition was observed with the isolate TC2 (77.25%) (Fig. 1). The isolate TN3, TK3 and TC3 were found to be more effective (collected from three districts), due to their more percentage inhibition. The results were in conformation with Krishnam Raju *et al.* (2008) who found that growth of *R. solani* in dual culture was suppressed by all the three *Trichoderma* spp. (*T. viride, T. harzianum* and *T. hamatum*). Highest inhibition was recorded in case of *T. harzianum* (76.47%), followed by *T.viride* (65.03%) and *T. hamatum* (63.43%). Inhibition zone with a yellow hallow (prevailed upto one week) was observed only in the case of *T. harzianum*, where as only mycoparasitism was observed in the case of *T. viride* and *T. hamatum*. The effect of non-volatile compounds produced by *Trichoderma* spp.(culture filtrates of antagonists) towards *R. solani* is aided by antibiotics which are released into the medium. The viability of sclerotia was decreased after 30 days of incubation. TN3 was able to inhibit the viability of sclerotia upto 62.04% (Fig. 2) followed by TK3 and TC3 equally (55.53%). In fact, in this investigation TN3 has shown to be an efficient mycoparasite of *R. solani*. The results were in agreement with De Melo and Faull, 2000 who observed that the viability of sclerotia was decreased after 30d of incubation. *T. harzianum*, Th-9 was able to reduce the germination of sclerotia in 72% and *T. koningii* in 43%. In fact, in this investigation *T. harzianum*, Th-9 has shown to be an efficient mycoparasite of *R. solani*. On the other hand, *T. koningii* has proved to be a good antibiotic producer. Ashraf Alikhan and Sinha (2007) who reported that the all the five isolates of *Trichoderma* spp. exhibited antibiotic potential against *R. solani* by inhibiting its mycelial growth. With the increase in concentration of culture filtrates of the bioagents, the radial growth of test pathogen was proportionally decreased, in general. Maximum inhibition (76.3%) of the mycelial growth of *R. solani* was observed with the culture filtrate of *T. harzianum* used at 50 % concentration. Krishnam Raju *et al.,* (2008) found that the cultures or cell free filtrates of all the *Trichoderma* spp. viz., *T. viride, T. harzianum* and *T. hamatum* suppressed the radial growth of *R. solani*. The bioagent, *T. harzianum* was found very effective in inhibiting the radial growth of test pathogen to an extent of 44.50% when 100% concentration of the culture filtrate of the antagonist was used. This was followed by *T. hamatum* (38.63%) and *T. viride* (35.37%). To be considered a successful biocontrol agent, a mycoparasite should be effective against resistant survival structures of plant pathogens. The results revealed that there was a reciprocal relationship between the culture filtrates of *Trichoderma* spp. and the radial growth of *R. solani*. Maximum inhibition of the mycelial growth of *R. solani* was observed with the TC3 (22.20%) followed by TK2 (18.80%) and TN2 (18.80%).

REFERENCES

Akhino S and Ogoshi A (1995). Pathogenecity and host specific in *Rhizoctonia solani*. In: Pathogenesis and Host specificity in Plant disease. Vol. **IInd** (Eds.) Keisuke Kohmoto, Uma, S Singh and Rudra P Singh. Programon Science. Ltd. Pub. U.K. 37-46.

Dennis C and Webster J (1971). Antagonistic properties of species groups of *Trichoderma* II Production of non metabolic antibiotics. *Transactions of British Mycological Society*. **57** 25-39.

Dugger BM (1915). *Rhizoctonia crocorum* (Pers) D C *Rhizoctonia solani* Kuhn (*Corticium vagum* B and C) with notes on other species. *Annual Missouri Botany Garden.* **2** 403-458.

Elad Y, Chet I, Boyle P and Henis Y.(1983). Parasitism of *Trichoderma* spp. on *Rhizoctonia solani* and *Sclerotium rolfsii*. Scanning electron microscopy and fluorescence microscopy. *Phytopathology* **73** 85-88.

Johnson LF, Curl EA, Bond JH and Fribourg HA (1959). Methods for studying soil microflora. Plant Disease Relationships. Burgers Publishing Company,Minneapolis, USA.

Khan AA and Sinha AP (2007). Biocontrol potential of *Trichoderma sps* against sheath blight of Rice. *Indian Phytopathology*. **2** 208 - 213.

Raju KS, Vijay Krishna Kumar K and Rajamannar M (2008). *In vitro* efficiency of volatile and non volatile metabolites of *Trichoderma sps* on Rice sheath blight pathogen, *Rhizoctonia solani* Kuhn. *Oryzae* **1** 84 – 86.

Rangaswami G and Mahadevan A (1999). Diseases of crop plants in India.(4th edition) Prentice Hall of India Pvt. Ltd. New Delhi, 60-79.