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Effect of volatile and non-volatile metabolites of antagonists on *Rhizoctonia solani* Kühn, the causal organism of Sheath Blight of rice

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Non-volatile and volatile metabolites of all the 29 test fungal antagonists were found to reduce the mycelial growth, sclerotial production and germination of the pathogen. Minimum mycelial growth, sclerotial production and germination was observed by *Trichoderma koningii 5201 followed by Chaetomium cochliodes 3319* in all the non-volatile and volatile metabolites. However, in non-volatile metabolites minimum size of sclerotia was observed in *Trichoderma virens* 4177 followed by *Trichoderma koningii* 5201 and in volatile metabolites minimum size was observed in *Trichoderma harzianum 5230.* In different combination of isolates also, the combination of *Trichoderma koningii 5201 + Chaetomium cochliodes* 3319 showed maximum inhibition of mycelial growth, sclerotial production, size and germination.

Key words : *Rhizoctonia. solani.* sheath blight, rice. metabolites, biocontrol agents

INTRODUCTION

Sheath blight caused by *Rhizoctonia solani* Kühn of rice is one of the major biotic constraints in rice production. The potential losses due to sheath blight of rice alone in India has been up to 51.3% (Rajan, 1987). There is an increasing attempt to introduce fungal biocontrol agents for managing plant pathogens, partly with a concern for environmental protection. There are reports on effect of volatile and non-volatile metabolites of antagonists of *R. solani.* They have reported that the culture filtrate of *T. lignorum* contained toxic metabolites which are effective on *R. solani.* The antagonist *G. virens* has been found to produce several antibiotic compounds. The species of *Trichoderma* produced a number of antifungal metabolites such as Trichodermin. Gliotoxin, a fungistatic metabolite produced by *Trichoderma* spp. has been found to cause bursting of hyphal tips of *R. solani.* A new metabolite Tricholin produced by T. *viride* has been found to be effective against *R. solani* (Weindling, 1932; Godfredsen and Vangedal, 1965 ; Taylor, 1986, Bruckner *et al*; 1990; Pietro *et al.* 1993; Lin *et al.*, 1994)

MATERIALS AND METHODS

Effect of non-volatile metabolites on mycelial growth of the pathogen

The 29 biocontrol isolates were grown in Potato Dextrose liquid broth for 8 days. The culture filtrates contaning non-volatile metabolites were passed through a sterile Whatman No. 1 filter paper into a 100 ml sterilized concial flask. From this filtrate, 10 ml was added to 90 ml of PDA, and mixed well. This amended medium was used for plating purpose. A mycelial disc of *R. solani* (5 mm diam) was inoculated in the centre of Petri-dish containing culture filtrate amended PDA medium. The plates were incubated at 28 ± 2°C. Suitable controls were maintained without amendments. The mycelial growth of the pathogen was recorded at 24, 48, 72 and 96 hrs after inoculation. Per cent inhibition of growh of the pathogen by non-volatile metabolites was calculated.

Effect of non-volatile and volatile metabolites on sclerotial production, size and germination

After measuring the mycelial growth of the pathogen, these sealed plates were kept in an incubator at 28±2°C for 4 days to allow production of sclerotia. Then, the number of sclerotia produced in the plates in each treatment were counted and per cent reduction over control was calculated. Further, the same Petri-plates from above experiment used to measure the size of the sclerotia. Ten matured sclerotia were taken from each plate and their size was measured in mm. For germination, ten matured

sclerotia of uniform size were placed on PDA with equal space and incubated for 48 hrs. Total germinated sclerotia were counted in each plate.

Effects of volatile metabolites of antagonists on mycelial growth

For testing the effect of volatile metabolites produced by the antagonistic agents on radial growth of the pathogen, a disc of 5 mm diameter was cut from the actively growing colony of antagonist and inoculated in the centre of the sterilized Petri-plate containing medium. Similarly a disc from the colony of the pathogen was placed in the centre of another Petri-plate of same diameter and inverted over the first plate. The junction of both the plates was then sealed with paraffin film. For each treatment three replications were maintained. The plates with pathogen alone served as control. The plates were then incubated at 28 ± 2°C and the data recorded for their growth at 24, 48 and 72 hrs after incubation and per cent inhibition of growth of the pathogen was calculated.

