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EFFECT OF FUNGAL METABOLITES AND AMENDMENTS ON MYCELIAL GROWTH OF *RHIZOCTONIA SOLANI*

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Abstract: A shift towards organic farming suggests amalgamation of organic resources against soil borne plant pathogens. The influence of metabolites of most ubiquitous *Aspergillus* spp., organic amendment extracts and their combined effect with *Trichoderma virens* were evaluated *in vitro* against *Rhizoctonia solani*. The minimum (36.1 mm) growth was attained by *R. solani* in co-culture with *A. niger*. The maximum (42.3 mm) inhibition of mycelial growth of the test organism was observed with culture filtrate of *A. ochraceous* followed by *A. niger*, *A. fumigatus*, *A. flavus* and *A. terreus*. Among organic amendment extractants, castor cake exhibited an additive effect on the growth of *T. virens,* however, the maximum (41.8 mm) suppressive effect on *R. solani* was observed with vermicompost. With the advance in time, the effect of organic amendment extracts increased markedly. Inhibition potential of culture filtrate mixturte of *A. niger* + *T. virens* and *A. ochraceous* + *T. virens* against *R. solani* was significantly higher in comparison to the other combinations.

Key words: *Aspergillus* spp., culture filtrate, organic amendment extracts, *Rhizoctonia solani*, *Trichoderma virens*

INTRODUCTION

Rhizoctonia solani Kühn, the causal agent of rice (*Oryza sativa*) sheath blight, is one of the notorious diseases of rice. Crop losses usually vary from negligible to 50 per cent depending on cultivars, cultural conditions, extent of severity of disease, crop growth stage and environmental conditions (Kannaiyan and Prasad 1976; Laha and Venkatraman 2001). Looking at the seriousness of hazards due to chemicals, eco-friendly approaches should have been followed to control the disease. Need of biological control was recognized since few decades because control of soil borne diseases through other control strategies has not resulted in promising means of management against these pathogens. Disease suppression is an inherent property of soil which is caused by the dominance and pre-dominance of *Aspergillus* spp., *Trichoderma* spp. and *Fusarium* spp. inhabiting soil, which was reported by various workers (Singh 2001; Valenzuela *et al.* 2001). *Trichoderma*, *Gliocladium*, *Aspergillus* etc. have been found effective in controlling sheath blight and extensively explored for control of soil borne plant pathogens (Khan and Sinha 2005).

At the same time the use of organic amendments as food base for biological control agents and their use well ahead of planting can be very effective in controlling many soil borne pathogens including *R. solani* has been already recognized (Hoitink and Boehm 1999). Compost prepared from lignolytic substances are predominantly colonized by *Trichoderma* spp. (Kuter *et al*. 1983) while *Aspergillus* and *Penicillium* isolates prefer waste of low cellulosic substance and high in sugar (Gorodecki and Hadar 1990). Parasitism is critical to biological control of *R. solani* in suppressive compost amended substrate (Nelson *et al.* 1983; Gorodecki and Hadar 1990) because *R. solani* has much narrower spectrum of biocontrol and this mycoflora does not consistently colonized composts (Grebus *et al.* 1994). The present investigation was undertaken to find out:

1. Probable way (individual presence or metabolite) of disease suppression by *Aspergillus* spp. predominantly found in to the soil after harvest of rice.
2. Suitable organic amendment supportive and suppressive to *T. virens* and *R. solani*, respectively.
3. Joint effect on parasitism of *T. virens* with metabolites of *Aspergillus* spp. and organic amendment extracts against test pathogens.

MATERIALS AND METHODS

Fungal culture

In the present investigation five *Aspergillus* spp. were evaluated against *R. solani*. These species were obtained from calciorthent (free $CaCo_3$ 33 per cent) soil of rice field under conservation tillage operation. *A. niger* (An), *A. fumigatus* (Afi), *A. flavus* (Af), *A. ochraceous* (Ao) and *A. terreus* (At) were isolated using dilution plate technique (Warcup 1960). *T. virens* was isolated from rhizospheric soil of guava plant (*Psidium guajava* L.).

