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In vitro efficacy of selected fungal isolates against *Colletotrichum gloeosporioides*, the causal agent of leaf spot disease of *Zingiber officinale* Rosc.

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Abstract

The present study was conducted to isolate the fungal pathogen *Colletotrichum gloeosporioides* causing leaf spot disease in *Zingiber officinale* Rosc. and to evaluate the efficacy of certain selected fungal isolates against the pathogen using Dual culture method and Inverted plate technique. 24 fungal species isolated from rhizospheric and non rhizospheric soil of *Zingiber officinale* Rosc. were selected for screening for antagonistic potentials against the pathogen. The fungal species are *Absidia cylindrospora, Aspergillus flavus, A.fumigatus, Cladosporium herbarum, Gliocladium catenulatum, G.roseum, Gliocladium sp, Paecilomyces lilacinus, Penicillium canescens, P.chrysogenum, P.citrinum, P.expansum, P.lanosum, P.oxalicum, P.paradoxum, P.sclerotiorum, P.simplicissimum, Sesquicillium candelabrum, Trichoderma harzianum, T.koningii ,T.koningiopsis, T.polysporum, T.pseudokoningii* and *T.viride.* The result from the dual culture revealed a varying degree of growth inhibition by all the fungal isolates tested. *Aspergillus flavus* exhibits the highest inhibition percentage (78.2%) and the least inhibition percentage was exhibited by *Cladosporium herbarum* (9.2%). Whereas in inverted plate technique the maximum inhibition by the volatile metabolites on the mycelial growth of *C.gloeosporioides* was exhibited by *T.pseudokoningii* (80%) and the least was exhibited by *Gliocladium catenulatum* (26.7%).

Keywords: *Colletotrichum gloeosporioides, Zingiber officinale* Rosc. , Inhibition, *In vitro*.

1. Introduction

Leaf spot of ginger is one of the phytopathological constraints in the cultivation of ginger in India. Among the diseases affecting aerial parts especially the foliage, leaf spot incited by *Colletotrichum gloeosporioides* is a major cause of concern in many of the ginger growing tracts (Dohroo 1997). *Colletotrichum* leaf spot was first reported by Sundararaman (1922) from the Godavari district of Andhra Pradesh and the species *C. zingiberi* was identified by Butler & Bisby (1931). Similarly, *Colletotrichum* leaf spot incited by species, *C. gloeosporioides* and *C. capsici* has been reported from South- East Asian countries (Xizhen *et al.* 2005). Darshana *et al* (2014) also reported the occurrence of *C.gloeosporioides* isolate from infected ginger leaves during survey from various districts of Karnataka and Kerela.

Colletotrichum gloeosporioides is the most widespread species on many host plants from among the genus *Colletotrichum* (Farr *et al.*, 1995). The disease affects the foliage, leading to extensive damage of the effective photosynthetically active surface area which in turn adversely affects the qualitative parameters of the growing rhizome. The disease symptoms start as small necrotic lesion. The disease is common in the month of July to September and prior to harvesting period. Symptoms are observed on leaves as oval to elongated spots. The spots are almost white in the centre and have a dark brown margin often with a yellowish halo surrounding the spot. The spots are usually isolated but may also become confluent resulting in big patches. The present study aims at screening certain selected fungal isolates from rhizospheric and non rhizospheric soil of *Zingiber officinale* Rosc. for *in vitro* efficacy evaluation against *colletotrichum gloeosporioides*.

2. Materials and Methods

2.1 Study site

The study was carried out in Mawjiej, West Khasi Hills District, Meghalaya, India, which is located at a geographical location of $25^0$47.272' N and $91^0$04.644' E.

2.2 Sampling

Soil sampling for isolation of antagonistic fungi was done at a monthly interval for a period of two year (2015 and 2016) for the crop growing season (April –December). For the purpose of rhizospheric soil sampling, the growing rhizome was uprooted and

the soil adhering to the rhizome was carefully collected in sterilized polythene bag whereas non rhizospheric soil was collected from the area surrounding the rhizome. The soil samples were stored at 4ºC until further analysis. Also the Infected leaves of ginger were collected in sterilized polythene bags from the farmer field.

2.3 Isolation of pathogen

For isolation of pathogen the infected leaves were cut into small segments and surface sterilized with 1% sodium hypochlorite solution for 1 minute. It was then rinsed for three times in sterile distilled water. After surface sterilization the leaf segments were placed on sterile filter paper to remove excess moisture. The leaves segments were then inoculated on Petri plates containing Potato Dextrose Agar medium (Jeffers and Martin, 2010).

2.4 Isolation of antagonistic fungi

Serial dilution plate method was followed for the isolation of fungi using Rose Bengal Agar medium. Three replicates were maintained for each sample. The inoculated Petri dishes were then incubated upside down at 25±1ºC for 5 days in a BOD incubator.

2.5 Identification of fungal species

The fungal species were identified on the basis of their morphology and reproductive structures by consulting monograph by Subramanian (1971), Barnett and Hunter(1972), Ellis (1971) and Domsch *et al.* (1980).

2.6 *In vitro* efficacy test

In vitro efficacy of selected fungal isolates was studied following the method of Skidmore and Dickinson (1976). 5mm diameter mycelial disc from actively growing 5days old fungal cultures of both the antagonist and pathogen were placed on the opposite end of the sterile Czapek Dox (CDA) plates. For each treatment three replicates were maintained. Plates with only the pathogen inoculated in it serves as control.

Percentage of inhibition was calculated using the formula:

% inhibition $=(C-T)/C*100$

Where, C = radial growth of the pathogen towards the opposite side in control plate.

T = radial growth of the pathogen towards the opponent antagonist in test plate.

Efficacy was categorized based on their ability to over grow and inhibit the growth of the pathogen by giving them a score as per modified Bell's scale. Where R1 = 100% over growth, R2 = 75% over growth, R3 = 50% over growth, R4 = locked at the point of contact.