Selection of efficient bio-control isolates

Based on the antagonon the antagonism tested through dual culture test of volatile and non-volatile metabolites, two isolates *Trichoderma koningii* 5201 and *Chaetomium cochliodes* 3319 were found to be more effective. They were further studied in *vitro* for their combined effect on the growth of pathogen. They were mass multiplied and used in pot culture and field experiments.

RESULTS AND DISCUSSION

Effect on non-volatile metabolites on mycelial growth, sclerotial production, size and germination of the pathogen

To know the inhibitory effect of non-volatile metabolites of the biocontrol agents, the pathogen was grown in 10 per cent culture filtrate of each of the biocontrol isolates. All the isolates were found to reduce the mycelial growth of the pathogen over the control plate. Of the test isolates. *T. konigii* 5201 recorded maximum growth inhibition of 49.2 per cent followed by *C. cochliodes* 3319 (44.8%) and *A. sydowii* 2148 (37.3%). The least effective were isolates of *Chaetomium globosum.* This suggested that the production of non-volatile metabolites is one of the reason by which antagonists viz. *Trichoderma, Cheatomium* and *Aspergillus* spp. inhibit the growth of the pathogen. The growth of *R. solani* was retarded by the culture filtrate of *T. viride* (Dubey and Dwivedi, 1988), and trichobrachin (Bruckner *et al.,* 1990)

In case of inhibitory effect of non-volatile metabolites on sclerotial production. *T. koningii 5210* isolate was found the best as it reduced sclerotial production up to 63.5 per cent followed by *C. cochliodes* 3319 (48.1%), *A sydowii* 2148 (42.5%) and *T. viride* 1433, 2211 (30.8%). In reducing the size of sclerotia, maximum efficient isolates were *T. virens* 4177 and T. koningii 5201 (69.4%) followed by *T. viride* 3798 and *T. harzianum* 4572, 5230 (61.5%). The present results also in line with Divakaran (1997) who reported the retardation of sclerotial production and size in *R. solani* and *T. harzianum* 4572, 5230 (61.5%). The present results also in line with Divakaran (1997) who reported the retardation of sclerotial production and size in *R. solani* and *Sclerotium rolfsii* by the culture filtrates of *T. viride* and *T. koningii.*

Antagonistic effect on sclerotial germination was studied using PDA medium. The sclerotia produced from the antagonist treated plates showed less number of sclerotial germination as compared to control. Minimum germination of sclerotia was observed in *T. koningii* 5201 and *C. cochliodes* 3319 treated plates and per cent reduction in germination was 88.5 and 82 respectively. These results once again confirmed the findings of Shanmugasundaram (1992) that the culture filtrate of *T. viride. T. koningii* and *T. harzianum* significantly reduced the sclerotial germination of *R. Solani.*

Effect of volatile metabolites on the mycelial growth, sclerotial production, size and germination of the pathogen

Many antagonists are known for the production of volatile compounds. The study on production of volatile compounds by the antagonists was undertaken.

All the antagonists showed inhibition over control plate. Average minimum growth was observed in *T. koningii* 5201 (26.1 mm), followed by *C. cochliodes* 3319 (28.3 mm) as against 54.3 mm in control. The growth of pathogen was maximum in *C. globosum* isolates recording least per cent inhibition over control.

Regarding their effect on sclerotial production, all bio-control isolates were found to reduce sclerotial density over control. *T. koningii* 5201 showed maximum reduction of sclerotial germination (50.3%) over control. However, the maximum reduction in

size of sclerotia was observed in *T. harzianum* 5230 (67.0%).

Among the twenty sclerotia kept for germination in PDA medium. *T. koningii* 5201 treated sclerotia had the least number of germinated sclerotia effecting highest per cent inhibition of 88.5 per cent, followed by *C. cochliodes* 3319 (82%), *A sydowii* 2148 (73.5%) and *T. viride* 2211 (65%). Volatile metabolites from *T. viride* had inhibitory effect on *R. solani* (Dennis and Webster, 1971). Papavizas (1985) reported that the propagules of *M. phaseolina. R. solani* and *Sclerotium rolfsii* were adversely affected by the volatile ammonia arising from decomposing organic matter from soil.