R. solani was obtained from naturally infected rice plant under conservation tillage practice. All fungal cultures were maintained on potato dextrose agar (PDA) medium for further use.

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Antagonism in dual culture

Dual culture method of Morton and Stroube (1955) on PDA was used for evaluating the antagonistic activities of *Aspergillus* spp. 20 ml sterilized melted PDA was poured in sterilized Petri plates, allowed to solidify and a 10 mm mycelial discs (from 7 days old culture) of *Aspergillus* spp. were placed against *R. solani* in each PDA plate. The distance between the two discs were maintained approximately at 6 cm away from each other. Four replications of each treatment were incubated at 25±1°C. Observations were recorded after 48 and 96 hours.

Effect of culture filtrate of *Aspergillus* spp. on *R. solani*

Effect of culture filtrate of *Aspergillus* spp. on growth of *R. solani* was studied by slightly modified to method of Dennis and Webster (1971). Filtrate of antagonist(s) culture in PDA broth for 10 days was collected after passing it twice through Whatman filter paper No. 1. These filtrates were used to amend Petri plates containing PDA at 5 per cent concentration and incubated at 25±1°C. An unamended Petri plate served as check (control). The observations of radial growth of test pathogen were recorded after 48 and 96 hours.

Effect of organic amendment on *R. solani* and *T. virens*

Four oil free cakes viz., neem (*Azadirachta indica*) cake (NC), karanj (*Pongamia pinnata*) cake (KC), mustard (*Brassica juncea*) cake (MC) and castor (*Ricinus communis*) cake (CC) and two composts of Farm yard manure (FYM) and vermicompost (VC) as organic amendment along with mixture of all cakes and compost extract were used individually to study their effect on mycelial growth of *R. solani* and *T. virens*. 10 g powder of different cakes and composts were mixed thoroughly in solution containing ethanol and water in 3 : 2 (v/v). The mixture was kept on shaker at 24°C for 24 hours at 150 rpm. The solution was filtered through muslin cloth and refiltered through Whatman filter paper No. 1. The obtained filtrate was concentrated through evaporating the ethanol on hot water bath for 10 min. This concentrated solution of each cake, compost and their mixture were used at the rate of 5 per cent in potato dextrose agar in each Petri plates. A centrally placed 10 mm^2 size of mycelial discs (from 7 days old culture) of *T. virens* and *R. solani* were placed individually on amended Petri plates in triplicate at 25±1°C along with control. The observations of mycelial growth of both fungi were recorded after time intervals of 48 and 96 hours.

Integrated effect of extractant of organic amendment and culture filtrate of *Aspergillus* spp. and *T. virens*

The extracts of two organic amendments (KC and CC), culture filtrate of five *Aspergillus* spp. and *T. virens* were used to find best combination against *R. solani*. Apart from extracts of two cakes, combination of culture filtrate of *T. virens* was made with culture filtrate of *Aspergillus* spp. and castor cake extracts in 1 : 1 (v/v). Mycelial disc of 10 mm size (from 7 days old culture) of *T. virens* and *R. solani* was placed at approximately 6 cm away from each other in 9 cm Petri plate containing different combinations in triplicate along with control. These Petri plates were incubated at 25±1°C and observations of mycelial growth were made after 96 hours.

The entire experiment was conducted under Completely Randomized Design (CRD) to analyse statistically with three replications under laboratory conditions at Rajendra Agriculture University, Pusa (Samastipur), Bihar, India during 2008–2009.

RESULTS

Antagonistic effect of *Aspergillus* spp. against *R. solani*

All five *Aspergillus* spp. exhibited growth suppressing ability against *R. solani* (Table 1). *A. niger* was found the most effective antagonist against of *R. solani* in suppressing the growth and proved statistically superior (39.8 mm) as compared to the *Aspergillus* spp. tested. The minimum (36.1 mm) growth was attained by *R. solani* in co-culture with *A. niger* followed by *A. flavus* (38.0 mm), *A. fumigatus* (39.5 mm), *A. ochraceous* (42.8 mm) and *A. terreus* (49.5 mm). This result indicates that *A. niger* is the best biocontrol agent against *R. solani*, however, *A. fumigatus* and *A. flavus* are identical but significantly higher antagonistic potentials to other *Aspergillus* spp.

