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ANTAGONISTIC POTENTIALITY OF SOME SOIL FUNGI AGAINST SIX FUNGAL PATHOGENS ISOLATED FROM COTTON (*GOSSYPIUM HIRSUTUM* L.) SEEDS

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Abstract

The antagonistic potentials of four soil filamentous fungi *viz*., *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and *Trichoderma viride* Pers. against six pathogens isolated from 14 varieties of cotton (*Gossypium hirsutum* L.) were evaluated by "dual culture colony interaction", volatile and non-volatile metabolites. In dual culture colony interaction, out of four soil fungi, *T. viride* was found be the most effective to control the growth of the cotton pathogens. *T. viride* showed the highest growth inhibition against *Curvularia lunata*, *Fusarium moniliforme*, *Mucor* sp. and *Rhizoctonia solani*. On the other hand *A. niger* showed the highest growth inhibition against *Fusarium nivale* and *A. fumigatus* showed the maximum growth inhibition against *C. gloeosporioides*. The highest inhibition of radial growth of *C. lunata*, *F. nivale* and *F. moniliforme* was observed might be due to the secretion of volatile metabolites of *T. viride* whereas, the maximum inhibition of radial growth of *C. gloeosporioides* was observed because of the volatile metabolites of *A. flavus*. *Mucor* sp. and *R. solani* were inhibited due to the volatile metabolites of *A. fumigatus*. The greatest radial growth inhibition of *C. lunata* and *F. moniliforme* were noticed in case of *T. viride* owing to the effect of non-volatile metabolites. On the other hand, the radial growth of *C. gloeosporioides* and *F. nivale* were inhibited highest amount for the effect of non-volatile metabolites of *A. niger*, whereas highest growth inhibition of *Mucor* sp. and *R. solani* was observed due to the non-volatile effect of *A. fumigatus*. The present investigation suggests that the isolates of *Aspergillus* and *Trichoderma* may be further exploited as potential biocontrol agents against the fungal pathogens of cotton in field trial.

Key words: Antagonistic potentiality, Fungal antagonists, Cotton pathogens.

Introduction

Cotton is the most renowned, reliable fiber yielding crops as well as cash crops around the world including Bangladesh. It is the major textile fiber used by man in the world and playing a key role in the economic and social welfare (Munro 1994).

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Each year, cotton production is being reduced due to the presence of some harmful fungal pathogens. Majority of cotton diseases are seed-borne *viz*., *Alternaria* blight, bacterial blight, *Fusarium* wilt, *Myrothecium* blight, *Cercospora* blight, *Exserohilum* blight etc. A number of seed-borne fungal pathogens such as *Alternaria, Fusarium, Rhizopus* and *Aspergillus* are frequently identified in cotton seeds (Minton and Garber 1983). *Aspergillus flavus*, *A. niger* (Type-I), *Curvularia lunata, Fusarium moniliforme* var. *subglutinans, F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for *Gossypium arboreum* in Bangladesh (Naznin and Shamsi 2018).

Application of antagonists to soil and seeds increased seed germination percentage and reduced the seed borne infection. 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. So, there is a need to screen newly available seed dressing molecules including bio-agents and botanicals for their efficacy in overcoming the seed-borne infections of fungi.

In Bangladesh, research on biological control of seed-borne fungi of cotton seeds through soil antagonists is very limited. So, for the sake of economy, more information on this regard is essential. Considering the importance of this valuable fiber, present investigation was undertaken to screen out the efficacy of antagonistic fungi against the fungal pathogens associated with cotton seeds.

Materials and Methods

Collection of samples: Seed samples of CB1-14 were collected from Cotton Research, Training and Seed multiplication Farm, Gazipur after harvesting and were kept in clean glass jars, labeled properly and preserved at room temperature for subsequent use. The experiment was conducted in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka.

Isolation and identification of fungi: The fungi were isolated from the collected seed samples following the "Tissue Planting Method" on PDA medium (CAB 1968), "Blotter method" and "Paper Towel Method" (ISTA 1996). Morphological identification of the isolates were determined following the standard literatures (Thom and Rapper 1945, Rapper and Thom 1949, Subramanian 1971, Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Ellis 1971, 1976, Sutton 1980) and molecular identification were done following Amer *et al.* 2011 with some modifications.