Effect of non-volatile metabolites of combined antagonists on the mycelial growth, sclerotial production, size and germination of the pathogen

To test inhibitory effect of non-volatile metabolites from the combined bio-control isolates, pathogen was grown in 10 per cent culture filtrate amended medium. The growth of the pathogen was recorded at different time intervals. All the combination of isolates found to reduce the mycelial growth of the pathogen over control plate (Table 1). Among them the combination of *T. koningii* 5201 + *C. cochliodes* 3319 isolates recorded the maximum growth inhibition of 61.4 per cent followed by *T. koningii* 5201 + *A. sydowii* 2148 (57.6%) compared to control plates after 96 hrs of incubation. The least inhibitory effect was shown by *A. sydowii* 2148 + *A. dydowii* 3285 combination (49.8%).

A combination of *T. koningii* 5201 + *C. cochliodes* 3319 showed maximum inhibition of sclerotial number (80.2%) followed by *T. koningii* 5201 + *A. sydowii* 2148 (75.8%) and *C. cochliodes* 3318 + *T. virens* 4177 (69.9%) over control. The abundant sclerotial formation in control plates and less number of sclerotia in treated plates clearly showed the effect of culture filtrate on sclerotial production. This result is in conformity with the findings of Claydon *et al.* (1987).

The diameter of sclerotia produced was minimum in *T. koningii* 5201 + *C. cochliodes* 3319 treated plates as 0.5 mm with 69.8 per cent reduction over control. This was followed by 0.6 mm diameter in *C.*

Table 1. Effect of non-volatile metabolites (combined) on mycelial growth, sclerotial formation, size and germination of *R. solani*.

Treatments (antagonists)	Mycelial growth (mm)		Sclerotial formation		Sclerotia size		Sclerotial germination*	
	Mean (mm)	Inhibition over control (%)	Sclerotia No. (%)	Reduction over control	Diameter (mm) (%)	Reduction over control	No. (%)	Inhibition over control
T. koningii 5201+*C. cochliodes* 3319	21.7	61.4	9.0	80.2	0.5	69.8	0.0	100.0
T. koningii 5201+*A. sydowii* 2148	23.8	57.6	11.0	75.8	0.8	50.0	1.0	90.0
T. koningii 5201+*T. virens* 4177	26.1	53.7	20.0	55.9	1.0	39.7	5.3	47.0
T. koningii 5201+*A. sydowii* 3285	26.7	52.5	22.0	51.5	1.1	30.1	5.0	50.0
C. cochliodes 3319+*A. sydowii* 2148	27.4	51.3	21.3	53.0	1.1	30.1	5.3	47.0
C. cochliodes 3319+*T.virens* 4177	24.3	56.8	13.6	69.9	0.6	60.2	3.3	67.0
C. cochliodes 3319+*A. sydowii* 3285	28.1	50.1	21.6	52.3	1.0	39.7	5.3	47.0
A. sydowii 2148+*T. virens* 4177	25.0	55.7	14.6	67.7	1.0	39.7	4.0	60.0
A. sydowil 2148 + A. sydowii 3285	28.3	49.8	19.0	58.1	1.1	30.1	4.3	57.0
T. virens 4177+*A. sydowii* 3285	25.5	54.7	18.6	58.9	1.0	39.7	4.6	54.0
Control	56.4	–	45.3	–	1.6	–	10.0	–
CD (P=0.05)	0.8		3.6		0.4		1.2	

* No. of sclerotia taken were 10

Table 2. Effect of volatile metabolites (combined) on mycelial growth, sclerotial formation, size and germination of *R. solani*.