The effect of culture filtrate of fungal antagonist on the growth of *R. solani* was found statistically significant

Table 1. Antagonistic effect of *Aspergillus* spp on *R. solani* through dual culture and poison food method

Aspergillus spp.	Time [hours]*		Growth of *R. solani* [mm]**		Mean
	48	96	dual	metabolite	
A. niger	41.1	38.5	36.1	43.5	39.8
A. terreus	40.1	62.8	49.5	53.5	51.5
A. ochraceous	34.6	50.5	42.8	42.3	42.5
A. fumigatus	41.1	48.7	39.5	50.4	44.9
A. flavus	39.0	51.5	38.0	52.5	45.2
Control	49.1	58.5	53.8	53.8	53.8
Mean	40.9	51.7	43.3	49.3	
CD (p = 0.05)	0.62		0.62		0.44

* interaction effect of *Aspergillus* spp. x time
**interaction effect of *Aspergillus* spp. x methods
CD – Critical difference

except of *A. terreus* and control. Maximum (42.3 mm) inhibition of mycelial growth of the test organism was observed with culture filtrate of *A. ochraceous*. Culture filtrate of *A. niger, A. fumigatus, A. flavus* and *A. terreus* inhibited radial growth of *R. solani* by 43.5, 50.4, 52.5 and 53.5 mm, respectively. Interaction between *Aspergillus* spp. and time was significant. Antagonistic effect of *A. ochraceous* was more conspicuous with time after 48 hours, whereas that of *A. niger* was observed after 96 hours. Dual culture *per se* resulted in remarkably higher suppression as compared to metabolites (Fig. 1).

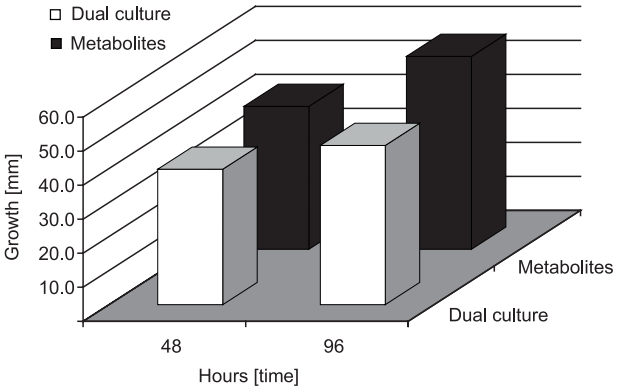

Fig. 1. Interaction effect on growth of *R. solani* with time

Effect of organic amendment extractant on *T. virens* and *R. solani*

All the seven amendments had significantly supportive effect on *T. virens* whereas detrimental effect on *R. solani*. The highest (20.2 mm) growth inhibitory effect was observed with mixed cake irrespective of the test organisms at different time intervals (Table 2). After 48 hours, maximum (36.7 mm) growth was supported by karanj cake followed by vermicompost (32.6 mm) and castor cake (32.4 mm), the later two had statistically identical effect. After 96 hours, castor cake was the most (79.2 mm) supporting amendment while the least effect (53.3 mm) had mustard cake. With the advance in time, effect of organic amendment increased markedly (Fig. 2). The highest (61.1 mm) growth of *T. virens* was supported by castor cake. FYM (47.8 mm) and vermicompost (47.4 mm) had identical effect on *T. virens*. Growth suppressive effect was observed for mustard cake in case of *T. virens*. Mixed cake was found statistically superior to other amendments in inhibiting the growth of *R. solani* being followed by vermicompost.