2.7 Effect of volatile metabolites

The effect of the volatile metabolites produced by the antagonistic fungi against the pathogen was evaluated by the inverted plate technique as described by Dennis and Webster (1971b). 5mm mycelial disc of both antagonists and the test pathogen was inoculated on separate Czapek Dox Agar plates and incubated at 25°C for 2 days. After completion of the incubation period the lid of the plate inoculated with the antagonist was replaced by the bottom of the plate inoculated with the pathogen and was sealed together using parafilm. The radial growth of the pathogen was recorded after 4days of incubation and the inhibition percentage was calculated by the above mentioned formula.

2.8 Statistical analysis

Each experiment was performed in triplicates. Statistical analysis was performed using SPSS 16 statistics software.

3. Results

3.1 Isolation of pathogen

The fungal pathogen was identified morphologically and through reproductive structures. The colony character and growth of the isolate varied on different media. Colour of the colony varied from white to dull grey, often concentric with orange conidial masses.

Conidial morphology

Conidia are borne on distinct well developed hyaline conidiophores. The acervuli were highly variable in size, shape and exude masses of conidia. The conidia are smooth walled. The conidial shapes were mostly cylindrical with tapering ends. Some are elliptical or dumb bell. (Plate 2). Colony characters and growth of the pathogen *Colletotrichum gloeosporioides* varied on different media. This might be due to the variations in the nutritional requirement of the fungus. Better growth and sporulation was observed in Czapek Dox agar medium. (Plate 3).

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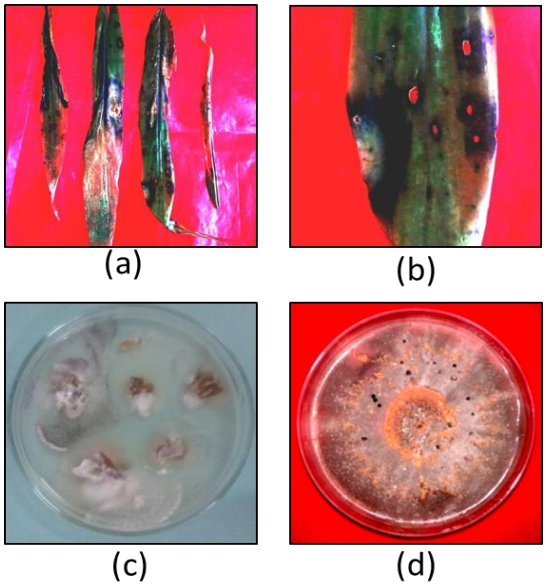

Plate 1: (a) Infected leaf showing disease symptom (b) Necrotic lesion on leaf surface (c) Isolation on Potato Dextrose Agar medium (d) Pure culture of *Colletotrichum gloeosporioides*.

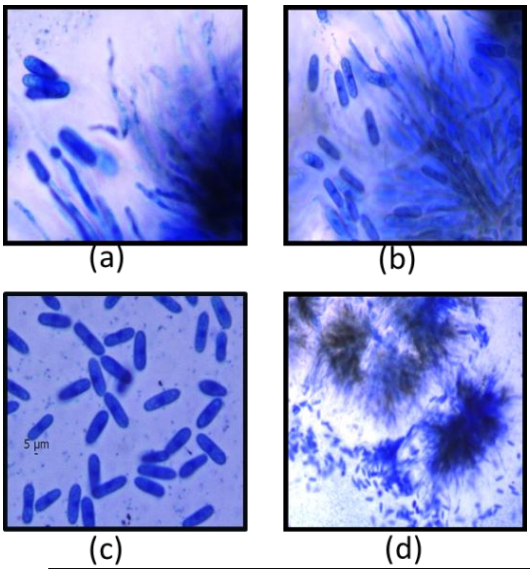

Plate 2: Microscopic view of the conidial morphology of *Colletotrichum gloeosporioides* (a) Conidiophores (b) Acervuli and conidia (c) Conidial mass (d) Setae.

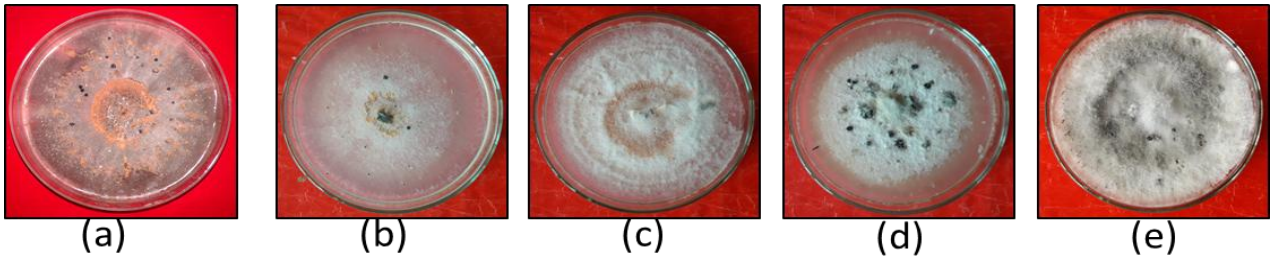

Plate 3: Colony morphology of *Colletotrichum gloeosporioides* isolated from infected leaves of *Zingiber officinale* Rosc. on different media (a) Czapek dox Agar (CDA) (b) Corn Meal Agar (CMA) (c) Potato Carrot Agar (PCA) (d) Potato Dextrose Agar (PDA) (e) Sabouraud Dextrose Agar(SDA).