Treatments (antagonists)	Mycelial growth		Sclerotial formation		Sclerotia size		Sclerotial germination*	
	Mean (mm)	Inhibition over control (%)	Sclerotia No.	Reduction over control (%)	Diameter (mm)	Reduction over control (%)	No.	Inhibition over control (%)
T. koningii 5201+*C. Cochliodes* 3319	19.5	64.6	8.0	80.1	0.8	50.0	0.0	100.0
T. koningii 5201+*A. sydowii* 2148	23.3	58.0	11.0	72.7	0.5	68.7	0.3	97.0
T. koningii 5201+*T. virens* 4177	24.3	56.0	19.0	52.8	1.1	31.2	3.6	64.0
T. koningii 5201+*A. sydowii* 3285	25.0	54.7	20.0	50.3	1.0	37.5	5.3	47.0
C.cochliodes 3319+*A. sydowii* 2148	27.3	50.6	13.6	66.2	0.6	62.5	4.6	54.0
C.cochliodes 3319+*T. virens* 4177	23.5	57.4	19.6	51.3	1.1	31.2	2.3	77.0
C.cochliodes 3319+*A. sydowii* 3285	26.7	51.7	21.6	46.4	1.0	37.5	4.6	54.0
A. sydowii 2148 +*T. virens* 4177	23.4	57.6	11.6	71.2	1.0	37.5	3.6	64.0
A. sydowii 2148 +*A. sydowii* 3285	27.2	50.8	16.0	60.2	1.1	31.2	4.3	57.0
T. virens 4177+*A. sydowii* 3285	24.1	56.4	19.6	51.3	1.0	37.5	4.3	57.0
Control	55.3	—	40.3	—	1.6	—	10.0	—
CD (P=0.05)	1.9		3.1		0.3		1.0	

* No. of sclerotia taken were 10

cochliodes 3319 + *T. virens* treated plates and 0.8 mm in *T. koningii* 5201 + *A. sydowii* 2148 treated plates.

Regarding germination of sclerotia among the ten sclerotia kept in PDA medium, none of them germinated that were taken from *T. koningii* 5201 + *C. cochliodes* 3319 treated plates and showed 100 per cent reduction over control. This was followed by *T. koningii* 5201 + *A. sydowii* 2148 treated plates showing 90 per cent reduction in germination (Table 1).

Effect of volatile metabolites of combined antagonists on the mycelial growth, sclerotial production, size and germination of the pathogen

Volatile metabolites produced individually by the bio-control isolates reduced the mycelial growth of the pathogen over control. To test whether the inhibitory effect increases or not when the bio-control isolates grown together, an experiment was carried out. The pathogen and antagonists were grown in the Petri-plates of same diameter and one plate was inverted over the other plate and the junction was sealed. All the combination of isolates found to reduce the radial growth of the pathogen over control plate (Table 2). Among them the combination of *T. koningii* 5201 + *C. cochliodes* 3319 showed maximum inhibition of 64.6 per cent followed by *T. koningii* 5201 + *A. sydowii* 2148 (58.0%) and *A. sydowii* 2148 + *T. viride* 4177 (57.6%) after 96 hrs of incubation. The least inhibition of 50.6 per cent was shown by *C.cochliodes* 3319 + *A. sydowii* 2148 combination.

It is interesting to recall that *T. koningii* 5201 when employed individually in dual culture with the pathogen its inhibition was only 51.9 per cent and in case of *C. cochliodes* 3319 it was only 47.8 per cent. But the maximum inhibition of 64.6 per cent was observed when they were both grown in combined form against the pathogen. The possible reason could be synergestic effect of these organisms against the pathogen. In general, all the ten combinations show higher inhibitory action on the pathogen than their individual action when they were grown independently. This confirms the earlier report on production of volatile metobolites by *Trichoderma* spp. isolates in dual culture by Zeppa *et al.* (1990), Claydon *et al.* (1991) and Martins *et al.* (1998).

The dual culture plates measured for the inhibition zone, were incubated at 28 ± 2°C for 10 days to induce the production of sclerotia. The number of sclerotia produced were counted in each plate and expressed as per cent reduction in sclerotial production over control. This can be seen from the Table 2, that the number of sclerotia produced in dual culture plates were significantly reduced as compared to the control plate. The maximum reduction in sclerotial production was obtained from *T. koningii* 5201 + *C. cochliodes* 3319 combination (80.1%) followed by *T. koningii* 5201 + *A. sydowii* 2148 (72.7%) and *A. sydowii* 2148 + *T. viride* 4177 (71.2%). Statistically they were at par with each other. The minimum reduction was observed in *C. cochliodes* 3319 + *A. sydowii* 3285 (46.4%) and *C. cochliodes* 3319 + *T. virens* 4177 (51.3%).

Regarding the size of sclerotia, the combination of *T. koningii* 5201 + *A. sydowii* 2148 showed maximum reduction of 68.7 per cent followed by *C. cochliodes* 3319 + *A. sydowii* 2148 (62.5%) and *T. koningii* 5201 + *C. cochliodes* 3319 (50%).