Table 2. Effect of organic amendment extract on *T. virens* and *R. solani*

Organic amendment extracts	Time [hours]*		Organisms**		Mean
	48	96	*T. virens*	*R. solani*	
Neem cake	29.6	67.7	52.4	45.0	48.7
Karanj cake	36.7	62.0	54.1	44.5	49.3
Mustard cake	27.2	53.3	33.5	47.0	40.2
FYM	29.0	66.5	47.8	47.5	47.7
Vermicompost	32.6	56.5	47.4	41.8	44.6
Mixed cake	14.1	26.4	20.6	19.9	20.2
Castor cake	32.4	79.2	61.1	50.5	55.8
Control	42.1	57.1	44.8	54.4	49.6
Mean	30.5	58.6	45.2	43.8	
CD (p = 0.05)	1.21		1.21		

*intercation effect of organic amendment extractant x time
** intercation effect of organic amendment extractant x organism
CD – Critical difference; FYM – Farma yard manure

Table 3. Effect of extract of cakes and metabolites of fungi on growth of *T. virens* and *R. solani* in dual culture

Treatments	*T. virens*	*R. solani*	Mean
Castor cake	62.0	26.6	44.3
Karanj cake	61.6	26.6	44.1
An + Tv + CC	58.5	30.0	44.2
An + Tv	61.1	23.1	42.1
Afi + Tv	52.8	36.6	44.7
Ao + Tv	53.8	23.5	38.6
Af + Tv	61.3	26.1	43.7
Control	54.5	49.1	51.8
Mean	58.2	30.2	
CD (p = 0.05)	2.33		1.64

An – *A. niger*; Tv – *T. virens*; CC – castor cake; Afi – *A. fumigatus*; Af – *A. flavus*; Ao – *A. ochraceous*; CD – Critical difference

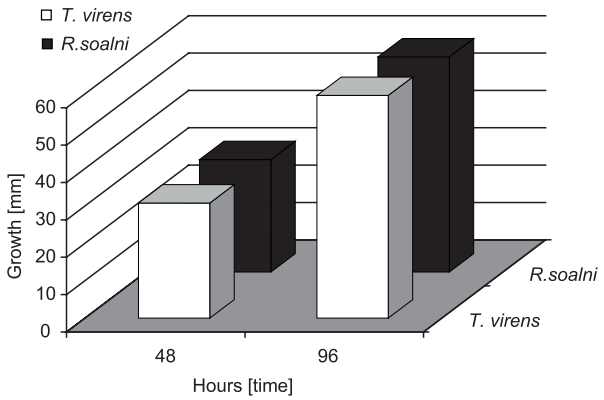

Fig. 2. Interaction effect on organisms with time

Combined effect of culture filtrate and organic amendment extractant on *T. virens* and *R. solani* in dual culture

Significant effect on growth of both species (beneficial and pathogenic) was observed in dual culture (Table 3). Organic amendment extractant, organic amendment extracts and metabolites (*A. niger* and *T. virens*), metabolites of *Aspergillus* spp. and *T. virens* had marked stimulatory and suppressive effect on growth of co-culture of *T. virens* and *R. solani*. Castor cake showed the higher (62.0 mm) stimulatory effect on *T. virens* followed by karanj cake (61.6 mm) and metabolites of *A. flavus* and *T. virens* (61.3 mm). All possible combinations in present study had inhibitory effect on growth of *R. solani*. However, inhibition potential of metabolites of *A. niger* + *T. virens* and *A. ochraceous* + *T. virens* was found statistically higher compared to other treatments. Castor cake, karanj cake and metabolites of *A. flavus* + *T. virens* had similar suppressive effect on *R. solani.*

DISCUSSION

Parasitism is the first step in biological control of pathogen where hyphal interaction between the pathogenic fungi and beneficial organism takes place. Under dual culture myco-parasitism was tested, the highest growth suppressing ability was observed with *A. niger* against *R. solani*. This may be attributed to fast growth and sporulation coupled with metabolites secreted from the sporulating fungi which results in lysis of the host cell. Sen *et al*. (1993) reported *A. niger*, an effective biological control agent against *R. solani* by way of antibiosis, overgrowth and hyperparasitism.

Aspergillus spp. studied under the present investigation is well known for secreting toxigenic compound around its surrounding. The highest growth inhibition of *R. solani* was observed with *A. ochraceous* which might be due to the presence of ochratoxin in the culture filtrate. Literature is scanty to support this finding, however, there is evidence that suggests the ochratoxin being injurious to human health but ochratoxin B derivative is non-toxigenic against human being (Dube 1999) which might get triggered on after interacting with enzymes of actively growing enzymatically active *R. solani*.

