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IN VITRO ANTAGONISM OF *TRICHODERMA VEREDE* AND *ASPERGILLUS* SPP. AGAINST A PATHOGENIC SEED BORNE FUNGUS OF SESAME

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ABSTRACT

Four soil fungi were isolated from the soil by serial dilution and were identified as *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and *Trichoderma viride* Pers. The soil fungi were selected to evaluate their antagonistic potential against seed borne fungus *Fusarium merismoides* isolated from sesame. In dual culture colony interaction *Trichoderma viride* showed the highest (45.88%) growth inhibiting effect on *F. merismoides* followed by *A. niger* (40.00%), *A. flavus* (36.37) and *A. fumigatus* (30.77%). Volatile metabolites from *T. viride* showed the highest growth inhibiting effect on *F. merismoides* (67.69%) and non-volatile metabolites from *T. viride* showed the highest growth inhibiting effect on *F. merismoides* (75.00%).

Key words: Antagonism, *Trichoderma viride, Aspergillus* spp., Seed borne fungus, Sesame seeds

INTRODUCTION

Sesame (*Sesamum indicum* L.) belongs to the family Pedaliaceae. It is also known as beniseed, gingelley and til. It is considered as 'The queen of oil' in the west. The origin of sesame is in Africa and secondary origin in India. Out of all the edible oil yielding plants sesame is the second biggest supplier of edible oil in our country next to mustard. The scarcity of edible oil both from plant and animal sources is an acute problem in Bangladesh and the same is increasing day by day (Kaul and Das 1986 and Rahman 1994). Sesame seed is a common ingredient in various cuisines. It is used whole in cooking for its rich, nutty flavor. The seed is used as nourishing and as flavoring agent. These tiny seeds are innately blessed with good protein levels. 100 grams of sesame seeds contain 18 grams of protein which makes up to 32% of RDA requirement. That is one of the key reasons why it could be used as an inevitable nut in kid's diet. Sesame seed is rich source of oleic acid, a monounsaturated fatty acid that can lower bad cholesterol and boost good cholesterol in body system, thus preventing coronary artery diseases and heart stroke. Sesame seeds contain magnesium, a mineral that has a rich anti-cancerous reputation and they aid in good digestive system and the colon, as they are good sources of fiber. Fiber helps in smoothening the functioning of intestine, facilitating the flushing out of waste, and thus offering guaranteed relief from constipation (Anonymous 2014). Sesame oilcake is good feed for cattle, goat and sheep (Khan *et al.* 2009). The Sesame oil may help to reduce high blood pressure and lower the amount of medication needed to control hypertension. Fungal infestation is one of the constrain for production of healthy seeds (Bakr *et al.* 2009).

Biological control is now increasingly considered as an alternative treatment to sustain agriculture. Biological control measures rely on the use of such organisms that are antagonistic to the target pathogens. Mechanisms by which antagonistic organisms act include mycoparasitism that may result from physical Interhypha l interference or by the production of volatile and nonvolatile metabolites.

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Study of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology because it has great potential to solve many agricultural and environmental problems. Dennis and Webstar (1971a, 1971b) observed effect of non volatile and volatile metabolites compounds produced by *Tricoderma* sp. The general term embracing all the mechanisms is designated as 'antagonism'. It includes all unfriendly relationships between antagonist and test pathogen. Antagonism embraces antibiosis, competition, predation and parasitism. Antibiosis is the inhibition of one organism by the metabolites of another. It occurs due to antibiotics, *viz*., aldehydes, alcohols, acetone, organic acids, volatile and non-volatile compounds etc. produced by microorganisms which have toxic effect on many plant parasitic fungi (Fries 1973, Fokema 1976, Bashar and Rai 1994 and Akter *et al*. 2014). Kakde and Chavan (2011) reported antagonistic properties of *T. viride* and *T. harzianum* against storage fungi. Tapwal and Chandra (2015) observed *in vitro* evaluation of *Trichoderma* species against seed. Thakur and. Harsh (2014) observed *in vitro* potential of volatile metabolites of phylloplane fungi of *Piper longumas.*

A number of workers have studied colony interaction between pathogenic and non-pathogenic fungi for the determination of antagonism of fungi isolated from different habitats (Skidmore and Dickinson 1976).