Antagonostic potentiality of fungi: Six pathogenic fungi, namely, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and

Rhizoctonia solani, isolated from 14 varieties of cotton seeds were selected as test pathogens against four antagonistic soil fungi.

Antagonistic fungi were isolated from rhizosphere soil of the host varieties following serial dilution method. Among the isolated fungi, *Aspergillus flavus, A. fumigatus, A. niger* and *Trichoderma viride* were selected to test their antagonistic potentials against the pathogens following "dual culture technique" described by Bashar and Rai (1994). The parameter used for the assessment of the colony interaction and per cent inhibition of radial growth was calculated by the formula of Fokkema (1976). Effects of volatile and non-volatile metabolites of the selected soil fungi against the test pathogens were also studied following the methods described by Bashar and Rai (1994).

Analysis of data: Data were evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Six fungal pathogens *viz., Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* were isolated from the seeds of 14 varieties of cotton seeds, which were found as virulent in the test of pathogenicity.

In colony interactions, antagonistic relationships among the soil fungi and test pathogens were grade 2 and 4. However, grade 2 was found to be the most commonly encountered type of colony interaction as 17 interactions were incorporated in this grade, which was followed by grade 4 (Table 1).

The intermingled zone between the soil fungi and test pathogens was very common. The maximum intermingled zone (0.3 cm) was observed in case of *A. fumigatus* and *T. viride* against *Mucor* sp. and *R. solani*. *T. viride* grew over the colony of the test pathogens but in case of *A. flavus, A. fumigatus* and *A. niger* inhibition zone was found and it was 0.2, 0.2 and 0.1, respectively.

All the tested soil fungi inhibited the growth of all the test pathogens to varied degrees in dual culture experiments on PDA plates. *A. fumigatus* showed the highest inhibition on radial growth of *C. gloeosporioides* (84.0%) followed by *A. niger* (80.0%), *A. flavus* (73.9%) and *T. viride* (63.1%). *T. viride* showed the highest (76.4%) growth inhibition on *Curvularia lunata* which was followed by *A. niger* (72.2%), *A. fumigatus* (68.4%) and *A. flavus* (59.0%). *T. viride* showed the highest growth inhibition on *F. moniliforme* (73.7%), which was followed by *A. flavus* (67.6%), *A. niger* (60.0%) and *A. fumigatus*

(53.8%). *A. niger* showed the highest growth inhibition (72.27%) on *F. nivale*, which was followed by *A. flavus* (68.1%), T. *viride* (66.6%) and *A. fumigatus* (45.4%). *T. viride* showed the maximum growth inhibition on *Mucor* sp. (60.0%) which was followed by *A. niger* (52.1%) *A. flavus* (50.0%), and *A. fumigatus* (44.1%). *T. viride* also showed the highest growth inhibition on *Rhizoctonia solani* (46.8%) which was followed by *A. niger* (30.2%) *A. flavus* (26.6%), and *A. fumigatus* (19.3%) (Table 1 and Fig. 1).

Table 1. Effects of dual culture between fungal antagonists and cotton pathogens.

Test pathogens		% inhibition of radial growth, intermingled and inhibition zone and type of reactions of the test pathogens			
		Aspergillus flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*
Colletotrichum gloeosporioides	% inhibition	73.9	84.0	80.0	63.1
	IMZ (cm)	0.1	0.2	0.2	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
Curvularia lunata	% inhibition	59.0	68.4	72.2	76.4
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.2	0.2	0.1	-
	Grade	4	4	4	2
Fusarium moniliforme	% inhibition	67.6	53.8	60.0	73.7
	IMZ (cm)	-	-	-	0.2
	IHZ (cm)	0.1	0.2	0.2	-
	Grade	4	4	4	2
Fusarium nivale	% inhibition	68.1	45.4	72.2	66.6
	IMZ (cm)	0.2	0.1	0.1	0.1
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2
Mucor sp.	% inhibition	50.0	44.1	52.1	60.0
	IMZ (cm)	0.2	0.3	-	0.3
	IHZ (cm)	-	-	0.1	-
	Grade	2	2	4	2
Rhizoctonia solani	% inhibition	26.6	19.3	30.2	46.8
	IMZ (cm)	0.2	0.3	0.2	0.3
	IHZ (cm)	-	-	-	-
	Grade	2	2	2	2

IMZ = Intermingling zone, IHZ = Inhibition zone, and '-' = not applicable.