The best supporting organic amendment extract for the growth of *T. virens* were that of castor and karanj cakes at different time intervals. A probable reason behind this finding might be due to the presence of organic nitrogen, carbon in complex form, humic acid and absence of toxic compounds in these substrates. *Trichoderma* spp. predominantly colonize the substrate prepared from lignocellulosic material like tree bark (Kuter *et al*. 1983). Mixed cake extracts resulted in higher suppression of growth of both fungi due to higher accumulation of nitrogen in amide and amino acid form. Lazarovits (2001) reported that organic amendments of high nitrogen content have a potential to suppress both the soil borne pathogen (and beneficial organisms) through the toxic effect of ammonia and nitrous acid. Vermicompost was found next to mixed cake that supported a poor growth of *R. solani*. Detrimental effect of vermicompost on *R. solani* may be ascribed to low nitrogen content, presence of heavy metal ions and low cellulose content. The pathogenic fungi *R. solani* is highly competitive as a saprophyte. It can utilize cellulose and colonize fresh organic material, but does not colonize mature, low cellulose compost (Hoitink *et al*. 1996).

Apart from castor and karanj cakes growth supportive effect on *T. virens* was also observed with mixture of metabolites of *A. niger* + *T. virens* while the same combination had opposite effect on *R. solani*. The maximum inhibitory effect against *R. solani* was found with mixed metabolites of *A. niger* + *T. virens* and *A. ochraceous* + *T. virens.* The probable reason behind this finding might be attributed to the production of antibiotics, humic acid like substances, maturity index of the amendment along with the hyphal interaction between *T. virens* and *R. solani* in dual culture on amended substrate. Apart from secreting aflatoxin, *A. niger* also produces humic acid substance. The constituents of humic acid are highly stable organic matter that does not break down further. Hence, nutrients for growth become a limiting factor against the test pathogen. In addition to competition, parasitism was also reported as a mechanism for suppressing *Rhizoctonia* spp. in growing media (Hoitink and Boehm 1999). During hyphal interaction it was observed that *T. virens* is interacting with *R. solani*. Initially, *T. virens* was running along the host hyphae but later *R. solani* exhibited disintegrated appearance which might be due to production of lytic enzyme by *T. virens*. Several traits that may contribute to antagonistic interaction of *T. virens* with disease causing fungi involve production of a peptide metabolite like gliotoxin which was reported by Wilhite *et al.* (2001).

CONCLUSION

The current study suggests that *A. niger* was the most effective antagonist against *R. solani*, culture filtrate of *A. ochraceous* had maximum inhibitory effect on growth of the test pathogen, mixed cake extract had detrimental effect on both beneficial and pathogenic fungi, karanj cake supported the maximum growth of *T. virens* while combination of *A. niger* + *T. virens* was most aggressive towards suppressing the growth of the test pathogen. Following the pattern of enhancement of soil native mycoflora, incorporation of organic amendment and bio-control agent is suggestive as achievement of sustainable disease management against sclerotia forming pathogen of rice.

REFERENCES

Dennis C., Webster J. 1971. Antagonistic properties of species group of *Trichoderma* III. Hyphal interaction. Trans. Br. Mycol. Soc. 57: 363–369.

Dube H.C. 1999. Mycotoxin and mycotoxicoses. In: "An Introduction to Fungi". Vikas Publishing House Pvt. Ltd., New Delhi, India, 608 pp.

Gorodecki B., Hadar Y. 1990. Suppression of *Rhizoctonia solani* and *Sclerotium rolfsi* in container media containing composted separated cattle manure and composted grape marc. Crop Protect. 9: 271–274.

Grebus N.E., Watson M.E, Hoitink H.A.J. 1994. Biological, chemical and physical properties of composted yard trimmings as indicators of maturity and plant disease suppression. Compost Sci. Util. 2 (1): 57–71.

Hoitink H.A.J., Boehm M.J. 1999. Biocontrol within the context of soil microbial communities: A substrate dependent phenomenon. Ann. Rev. Phytopathol. 37: 427–446.