3.2 Isolation of antagonistic fungal species

24 indigenous fungal species isolated from rhizospheric and non rhizospheric soil of *Zingiber officinale* Rosc. were selected for evaluation of antagonistic efficiencies against the leaf spot pathogen *Colletotrichum gloeosporioides* .The fungal species selected are the monthly dominant species and the known antagonists isolated during the two years study period (2015 and 2016). The fungal species are *Absidia cylindrospora, Aspergillus flavus, A.fumigatus, Cladosporium herbarum ,Gliocladium catenulatum, G. roseum, Gliocladium sp, Paecilomyces lilacinus, Penicillium canescens, P. chrysogenum, P.citrinum, P.expansum, P.lanosum, P.oxalicum, P.paradoxum, P. sclerotiorum P.simplicissimum, , Sesquicillium candelabrum, Trichoderma harzianum , T. koningii, T. koningiopsis, T.polysporum, T.pseudokoningii* and *T.viride* .

3.3 Evaluation of antagonistic potentials by the fungal isolates in Dual culture method.

The result from the dual culture of the 24 fungal isolates and the pathogen *Colletotrichum gloeosporioides* revealed a varying degree of growth inhibition of the pathogen by all the fungal isolates. *Aspergillus* species evaluated in the study viz., *Aspergillus flavus* and *A.fumigatus* showed high degree of growth inhibition of the pathogen with *Aspergillus flavus* exhibiting the highest inhibition percentage of 78.2%. Other organisms which showed high degree of inhibition of growth of the pathogen with over 70% growth inhibition includes *Trichoderma koningii* (77%), *Aspergillus fumigatus* (74.7%), *Penicillium citrinum* (74.1%) *T.pseudokoningii* (73%) and *Trichoderma viride* (70%) respectively. Distinct zone of inhibition is displayed in some of the dual culture plates of the pathogen and the antagonists. An inhibition zone of 0.5cm is observed in dual cultures of the pathogen

and *Trichoderma koningii, T.polysporum* and *Penicillium canescens* and *P. paradoxum.* In the interaction between the pathogen and *C.herbarum, G.roseum, P.sclerotiorum* and *S.candelabrum* a clear zone of 0.4cm is observed. Similarly a clear zone of 0.3cm is also observed in interaction of the pathogen with *Trichoderma harzianum* , *Absidia cylindrospora* and *Gliocladium sp.* as well. An intermingled zone is observed in some of the interaction viz; *Penicilllium expansum* (0.2cm), *Trichoderma koningiopsis* (0.2cm), *T.pseudokoningii* (1cm) and *T.viride* (0.5cm). Other isolates evaluated in the study also showed a fairly good inhibition against the growth of the pathogen. The least inhibition percentage was exhibited by *Cladosporium herbarum* (9.2%) followed by *Sesquicillium candelabrum* (10.3%) and *Gliocladium roseum* (14.9%). (Table 1, Plate 4).

Table 1: Effect of the test fungal isolates on the radial growth of the pathogen *Colletotrichum gloeosporioides* in dual culture method. (Skidmore and Dickinson, 1976).

Sl.no	Dual culture	Radial growth (in cm)		% of I	IZ	IM	Bell's Ranking
		C	T				
1	*C. gloeosporioides* and *Absidia cylindrospora*	5.8	3.2	44.3	0.3	-	R3
2	*C. gloeosporioides* and *Aspergillus flavus*	5.8	1.3	78.2	-	-	R1
3	*C. gloeosporioides* and *A.fumigatus*	5.8	1.5	74.7	-	-	R1
4	*C. gloeosporioides* and *Cladosporium herbarum*	5.8	5.3	9.2	0.4	-	R4
5	*C .gloeosporioides* and *Gliocladium catenulatum*	5.8	3.9	32.2	-	-	R4
6	*C. gloeosporioides* and *G.roseum*	5.8	4.9	14.9	0.4	-	R4
7	*C.gloeosporioides* and *Gliocldium sp.*	5.8	4.6	20.7	0.3	-	R4
8	*C. gloeosporioides* and *Paecilomyces lilacinus*	5.8	3.9	33.3	-	-	R4
9	*C. gloeosporioides* and *Penicillium canescens*	5.8	3.9	32.8	0.5	-	R3
10	*C .gloeosporioides* and *P.chrysogenum*	5.8	2.0	65.5	-	-	R2
11	*C.gloeosporioides* and *P. citrinum*	5.8	1.5	74.1	-	-	R1
12	*C. gloeosporioides* and *P.expansum*	5.8	2.1	63.2	-	0.2	R2
13	*C .gloeosporioides* and *P.lanosum*	5.8	2.7	52.9	-	-	R3
14	*C .gloeosporioides* and *P.oxalicum*	5.8	2.4	58.0	-	-	R2
15	*C.gloeosporioides* and *P.paradoxum*	5.8	4.6	20.1	0.5	-	R4
16	*C. gloeosporioides* and *P.sclerotiorum*	5.8	2.7	53.4	0.4	-	R4
17	*C. gloeosporioides* and *P.simplicissimum*	5.8	4.4	24.7	-	-	R3
18	*C. gloeosporioides* and *Sesquicillium candelabrum*	5.8	5.2	10.3	0.4	-	R4
19	*C. gloeosporioides* and *T.harzianum*	5.8	2.2	61.5	0.3	-	R2
20	*C. gloeosporioides* and *Trichoderma koningii*	5.8	1.3	77.0	0.5	-	R1
21	*C. gloeosporioides* and *T.koningiopsis*	5.8	2.4	58.6	-	0.2	R2
22	*C .gloeosporioides* and *T.polysporum*	5.8	3.1	47.1	0.5	-	R3
23	*C. gloeosporioides* and *T.pseudokoningii*	5.8	1.6	73.0	-	1	R1
24	*C. gloeosporioides* and *T.viride*	5.8	1.7	70.7		0.5	R2

Note: 'I'- Inhibition, 'IZ'- Inhibition Zone, 'IM' –Intermingled Zone, C-Radial growth of the pathogen in control plate, T-Radial growth of the pathogen in test plate.
*Each value is a mean of three replicate.