Present investigation was undertaken to find out the antagonistic potential of soil fungi *Aspergillus flavus, A. fumigates,, A. niger* and *Trichoderma viride* against seed borne fungus *Fusarium merismoides* isolated from sesame.

MATERIALS AND METHODS

Dual culture assays: Four soil fungi were isolated from the soil by serial dilution and were identified as *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride*. The soil fungi were selected to evaluate their antagonistic potential against seed borne fungus *Fusarium merismoides in vitro* dual culture assays on potato dextrose agar (PDA)

Colony interactions between the test pathogens and the selected soil fungi were studied in dual cultures on Potato dextrose agar medium. A Petri plate with 15 ml solidified PDA medium was inoculated with 5 mm mycelial agar disc of a pathogen and a soil fungus, 30 mm apart from each other. Three replications were maintained in each case. The inoculated plates were incubated at 25±2°C temperatures for 5 days. The colony growth of the pathogen was measured at the both sides, that is, towards and opposing each other from their central loci. Ten replicates were made for each treatment. The radial growth was measured after 3 and 5 days. Intermingled and inhibition zone was also measured during the same period. Assessments of colony interaction between the test pathogens and the soil fungi were done in terms of grades which were determined by the model of Skidmore and Dickinson (1976).

The grades and types are as follows:

Grade 1 (Type A): Mutually intermingling growth where both. fungi grew into one another without any microscopic sign of interaction.

Grade 3 (Type Bi): Intermingling growth where the fungus being observed into the opposed fungus either above or below its colony.

Grade 2 (Type Bii): Intermingling growth. where the fungus under observation has ceased the growth and is being overgrown by another colony.

Grade 4 (Type C): Slight inhibition with a narrow demarcation line (1-2mm).

Grade 5 (Type D): Mutual inhibition at a distance more than 2 mm. The parameter used for the assessment of the colony interaction were the width of inhibition zone, intermingled zone

and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976).

$$\text{Antagonistic effect} = \frac{R1 - R2}{R1} \times 100$$

Where,

R1= the radial growth of the pathogens towards the opposite side

R2 = denotes the radial growth of the pathogen towards the antagonist

The same method was followed for all possible combinations amongst the pathogens and selected four soil fungi.

Ability of the antagonists to produce volatile compounds on PDA plates:

PDA plates were inoculated in the centre with a 0.5 cm diameter mycelial disc containing both antagonists and pathogen. Fungal isolate *F. merismoides* was separately cultivated on PDA per plate. The lids were removed and two plates containing test pathogen *F. merismoides* and on soil fungus, and one plate was inverted and placed on top of the other plate. The two plate bases were then sealed with a double layer of parafilm. All plates were randomized and placed at room temperature. Controls were prepared using the same experimental setup, except that a PDA disc was used instead of the antagonist culture. Ten replicates per treatmen were used.

The formula of percent growth inhibition is given below:

$$I = \frac{C - T}{C} \times 100$$

Where, I= Per cent growth inhibition

C= Growth in control

T= Growth in treatment

The results were statistically analyzed by "t" test following Steel and Torrie (1960).

Effect of culture filtrates (Non-volatile metabolites) of the soil fungi on the radial growth of the test pathogen.

The test pathogens selected for the present study were the same as in the experiment number. Three equal size blocks each of individual fungus, cut from the actively growing margins of 5 days old cultures, were inoculated separately into the 250 mL conical flasks each containing 100 mL sterilized Potato dextrose broth medium. After 10 days of incubation at 25 ± 2°C, the culture of a soil fungus was filtered first through a Whatman filter paper and then centrifuged at 3000 rpm for 20 minutes.