Similar observation was also noticed in the study of Akter *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019 where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

In dual culture technique, maximum growth inhibition was recorded for *Trichoderma* spp. against different pathogenic fungi in the research of Tapwal *et al*. 2015, Patel and Joshi 2001, Sunitha and Kurundkar 2007, Al-Ameen *et al*. 2017, Goswami and Islam 2002.

Grade 2 = Mutual intermingling growth where the growth of the fungus is ceased and being over growth by the opposed fungus. Grade 4 = Slight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm) based on Skidmore and Dickinson (1976).

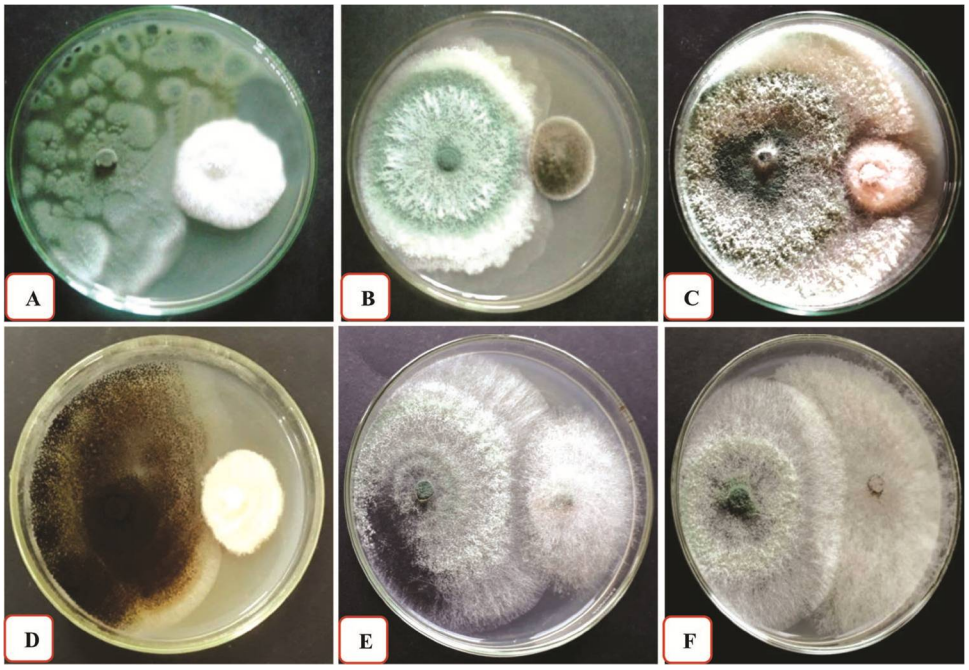

Fig. 1. Photographs showing colony interactions between pathogenic fungi and antagonists. A. *Colletotrichum gloeosporioides* and *Aspergillus fumigatus*; B. *Curvularia lunata* and *Trichoderma viride*; C. *Fusarium moniliforme* and *T. viride*; D. *Fusarium nivale* and *Aspergillus niger*; E. *Mucor* sp. and *T. viride*; F. *Rhizoctonia solani* and *T. viride.*

The effect of volatile metabolites of antagonistic fungi against cotton seed pathogens are presented in Table 2. The maximum inhibition of radial growth of *C. gloeosporioides* was observed in *A. flavus* (59%), which was followed by *A. niger* (48.7%), *A. fumigatus* (38.2%) and *T. viride* (35.9%) due to the volatile metabolites. The maximum inhibition of radial growth of *Curvularia lunata* was observed in *T. viride* (71.4%) followed by *A. fumigatus* (47.6%), *A. flavus* (38.1%) and *A. niger* (33.3%) owing to volatile metabolites. The maximum inhibition of radial growth of *Fusarium nivale* was also observed in *T. viride* (64.2%), which was followed by *A. niger* (54.7%), *A. fumigatus* (47.6%) and *A. flavus* (40.4%). Highest inhibition of radial growth of *F. moniliforme* was found by *T. viride* (51.1%) followed by *A. fumigatus* (42.2%), *A. niger* (40.0%) and *A. flavus* (35.5%). Whereas, *A. fumigatus* showed 58.2% inhibition of radial growth of *Mucor* sp. which was followed by *T. viride* (55.5%), *A. flavus* (47.2%) and *A. niger* (36.3%). At last, maximum inhibition of radial growth of *Rhizoctonia solani* was noticed in *A. fumigatus* (72.4%), which was followed by *T. viride* (55.5%), *A. niger* (53.3%) and *A. flavus* (52.2%) (Table 2 and Fig. 2).