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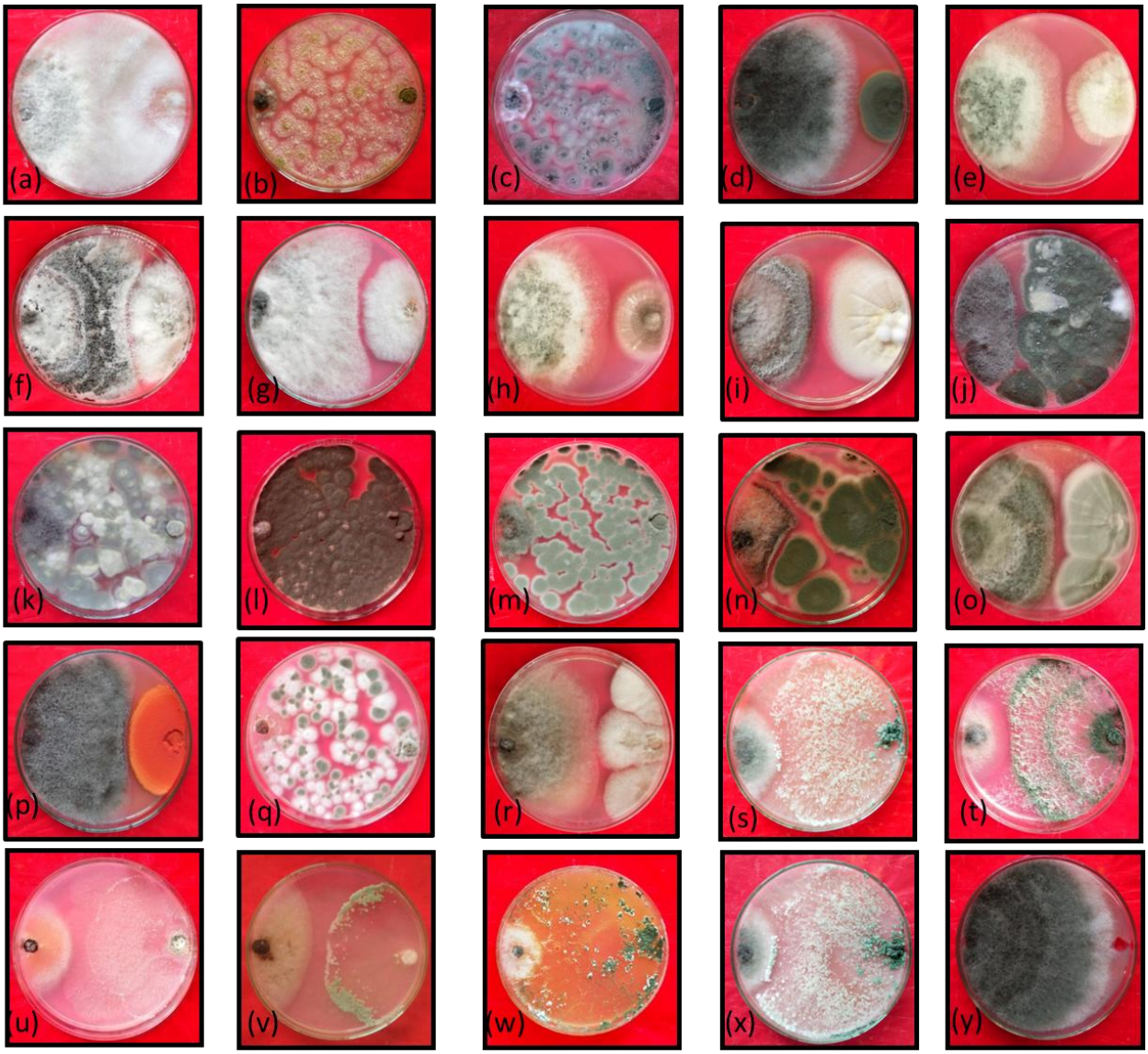

Plate 4: Dual culture method showing colony interaction between pathogen **Colletotrichum gloeosporioides* and antagonists (a) *Absidia cylindrospora* (b) *Aspergillus flavus* (c) *A.fumigatus* (d) *Cladosporium herbarum* (e) *G.catenulatum* (f) *Gliocladium roseum* (g) *Gliocladium sp.* (h) *Paecilomyces lilacinus* (i) *Penicillium canescens* (j) *P. chrysogenum* (k) *P. citrinum* (l) *P.expansum* (m) *P. lanosum* (n) *P.oxalicum* (o) *P.paradoxum* (p) *P. sclerotiorum.* (q) *P. simplicissimum* (r) *Sesquicillium candelabrum* (s) *Trichoderma harzianum* (t) *T. koningii* (u) *T. koningiopsis* (v) *T. polysporum* (w) *T. pseudokoningii* (x) *T. viride* (y) Control.
(Note:*Left side of the plate in all the above pictures)

3.4 Effect of volatile metabolites

The volatile metabolites produced by the antagonists exhibit a significant reduction on the growth of the pathogen *Colletotrichum gloeosporiodes* . Maximum growth inhibition was exhibited by volatile metabolites of *Trichoderma pseudokoningii* (80%) followed by *Aspergillus flavus* (78%), *T.viride* (75%) and *T.koningii* (68%). *Penicillium species* evaluated in the study also exhibited a fairly good inhibition percentage against the pathogen with the maximum growth inhibition of the pathogen exhibited by *Penicillium chrysogenum* (66%), and *P. sclerotiorum* (59.2%) and *P.citrinum* (58.3%). The least inhibition percentage was exhibited by *Gliocladium catenulatum* (26.7%) (Fig.1, Plate 5).