The 5, 10, 15 and 20 mL metabolites of each fungus were added in 95, 90, 15 and 80 mL sterilized PDA medium separately. The conical flask containing the PDA medium and culture filtrates was moved in different directions gently on the Laminar air flow table to get the homogenous distribution of the supplemented medium. This concentration was found to be most suitable for such studies by Singh (1978). Each Petri plate contained 15 mL of PDA medium and metabolites with an addition of 1 drop (ca 0.03) of lactic acid which was used to check the bacterial growth. The 5, 10, 15 and 20 mL of supplemented medium was poured in a sterilized Petri plate and was allowed to solidify. Each Petri plate was inoculated centrally with a 5 mm agar disc, cut from the margin of actively growing culture of a test pathogen. In the control, Petri plate containing PDA medium without culture filtrates were inoculated with a test pathogen as described above. In control set, equal amount of sterilized water was added with the PDA medium instead of culture filtrate. Ten replications of each treatment were maintained. All the Petri plates were incubated at 25 ± 2°C. The radial growth of the colonies was measured after 4 and 7 days of incubation. The per cent inhibition of each test pathogen was calculated with the formula mentioned above.

RESULTS AND DISCUSSION

The results of colony interactions have been summarized in Table 1. Different antagonistic effects of the soil fungi were noted against the test pathogens. Bii type (grade 2) was found to be the most commonly encountered type of colony interaction. From the results it is evident that out of four soil fungi examined only *Aspergillus flavus*, *A. niger* and *T. viride* exhibited strong antagonistic effect against the test fungi.

In dual culture colony interaction *Trichoderma viride* showed the highest (45.88%) growth inhibiting effect on *Fusarium merismoides* followed by *Aspergillus niger* (40.00%), *A. flavus* (36.37%) and *A. fumigatus* (30.77%) (Table 1).

The maximum intermingled zone was observed in case of *Trichoderma viride* (0.4). *Trochoderma viride* grew over the colony of the *F. merismoides.*

In contrast to the present study, Bashar and Chakma (2014) reported that in dual culture colony interaction *Aspergillus niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 65.21, 64.24, 57.14 and 34.78% growth inhibition on *F. oxysporum*, respectively. Again *Aspergillus niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 56.25, 50.00, 43.33 and 34.78% growth inhibition on *F. solani*, respectively. Aktar *et al.* (2014) reported that in dual culture colony interaction *Aspergillus flavus*, *A. niger*, *T. viride* and *A. fumigatus* showed 65.62, 55.74, 55.35 and 51.50% growth inhibition on *F. oxysporum*, respectively. The same antagonists also showed different effects on different fungi in the present investigation. This variation might be due to selection of different test pathogens.

The results of effect of volatile metabolites of antagonistic fungi against sesame pathogen are presented in Table 2. The maximum inhibition of radial growth of *F. merismoides* was observed in case of *T. viride* (67.69%) followed by *Aspergillus niger* (64.62%), *A. flavus* (61.54%) and *A. fumigatus* (56.57%) due to the volatile metabolites after 5 days of incubation at 25±2˚C.

Table 1. Colony interaction between *Fusarium merismoides* and four antagonists

Name of antagonist	Grade*	Type	% inhibition of colony of the pathogen	Intermingled zone (cm)	Inhibition zone (cm)
Aspergillus flavus	2	Bii	36.37	0.1	_
A. fumigatus	2	Bii	30.77	0.3	_
A. niger	2	Bii	40.00	0.2	_
Trichoderma viride	2	Bii	45.88	0.4	_

'–'= absent

* = Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), based on Skidmore and Dickinson (1976).

Bii = Intermingling growth where the fungus under observation has ceased the growth and is being overgrown by another colony (2).

Table 2. Per cent inhibition of radial growth of *Fusarium merismoides* by volatile metabolites of four antagonistic fungi

Sl. No.	Name of the antagonists	% inhibition of radial growth of thee test pathogen
1.	*Aspergillus flavus*	61.54[a]
2.	*Aspergillus fumigatus*	56.57[a]
3.	*Aspergillus niger*	64.62[a]
4.	*Trichoderma viride*	67.69[a]

Remarks efficiency of antagonists: *T. viride* > *A. niger* > *A. flavus* > *A. fumigatus*.
a indicates significance at 0.1% level.