Hoitink H.A.J., Stone A.G., Grebus M.E. 1996. Suppression of plant diseases by composts. p. 373–381. In: "The Science of Composting" (M. De Bertoldi, P. Sequi, B. Lemmes, T. Papi, eds.). Blackie Academic & Professional, Glasgow. Vol. 1, 1055 pp.

Kannaiyan S., Prasad U.N. 1976. Efficacy of certain fungicides on control of sheath blight. Int. Rice Res. Newsletter 1, p. 19.

Khan A.A., Sinha A.P. 2005. Influence of different factors on the activity of fungal bioagents to manage sheath blight of rice in nursery. Ind. Phytopathol. 58: 289–293.

Kuter G.A., Nelson E.B., Hoitink H.A.J., Madden L.V. 1983. Fungal populations in container media amended with composted hardwood bark suppressive and conducive to *Rhizoctonia* damping-off. Phytopathology 73: 1450–1456.

Laha G.S., Venkatraman S. 2001. Sheath blight management in rice with biocontrol agents. Ind. Phytopathol. 54: 461–464.

Lazarovits G. 2001. Management of soilborne plant pathogens with organic soil amendments: A disease control startegy salvaged from the past. Can. J. Plant Pathol. 23: 1–7.

Morton D.J., Stroube W.H. 1955. Anatagonistic and stimulating effects of soil micro-organisms upon *Sclerotium*. Phytopathology 45: 417–420.

Nelson E.B., Kuter G.A., Hoitink H.A.J. 1983. Effect of fungal antagonist and compost age on suppression of *Rhizoctonia* damping-off in container media amended with composted with hardwood bark. Phytopathology 73: 1457–1462.

Sen B., Sharma J., Asalmol M.N., Chattopadhyay C., Patibanda A.K. 1993. *Aspergillus niger* - A potential biocontrol agent for soil borne pathogens. Ind. Phytopathol. 46 (3): 275–278.

Singh A. 2001. Studies on Fungal Decomposition of Sunhemp (*Crotolaria juncea* L.) in Soil. Ph.D. Thesis, Banaras Hindu University, Varanasi, India, 83 pp.

Valenzuela E., Leiva S., Godoy R. 2001. Seasonal variation and enzymatic potential of microfungi associated with the decomposition of *Northofagus pumilio* leaf litter. Rev. Chil. Hist. Nat. 74 (4): 737–749.

Warcup J.H. 1960. Method for isolation and estimation of activity of fungi in soil. p. 3–21. In: "Ecology of Soil Fungi" (D. Parkinson, J.S. Waid, eds.). The University Press, Liverpool.

Wilhite S.E., Lumsden R.D., Straney D.C. 2001. Peptide synthatase gene in *T. virens*. Appl. Environ. Microbiol. 67 (11): 5055–5062.

POLISH SUMMARY

DZIAŁANIE METABOLITÓW GRZYBOWYCH I WYCIĄGÓW ORGANICZNYCH NA WZROST GRZYBNI *RHIZOCTONIA SOLANI*

W produkcji ekologicznej sugeruje się połączenie organicznych źródeł przeciwko patogenom odglebowym. Oceniono wpływ metabolitów powszechnie występujących gatunków *Aspergillus*, organicznych wyciągów i ich łączne działanie z *Trichoderma*, przeciwko *Rhizoctonia solani*. Najmniejszy wzrost (36,1 mm) *R. solani* odnotowano w podwójnej kulturze z *A. niger*. Maksymalną inhibicję (42,3 mm) wzrostu grzybni testowego mikroorganizmu zaobserwowano w przypadku filtratu kultury z *A. ochraceous*, a następnie z *A. niger, A. fumigatus, A. flavus* i *A. terreus*. Spośród wyciągów, te z rącznika wykazywały stymulujące działanie na wzrost *T. virens*, a maksymalne (41,8 mm) supresyjne działanie na *R. solani* obserwowano w przypadku wermikompostu. Z czasem, działanie wyciągów z organicznych dodatków znacznie wzrastało. Potencjał inhibicyjny mieszaniny filtratu kultury *A. niger* + *T. virens* i *A. ochraceous* + *T. virens* przeciwko *R. solani* był istotnie wyższy w porównaniu z innymi kombinacjami.