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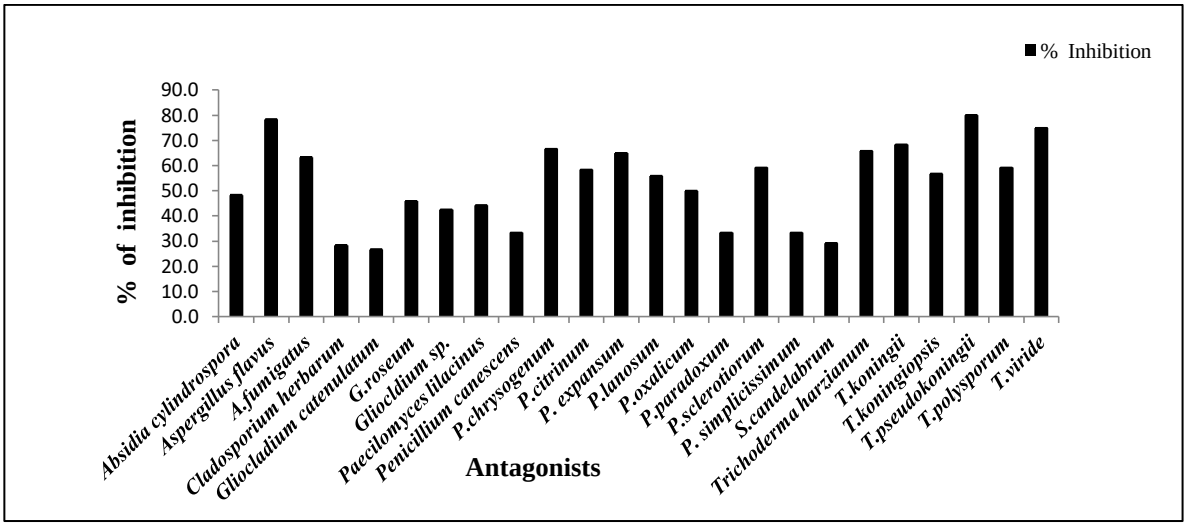

Fig.1. Inhibitory effect (%) of the volatile metabolites of the fungal isolates against *Colletotrichum gloeosporioides*.

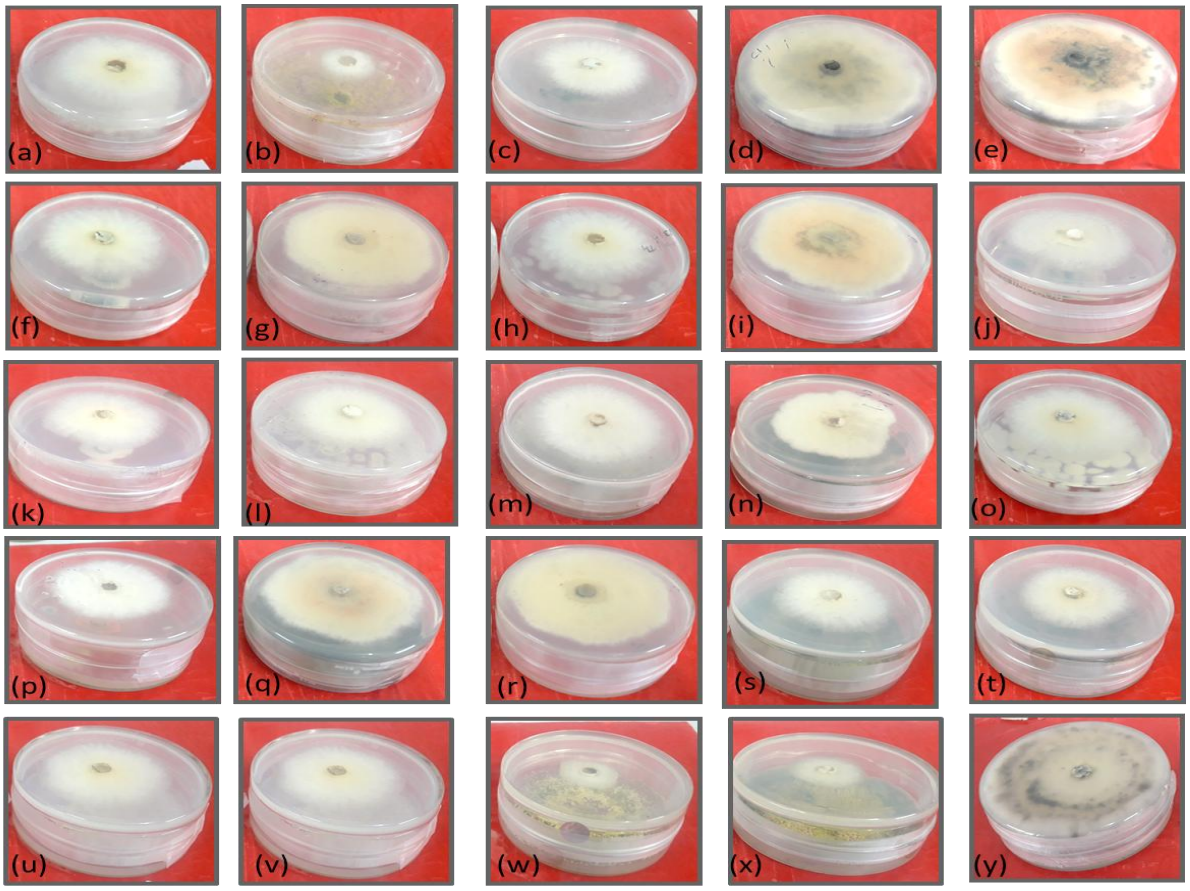

Plate 5: Inverted plate technique (Dennis and Webster, 1971b) to evaluate the effect of volatile metabolites on the pathogen **Colletotrichum gloeosporioides* by (a) *Absidia cylindrospora* (b) *Aspergillus flavus* (c) *A.fumigatus* (d) *Cladosporium herbarum* (e) *G.catenulatum* (f) *Gliocladium roseum* (g) *Gliocladium sp.* (h) *Paecilomyces lilacinus* (i) *Penicillium canescens* (j) *P. chrysogenum* (k) *P. citrinum* (l) *P.expansum* (m) *P. lanosum* (n) *P.oxalicum* (o) *P.paradoxum* (p) *P.sclerotiorum.* (q) *P.simplicissimum* (r) *Sesquicillium candelabrum* (s) *Trichoderma harzianum* (t) *T koningii*. (u) *T. koningiopsis* (v) *T. pseudokoningii* (w) *T. polysporum* (x) *T. viride* (y) Control.
(Note:*upper portion of the plate in all the above pictures).