Table 2. Percent inhibition of radial growth of the test pathogens owing to volatile metabolites of antagonistic fungi.

Antagonistic fungi	% inhibition of radial growth of the test pathogens					
	Colletotrichum gloeosporioides	*Curvularia lunata*	*Fusarium nivale*	*Fusarium moniliforme*	*Mucor* sp.	*Rhizoctonia solani*
Aspergillus flavus	58.9[a]	38.1[c]	40.4[d]	35.5[d]	47.2[c]	52.2[d]
A. fumigatus	38.4[c]	47.6[b]	47.6[c]	42.2[b]	58.1[a]	72.4[a]
A. niger	48.7[b]	33.3[d]	54.7[b]	40.0[c]	36.3[d]	53.3[c]
Trichoderma viride	35.9[d]	71.4[a]	64.2[a]	51.1[a]	55.5[b]	55.5[b]
CV%	0.0220	0.0210	0.0193	0.0237	0.0234	0.0171

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Similar observation was also noticed in the study of Aktar *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019, where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

Al-Ameen *et al.* (2017) reported that some volatile metabolites released from *T. viride* cultures might be responsible for extending the inhibitory activity against some pathogens such as *Colletotrichum* and *Fusarium* species isolated from banana (*Musa sepientum* L.)

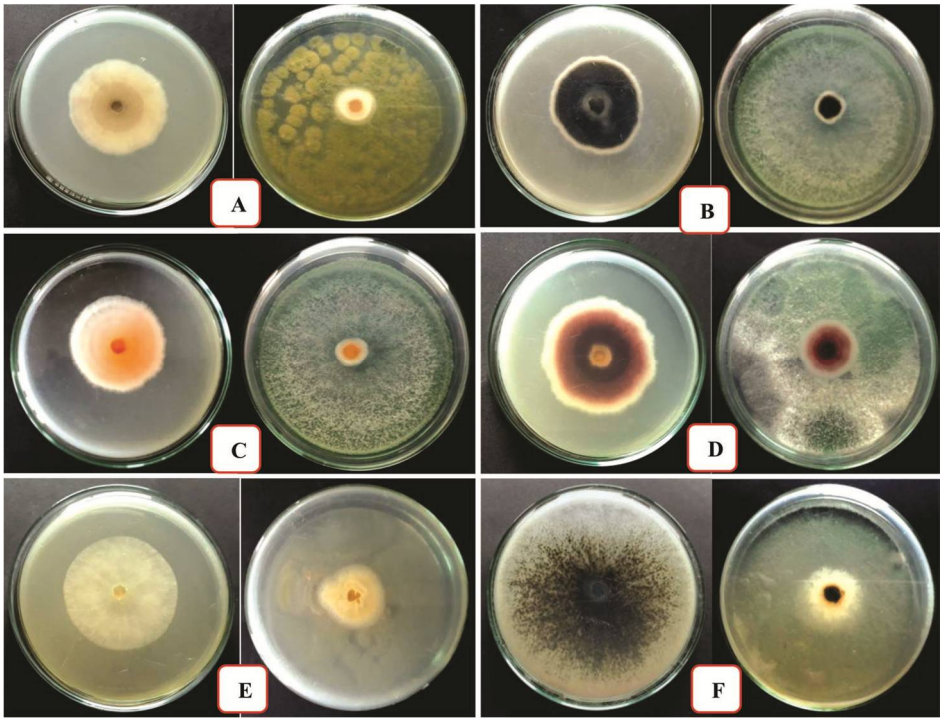

Fig. 2. Growth inhibition of pathogenic fungi owing to volatile metabolites of the antagonists. A. *A. flavus*: *Colletotrichum gloeosporioides*; B. *T. viride*: *Curvularia lunata*; C. *T. viride*: *Fusarium nivale*; D. *T. viride*: *Fusarium moniliforme*; E. *A. fumigatus*: *Mucor* sp. and F. *A. fumigatus*: *Rhizoctonia solani.*