Table 3. Per cent inhibition of radial growth of *Fusarium merismoides* by non-volatile metabolites of antagonistic fungi

SI. No.	Name of the antagonists	% inhibition of radial growth at different concentrations (%)			
		5	10	15	20
1.	*Asprergillus flavus*	4.55[NS]	10.61[NS]	34.09[b]	56.82[b]
2.	*Aspergillus fumigatus*	25.00[c]	47.73[b]	54.55[b]	60.61[b]
3.	*Aspergillus niger*	10.61[NS]	35.61[b]	40.91[b]	54.55[b]
4.	*Trichoderma viride*	64.58[b]	66.67[b]	70.83[b]	75.00[b]

Remarks efficiency gradient: *T.viride*> *A. fumigatus*> *A. niger*> *A. flavus*
a, b and c indicate significance at 0.1, 1 and 5% level, respectively. In a row, figures with same letter do not differ significantly whereas figures with dissimilar lettet differ significantly (as per DMRT), NS = Not significant.

In contrast to the present study, Bashar and Chakma (2014) reported that volatile substances produced by *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* showed 29.75, 20.15, 15.78 and 12.25% growth inhibition on *F. oxysporum*, respectively. Again, *T. viride, A. niger, A. flavus* and *A. fumigatus* showed 34.24, 26.33, 14.79 and 13.06% growth inhibition on *F. solani*, respectively.

Aktar *et al*. (2014) reported that volatile metabolites produced by an isolate of *A. flavus, A. niger, A. fumigatus* and *T. viride* inhibited mycelial growth of *F. oxysporum* by 18.00, 14.50, 12.00 and 10.25%, respectively. Aktar *et al*. (2014) also reported that volatile metabolites produced by an isolate of *T. viride* and *A. niger* inhibited mycelial growth of *Curvularia lunata* by 20.86 and 14.85%, respectively. Difference in percent inhibition with the present study might be due to the difference in organism involved in the interaction.

Table 3 shows the effect of non-volatile metabolites on the growth of *F. merismoides*. The selected antagonists showed varied degree of growth inhibition of the pathogen at different concentrations.

The maximum inhibition of radial growth of *Fusarium merismoides* was observed with the culture filtrates of *T. viride* (75.00%) followed by *A. fumigatus* (60.61%), *A. flavus* (56.82%) and *A. niger* (54.55%) at 20% concentration after 4 days of incubation at 25±2˚C.

Trichoderma viride showed 64.58, 66.67 and 70.83% growth inhibition of *F. merismoides* at 5, 10 and 15% concentrations due to non-volatile metabolites, respectively. *A. niger* showed 10.61, 35.61 and 40.91% growth inhibition of *F. merismoides* at 5, 10 and 15% concentrations due to non- volatile metabolites, respectively. *Aspergillus fumigatus* showed 25.00, 47.73 and 54.55% growth inhibition of *F. merismoides* at 5, 10 and 15% concentrations due to non- volatile metabolites, respectively. *A. flavus* showed 4.55, 10.61 and 34.09% growth inhibition of *F. merismoides* at 5, 10 and 15% concentrations due to non- volatile metabolites, respectively.

In contrast to the present study, Bashar and Chakma (2014) reported that culture filtrates of *T. viride*, *A. fumigatus*, *A. niger and A. flavus* showed 82.05, 80.56, 72.22 and 66.66% growth inhibition of *F. oxysporum* at 20% concentration. Again, *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* showed 79.49, 74.36, 74.36 and 71.36% growth inhibition of *F. solani* at 20% concentration, respectively.

In contrast to the present study, Aktar *et al*. (2014) reported that culture filtrates of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* showed 58.25, 52.00, 45.75 and 39.00% growth inhibition on *F. oxysporum* at 20% concentration, respectively. Aktar *et al*. (2014) also reported that non-volatile metabolites produced by an isolate of *T. viride* and *A. niger* inhibited mycelial growth of *Curvularia lunata* by 60.07% and 52.50%, respectively.

Madhanraj *et al*. (2010) reported that culture filtrates of *T. viride* and *A. niger* inhibited the mycelial growth of *F. solani* by 85% and 70% at 20 per cent concentration, respectively.

Hosen *et al*. (2016) reported that non-volatile metabolites produced by an isolate of *T. viride* and *A. niger* inhibited mycelial growth of *Colletotrichum gloeosporioides* by 55.35% and 53.57% at 20% concentration, respectively. Differences in per cent inhibition with the present study might be due to the difference in organism involved in the interaction.