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3.5 Statistical analysis

One way analysis of variance showed a significant variation of 4.61 x 10 $^{-42}$ ($p<0.05$) in the colony interaction between the antagonistic fungi whereas a significant variation of 6.50 x 10^{-23}($p<0.05$) was observed in the effect of volatile metabolites secreted by the fungal isolates evaluated in the study.

Also Tukey's Post hoc test that was performed revealed significant variations between the antagonists. (Table 3 and 4)

Table 2: One way Analysis of Variance (ANOVA) of colony interaction and effect of volatile metabolites between antagonistic fungi ($p<0.05$).

Parameters	Colony interaction		Volatile metabolites	
	F-ratio	p level	F-ratio	p level
Antagonistic Fungi	242.89	4.61 x 10 $^{-42}$	229.92	6.50 x 10^{-23}

Table 3(a) and 3(b) Tukey's Post Hoc Test of colony interaction between antagonistic fungi ($p<0.05$)

3(a)

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.13x10^{-4}$	$1.93x10^{-11}$	$2.03x10^{-11}$	$7.32x10^{-4}$	$2.91x10^{-4}$	$5.15x10^{-11}$	$1.93x10^{-11}$	$1.27x10^{-9}$	0.02
2		0.98	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$4.48x10^{-5}$	0.94	$9.57x10^{-7}$	$1.94x10^{-11}$
3			$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	0.01	NS	$2.92x10^{-4}$	$3.25x10^{-11}$
4				$2.16x10^{-11}$	0.48	$2.94x10^{-4}$	$1.97x10^{-11}$	$2.03x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
5					$2.09x10^{-8}$	$2.94x10^{-4}$	NS	NS	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$9.8x10^{-11}$
6						0.48	$3.16x10^{-9}$	$8.1x10^{-9}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
7							$4.46x10^{-5}$	$1.15x10^{-4}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
8								NS	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$5.11x10^{-10}$
9									$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$2.15x10^{-10}$
10										0.02	NS	$4.46x10^{-5}$
11											$7.35x10^{-4}$	$5.15x10^{-11}$
12												0.001
13												

3(b)

	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	$6.64x10^{-6}$	$1.97x10^{-11}$	$5.06x10^{-10}$	0.01	$1.93x10^{-11}$	$2.10x10^{-8}$	$1.93x10^{-11}$	$2.54x10^{-6}$	0.99	$1.93x10^{-11}$	$1.93x10^{-11}$
2	$2.15x10^{-10}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.95x10^{-11}$	$1.93x10^{-11}$	$5.37x10^{-8}$	NS	$5.09x10^{-10}$	$1.93x10^{-11}$	0.67	0.09
3	$5.04x10^{-8}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$5.13x10^{-11}$	$1.93x10^{-11}$	$1.72x10^{-5}$	NS	$1.40x10^{-7}$	$1.93x10^{-11}$	NS	0.94
4	$1.93x10^{-11}$	$7.35x10^{-4}$	$3.68x10^{-7}$	$1.93x10^{-11}$	NS	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
5	$1.93x10^{-11}$	$1.15x10^{-4}$	0.096	$5.13x10^{-11}$	$3.25x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$9.5x10^{-7}$	$1.93x10^{-11}$	$1.93x10^{-11}$
6	$1.93x10^{-11}$	0.67	0.004	$1.93x10^{-11}$	0.84	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
7	$1.93x10^{-11}$	NS	0.94	$1.93x10^{-11}$	0.001	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
8	$1.95x10^{-11}$	$1.72x10^{-5}$	0.02	$2.16x10^{-10}$	$2.16x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.94x10^{-11}$	$6.57x10^{-6}$	$1.93x10^{-11}$	$1.93x10^{-11}$
9	$1.94x10^{-11}$	$4.48x10^{-5}$	0.047	$9.84x10^{-11}$	$2.48x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$2.5x10^{-6}$	$1.93x10^{-11}$	$1.93x10^{-11}$
10	0.09	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.14x10^{-4}$	$1.93x10^{-11}$	0.94	$2.94x10^{-4}$	0.17	$3.18x10^{-9}$	0.096	0.67
11	$1.41x10^{-7}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$9.84x10^{-11}$	$1.93x10^{-11}$	$4.48x10^{-5}$	0.99	$3.68x10^{-7}$	$1.93x10^{-11}$	NS	0.98
12	0.67	$1.93x10^{-11}$	$1.93x10^{-11}$	0.004	$1.93x10^{-11}$	NS	$6.57x10^{-6}$	0.84	$1.42x10^{-7}$	0.004	0.09
13	0.67	$1.93x10^{-11}$	$1.93x10^{-11}$	NS	$1.93x10^{-11}$	0.02	$1.97x10^{-11}$	0.48	0.48	$2.15x10^{-10}$	$8.10x10^{-9}$
14		$1.93x10^{-11}$	$1.93x10^{-11}$	0.84	$1.93x10^{-11}$	0.98	$1.25x10^{-9}$	NS	$7.39x10^{-4}$	$9.62x10^{-7}$	$4.48x10^{-5}$
15			0.84	$1.93x10^{-11}$	0.004	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
16				$1.93x10^{-11}$	$2.53x10^{-6}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$2.47x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
17					$1.93x10^{-11}$	0.047	$2.03x10^{-11}$	0.67	0.31	$5.09x10^{-10}$	$2.08x10^{-8}$
18						$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$	$1.93x10^{-11}$
19							$3.66x10^{-7}$	0.99	$2.54x10^{-6}$	$2.92x10^{-4}$	0.01
20								$3.16x10^{-9}$	$1.93x10^{-11}$	0.94	0.31
21									$2.95x10^{-4}$	$2.51x10^{-6}$	$1.15x10^{-4}$
22										$1.93x10^{-11}$	$2.03x10^{-11}$
23											NS