Table 3 shows the effect of non-volatile metabolites on the growth of *C. gloeosporioides*, *C. lunata*, *F. nivale*, *F. moniliforme*, *Mucor* sp. and *R. solani*. The maximum inhibition of radial growth of *C. gloeosporioides* was observed with the culture filtrates of *A. niger* (76.3%), which was followed by *T. viride* (70.9%), *A. fumigatus* (58.4%) and A. flavus (57.7%) at 20% concentration. The maximum inhibition of radial growth of *Curvularia lunata* was observed with the culture filtrates of *Trichoderma viride* (78.2%) which was followed by *A. niger* (73.1%), *A. flavus* (72.3%), and *A. fumigatus* (57.4%) at 20% concentration. The highest inhibition of radial growth of *Fusarium moniliforme* was

observed with the culture filtrates of *T. viride* (76.3%), which was followed by *A. niger* (74.0%), *A. fumigatus* (67.2%) and *A. flavus* (56.3%) at 20% concentration.

Table 3. Percent inhibition of radial growth of test pathogens by non-volatile metabolites of antagonistic fungi.

Test pathogens	Concentration (%)	% inhibition of radial growth of test pathogens by non-volatile metabolites owing to different antagonists				CV (%)
		Aspergillus flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*	
Colletotrichum gloeosporioides	5	28.9^{c}	27.6^{d}	47.2^{a}	41.8^{b}	0.0275
	10	48.8^{c}	42.5^{d}	58.1^{a}	49.0^{b}	0.0201
	15	55.5^{c}	51.0^{d}	66.0^{a}	65.4^{b}	0.0168
	20	57.7^{d}	58.4^{c}	76.3^{a}	70.9^{b}	0.0152
Curvularia lunata	5	36.1^{d}	38.1^{c}	56.3^{a}	45.6^{b}	0.0227
	10	48.9^{c}	44.6^{d}	65.4^{a}	65.2^{b}	0.0178
	15	63.8^{c}	53.1^{d}	72.7^{a}	69.5^{b}	0.0154
	20	72.3^{c}	57.4^{c}	73.1^{b}	78.2^{a}	0.0142
Fusarium moniliforme	5	30.9^{d}	38.1^{b}	33.3^{c}	43.6^{a}	0.0685
	10	43.6^{d}	52.7^{c}	55.5^{a}	54.5^{b}	0.0224
	15	50.9^{d}	58.1^{c}	62.9^{b}	65.4^{a}	0.0168
	20	56.3^{d}	67.2^{c}	74.0^{b}	76.3^{a}	0.0146
Fusarium nivale	5	33.3^{b}	30.9^{d}	32.7^{c}	50.0^{a}	0.0272
	10	40.4^{c}	38.1^{d}	50.9^{b}	64.0^{a}	0.0207
	15	47.6^{d}	50.0^{c}	67.2^{b}	68.1^{a}	0.0871
	20	64.2^{d}	71.4^{c}	81.8^{a}	74.0^{b}	0.0137
Mucor sp.	5	27.5^{d}	36.4^{b}	35.0^{c}	38.6^{a}	0.0290
	10	38.8^{d}	44.7^{a}	43.7^{c}	44.0^{b}	0.0234
	15	42.3^{d}	55.3^{a}	53.7^{c}	54.6^{b}	0.0194
	20	47.0^{d}	64.7^{a}	57.5^{c}	60.0^{b}	0.0581
Rhizoctonia solani	5	20.0^{a}	10.0^{d}	18.8^{b}	14.1^{c}	0.0634
	10	37.7^{b}	22.2d	31.1^{c}	54.1^{a}	0.0275
	15	45.5^{b}	30.0^{d}	37.7^{c}	58.8^{a}	0.0232
	20	55.5^{d}	72.2^{c}	58.8^{b}	62.8^{a}	0.0211

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

The highest inhibition of radial growth of *F. nivale* was observed with the culture filtrates of *A. niger* (81.8%), which was followed by *T. viride* (74.0%), *A. fumigatus* (71.4%) and *A. flavus* (64.2%) at 20% concentration. The maximum inhibition of radial growth of *Mucor* sp. was observed with the culture filtrates of *A. fumigatus* (64.7%)

which was followed by *T. viride* (60.0%), *A. niger* (57.5%) and *A. flavus* (47.0%) at 20% concentration. The maximum inhibition of radial growth of *Rhizoctonia solani* was observed with the culture filtrates of *A. fumigatus* (72.2%), which was followed by *T. viride* (62.8%), *A. niger* (58.8%) and *A. flavus* (55.5%) at 20% concentration (Table 3). Differences in percent inhibition with the present study might be due to the differences in organism strains involved in the interaction (Table 3 and Fig. 3).