Among the ten sclerotia kept for germination in PDA medium, 100 per cent reduction in sclerotial germination was observed in *T. koningii* 5201 + *C. cochliodes* 3319 treated plates. This was followed by 97 per cent reduction obtained from *T. koningii* 5201 + *A. sydowii* 2148 treated plates (Table 2). This clearly indicates that the volatile metabolites produced by the antagonists had toxic effect on sclerotia and reduced its germinability or in some cases even killed. Many of the antifungal compounds produced by *Trichoderma* spp, *Chaetomium* spp against *R. solani* are butenolide (Almassi *et al.*, 1991) and harziandione (Hockless *et al.*, 2005).

REFERENCES

Almassi, F., Ghisalberti, E. L. Narbey, M.J. and Sivasithambaram, K. 1991. New antibiotics from strains of *Trichoderma harzianum. J. Nat. Prod.* **54** : 396-402.

Bruckner, H., Reinkecke, C., Kripp, T. and Kieb, M. 1990. Screening, isolation and sequence determination of a unique group of polypeptide antibiotics filamentous fungi. *Proc. Interaction Mycol. Cong.* Regensburg. Federation Republic Germany, P. 224.

Claydon, N., Allan, M., Hanson, J. R. and Avent, A.G. 1987. Antifungal alkyl pyrones of *Trichoderma harzianum. Trans Brit, Mycol. Soc.*, **88** : 503–513.

Dennis, C and Webster, J. 1971. Antagonistic properties of species of groups of *Trichoderma. Trans Brit, Mycol. Soc.* **57** : 41-48.

Divakaran, D. 1997. *Biological control of blackgram root rot caused by Macrophomina phaseolina (Tassi.) Goid.* Ph.D. thesis, TNAU, Combatore, India, p. 223.

Dubey, R. C. and Dwivedi, R. S. 1988 Antagonism between *Macrophomina phaseolina* and some rhizosphere micro fungi of soybean : *Int. J. Trop. Pl. Dis.* **6** : 89-94.

Godtfredsen, W.O. and Vangedal, S. 1965. Trichodermin, a new sesquiterpene antibiotic. *Acta Chem. Scand.* **19** : 1088-1102.

Hockless, D.C.R., Rowland, C. and White A.H. 2005. Harziandione, A new class of diterpene from *Trichoderma harzianum. J. Nat. Prod.* **55** : 1690-1694.

Lin, A., Lee, T.M. and Rern, J. C. 1994. Tricholin, a new antifungal agent from *Trichoderma viride* and its action in biological control of *Rhizoctonia solani. J. Antibiot.* (Tokyo), **47** : 799-805.

Martins, C.M.P., Melo, I.S. and De, M.I.S. 1998. *In vitro* antagonism of *Trichoderma* spp against *Verticillium dahliae, Scientia Agricola,* **55** : 1-7.

Papavizas, G.C. 1985 *Trichoderma* and *Gliocladium* biology, ecology and the potential for biocontrol. *Ann. Phytopathol.*, **23** : 23-54.

Pietro, A.Di., Lorito, M., Mayes, C.K., Broadway, R.M. and Harman, G.E. 1993. Endochitinase from *Gliocladium virens* isolation, characterisation and synergistic antifugual activity in combination with gliotoxin. *Phytopathology,* **83** : 208-313.

Rajan, C.P.D. 1987. Estimation of yield losses due to sheath blight of rice. *Indian Phytopath* **40** : 174-177.

Shanmugasundaram, P. 1992. Studies on charcoal rot of sunflower *(Helianthus annus* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. M. Sc. (Ag.) Thesis, TNAU, Coimbatore, India p 74.

Taylor, A. 1986. Some aspects of the chemistry and biology of the genus *Hypocrea* and its anamorphs *Trichoderma* and *Gliocladium. Proc. N.S. Inst. Sci.,* **36** : 27-58.

Weindling, R. 1934. Studies on a lethal principal effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathology,* **24** : 1153-1179.

Zeppa, G., Allegrone, G. Barbeni, M. and Guarda, P.A. 1990. Variability in the production of volatile metabolites by *Trichoderma viride. Annali di Microbiologia Entamologia,* **90** : 11-176.

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