Note*:* **1**- *Absidia cylindrospora*, **2**- *Aspergillus flavus*, **3**- *A.fumigatus*, **4**-*Cladosporium herbarum*, **5**-*Gliocladium catenulatum*, **6**-*G.roseum*,**7**-*Gliocladium sp*, **8**- *Paecilomyces lilacinus*, **9**-*Penicillium canescens*, **10**- *P.chrysogenum*, **11**- *P.citrinum*, **12**-*P.expansum*, **13**-*P.lanosum*,**14** *P.oxalicum*, **15**- *P.paradoxum*, **16**- *P.sclerotiorum*, **17**- *P.simplicissimum*, **18**- *Sesquicillium candelabrum*, **19**- *Trichoderma harzianum*,**20**-*T.koningii*, **21**-*T. koningiopsis*, **22**-*T.polysporum*, **23**-*T.pseudokoningii* and **24**-*T.viride.*

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Table 4(a) and 4(b) Tukey's Post Hoc Test of effect of volatile metabolites on pathogen between antagonistic fungi ($p<0.05$)

4(a)

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	$1.95x10^{-11}$	$1.43x10^{-7}$	$4.44x10^{-10}$	$9.6x10^{-11}$	0.98	0.082	0.51	$1.41x10^{-7}$	$2.62x10^{-9}$	$1.6x10^{-4}$	$1.8x10^{-8}$	0.007
2		$1.42x10^{-7}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.36x10^{-5}$	$4.07x10^{-10}$	$1.29x10^{-6}$	$5.37x10^{-11}$
3			$1.95x10^{-11}$	$1.95x10^{-11}$	$6.8x10^{-9}$	$1.99x10^{-10}$	$1.06x10^{-9}$	$1.95x10^{-11}$	0.83	0.23	NS	0.007
4				NS	$6.7x10^{-9}$	$4.21x10^{-7}$	$4.97x10^{-8}$	0.23	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.99x10^{-11}$
5					$1.05x10^{-9}$	$4.97x10^{-8}$	$6.7x10^{-9}$	0.02	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.96x10^{-11}$
6						0.83	NS	$4.06x10^{-6}$	$1.98x10^{-10}$	$4.09x10^{-6}$	$1.05x10^{-4}$	$1.61x10^{-4}$
7							NS	$5.68x10^{-4}$	$2.66x10^{-11}$	$4.97x10^{-8}$	$5.37x10^{-11}$	$1.29x10^{-6}$
8								$4.5x10^{-5}$	$5.37x10^{-11}$	$4.21x10^{-7}$	$1.9x10^{-10}$	$1.3x10^{-5}$
9									$1.95x10^{-11}$	$2.28x10^{-11}$	$1.95x10^{-11}$	$5.37x10^{-11}$
10										0.002	NS	$4.53x10^{-5}$
11											0.025	0.98
12												$5.68x10^{-4}$
13												

4(b)