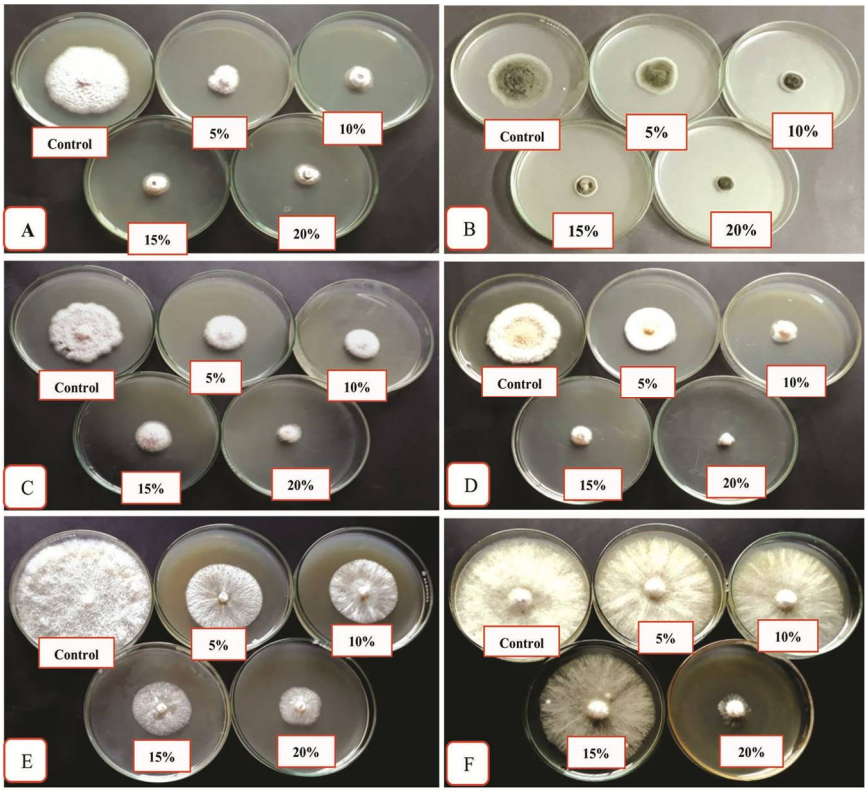

Fig. 3. Growth inhibition of pathogenic fungi owing to non-volatile metabolites of antagonists at 5, 10, 15 and 20% concentrations. A. *Colletotrichum gloeosporioides*: *Aspergillus niger*; B. *Curvularia lunata*: *Trichoderma viride*; C. *Fusarium moniliforme: T. viride*; D. *Fusarium nivale: A. niger*; E. *Mucor* sp.: *Aspergillus fumigatus* and F. *Rhizoctonia solani*: *A. fumigatus.*

Similar result was found in the research of of Akter *et al*. 2014, Bashar and Chakma 2014, Helal and Shamsi 2019, where *A. flavus, A. fumigatus, A. niger* and *T. viride* showed significant growth inhibition against *Colletotrichum* spp., *Curvularia lunata* and *Fusarium* spp.

The non-volatile metabolites produced from the culture filtrates of *T. viride* and *A. niger* were responsible for maximum inhibition against different pathogenic species of *Colletotrichum* and *Fusarium* according to Al-Ameen *et al*. 2017 and Madhanraj *et al.* 2010.

Tapwal *et al.* (2015) also reported that, culture filtrates of *T. viride* showed major growth inhibition on *C. gloeosporioides.* A number of *Trichoderma* species are effective agents for the control of plant pathogenic fungi, such as *Fusarium* spp. (Sivan and Chet 1986), *Pythium* spp. (Naseby *et al.* 2000) and *Rhizoctonia* spp. (Lewis and Papavizas 1987).

The present investigation suggests that the isolates of *Aspergillus* and *Trichoderma* may be further exploited as potential biocontrol agents against the fungal pathogens of cotton in field trial.

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