Present findings show that soil fungi have significant antagonistic activity against test pathogen *F. merismoides.*

REFERENCES

Aktar, M.T., K.S. Hossain and M. A. Bashar. 2014. Antagonistic potential of rhizosphere fungi against leaf spot rand fuit rot pathogens of brinjal. *Bangladesh J. Bot.* **43**(2): 213-217.

Anonymous 2014. 31 Amazing benefits and uses of sesame seeds. https:// www. healthbeckon.com/sesame- seeds-benefits/

Bakr, M. A., M. A. Hossain and M. M. Karim. 2009. Gradient of oil seed crop disease management, fungal associations and mycotoxin contamination. Advances in Oilseed Researches in Bangladesh. Oilseed Research Centr. BARI, Gazipur, Bangladesh. pp180.

Bashar, M. A. and B. Rai. 1994. Antagonistic potential of root-region microflora of chickpea against *Fusarium oxysporum* f. sp. *ciceri*. *Bangladesh J. Bot.* **23**(1): 13-19.

Bashar, M.A. and M. Chakma 2014. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. *Dhaka Univ. J. Biol. Sci*. **23**(1): 53-60.

Dennis, C. and J. Webster. 1971a. Antagonistic properties of species groups of *Trichoderma.*I. Production of non-volatile antibiotics. *Trans. Brit. Mycol. Soc.* **57:** 25-29.

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

Fokkema, N. J. 1976. Antagonism between fungal saprophytes and pathogens on aerial plant surfaces. *In*: Microbiology of Aerial Plant Surfaces (eds. Dickinson CH and Preece TF), Academic Press, London. pp. 487-505.

Fries, N. 1973. Effect of volatile organic compounds on the growth and development of fungi. Trans. Brit. Mycol. Soc. **60:** 1-21.

Hosen S, S Shamsi and M.A. Bashar. 2016. *In vitro* biological control of *Colletotrichum gloeosporioides* (Penz.) Sacc. And *Sclerotium rolfsi* Sacc., causal agent of anthracnose and soft rot of *Corchorus capsularis* L. *Bangladesh J. Bot*. **45**(1): 175-180.

Kakde, R.B. and A.M. Chavan. 2011. Antagonistic properties of *Trichoderma viride* and *Trichoderma harzianum* against storage fungi. *Elixir Appl. Botany*. **41**: 5774-5778.

Kaul,. A.K. and M.L. Das. 1986. *Oil Seeds in Bangladesh.* Ministry of Agriculture. Government of the People's Republic of Bangladesh. Dhaka. p. 63.

Khan, M.A. H., N.A. Sultan, M.N. Islam and M. Hasanuzzaman. 2009. Yield and yield contributing characters of sesame as affected by different management practices. *American-Eurasian Journal of Scientific Research* **4**(3): 195-197.

Madhanraj, P., V. Ambikapathy and A. Panneerselvam. 2010. Biological control of banana wilt caused by *Fusarium solani* (Mart.) Sacc. *International Journal of Applied Biology and Pharmaceutical Technology* **3**(1): 1032-1039.

Skidmore, A.M. and C. H. Dickinson. 1976. Colony interaction and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Trans. Brit. Mycol. Soc.* **66**: 57-64.

Steel, R.G.D. and J.H. Torrie. 1960. *Principles and Procedures of Statistics, with special reference to the Biological Sciences*. McGraw-Hill Book Co., New York pp. xvi+481.

Tapwal, A, A. Tyagi, G. Thakur and S. Chandra. 2015. *In vitro* evaluation of *Trichoderma* species against seed borne pathogens. IJCBS Research Paper **1**(10).

Rahman, M.M., M.G. MauIa, S. Begum and M.A. Hossain. 1994. Maximization of yield of sesame through management practices. Central Annual Research. BARI, Joydebpur, Gazipur. pp. 53-56.

Thakur, S, and N.S.K. Harsh. 2014. *In vitro* potential of volatile metabolites of phylloplane fungi of *Piper longumas* biocontrol agent against plant pathogen. *I.J.S.N*. **5**(1): 33-36.

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