	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	NS	$1.41x10^{-7}$	$4.6x10^{-5}$	$1.41x10^{-7}$	$1.05x10^{-9}$	$6.7x10^{-9}$	$4.47x10^{-10}$	0.002	$1.95x10^{-11}$	$4.6x10^{-5}$	$2.03x10^{-11}$
2	$1.97x10^{-11}$	$1.95x10^{-11}$	1.0510^{-9}	$1.95x10^{-11}$	$1.95x10^{-11}$	$4.12x10^{-6}$	$1.6x10^{-4}$	$9.7x10^{-11}$	NS	$1.05x10^{-9}$	0.84
3	$1.29x10^{-6}$	$1.95x10^{-11}$	0.51	$1.95x10^{-11}$	$1.95x10^{-11}$	0.98	0.23	0.02	$1.7x10^{-8}$	0.51	$1.34x10^{-5}$
4	$9.7x10^{-11}$	0.23	$1.95x10^{-11}$	0.23	NS	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.97x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$
5	$3.49x10^{-11}$	0.025	$1.95x10^{-11}$	0.02	0.98	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$
6	0.51	$4.06x10^{-6}$	$1.29x10^{-6}$	$4.06x10^{-6}$	$1.79x10^{-8}$	$4.4x10^{-10}$	$5.3x10^{-11}$	$4.5x10^{-5}$	$1.95x10^{-11}$	$1.29x10^{-6}$	$1.96x10^{-11}$
7	0.007	$5.68x10^{-4}$	$1.8x10^{-8}$	$5.6x10^{-4}$	$1.28x10^{-6}$	$3.4x10^{-11}$	$2.1x10^{-11}$	$4.1x10^{-7}$	$1.95x10^{-11}$	$1.8x10^{-8}$	$1.95x10^{-11}$
8	0.08	$4.53x10^{-5}$	1.4310^{-7}	$4.53x10^{-5}$	$1.41x10^{-7}$	$9.7x10^{-11}$	$2.6x10^{-11}$	$4.0x10^{-6}$	$1.95x10^{-11}$	$1.43x10^{-7}$	$1.95x10^{-11}$
9	$1.79x10^{-8}$	NS	$2.1x10^{-11}$	NS	0.517	$1.95x10^{-11}$	$1.95x10^{-11}$	$3.48x10^{-11}$	$1.95x10^{-11}$	$2.1x10^{-11}$	$1.95x10^{-11}$
10	$1.79x10^{-8}$	$1.95x10^{-11}$	0.007	$1.95x10^{-11}$	$1.95x10^{-11}$	NS	NS	$1.6x10^{-4}$	$1.29x10^{-6}$	0.007	0.002
11	0.002	$2.28x10^{-11}$	NS	$2.28x10^{-11}$	$1.96x10^{-11}$	0.007	$1.6x10^{-4}$	NS	$9.70x10^{-11}$	NS	$1.80x10^{-8}$
12	$1.42x10^{-7}$	$1.95x10^{-11}$	0.08	$1.95x10^{-11}$	$1.95x10^{-11}$	NS	0.84	0.002	$1.42x10^{-7}$	0.08	$1.60x10^{-4}$
13	0.08	$5.37x10^{-11}$	0.83	$5.37x10^{-11}$	$2.03x10^{-11}$	$1.58x10^{-4}$	$4.09x10^{-6}$	NS	$2.66x10^{-11}$	0.83	$1.05x10^{-9}$
14		$1.79x10^{-8}$	$5.73x10^{-4}$	$1.79x10^{-8}$	$1.98x10^{-10}$	$4.97x10^{-8}$	$2.63x10^{-9}$	0.025	$1.95x10^{-11}$	$5.73x10^{-4}$	$2.28x10^{-11}$
15			$2.11x10^{-11}$	NS	0.51	$1.95x10^{-11}$	$1.95x10^{-11}$	$3.48x10^{-11}$	$1.95x10^{-11}$	$2.11x10^{-11}$	$1.95x10^{-11}$
16				$2.11x10^{-11}$	$1.95x10^{-11}$	0.025	$5.73x10^{-4}$	0.98	$1.98x10^{-10}$	NS	$4.97x10^{-8}$
17					0.51	$1.95x10^{-11}$	$1.95x10^{-11}$	$3.48x10^{-11}$	$1.95x10^{-11}$	$2.11x10^{-11}$	$1.95x10^{-11}$
18						$1.95x10^{-11}$	$1.95x10^{-11}$	$1.99x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$	$1.95x10^{-11}$
19							0.98	$5.73x10^{-4}$	$4.21x10^{-7}$	0.025	$5.73x10^{-4}$
20								$1.36x10^{-5}$	$1.34x10^{-5}$	$5.73x10^{-4}$	0.025
21									$3.5x10^{-11}$	0.98	$2.63x10^{-9}$
22										$1.98x10^{-10}$	0.23
23											$1.97x10^{-8}$

Note: **1**- *Absidia cylindrospora*, **2**- *Aspergillus flavus*, **3**- *A.fumigatus*, **4**-*Cladosporium herbarum*, **5**-*Gliocladium catenulatum*, **6**-*G.roseum*,**7**-*Gliocladium sp*, **8**- *Paecilomyces lilacinus*, **9**-*Penicillium canescens*, **10**- *P.chrysogenum*, **11**- *P.citrinum*, **12**-*P.expansum*, **13**-*P.lanosum*,**14** *P.oxalicum*, **15**- *P.paradoxum*, **16**- *P.sclerotiorum*, **17**- *P.simplicissimum*, **18**- *Sesquicillium candelabrum*, **19**- *Trichoderma harzianum*,**20**-*T.koningii*, **21**-*T. koningiopsis*, **22**-*T.polysporum*, **23**-*T.pseudokoningii* and **24**-*T.viride.*

4. Discussion

Overall from among the species evaluated in the study species of *Trichoderma* , *Penicillium* and *Aspergillus* showed virulent activity against the pathogen under *in vitro* condition. The highest growth inhibition of the pathogen *Colletotrichum gloeosporioides* by *Aspergillus flavus* could be attributed to the production of secondary metabolites. The fast growing antagonists caused more growth inhibition of the pathogens may be due to mycoparasitism and competition for space and nutrients. Evueh & Ogbebor (2008) had also reported that *Aspergillus sp.* lysed the cytoplasm of *C. gloeosporioides* on Potato Dextrose Agar. This could be a result of antagonism due to parasitism and (or) antibiosis as lytic activity. Similarly the inhibitory effect by *Aspergillus sp.* to several plant pathogens has also been reported by Gachomo and Kotchoni (2008). *Trichoderma* species evaluated in the study showed excellent growth inhibition against the pathogen. The plausible mechanism of antagonism employed by *Trichoderma* species includes nutrient and niche competition, antibiosis by producing volatile components and non-volatile antibiotics (Behzad *et al.,* 2008). *Penicillium species* tested in the study also showed high efficacy growth retardation of the pathogen. *Penicillium* are well known for their antagonistic activity against pathogen by producing antibiotics and induce resistance in plants by activating multiple defense signals (Hossain *et al.* 2007).

Some interactions resulted in production of a zone of inhibition, contact inhibition or no inhibition at all. The zones of inhibition produced might be due to the production of antifungal metabolites by the test antagonists (Adejumo *et al*., 1999). The mutual intermingling growth of two organisms without any zone of inhibition indicates the failure of the production of antibiotics either by the pathogen (or) by the antagonist whereas; formation of zone of inhibition is an indication for the production of antibiotic substances either by the pathogen against antagonistic fungi or vice versa (Gomathi & Ambikapathy 2011). The study is also in agreement with the findings of Begashaw (2003) who reported positive antagonistic activity of *Trichoderma, Aspergillus, Pencillium, Neosartorya* and *Fusarium* against *Colletotrichum gloeosporioides* using dual culture assay.

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5. Conclusion

The present study further highlights the effectiveness of *Trichoderma* , *Aspergillus* and *Penicillium* species as biocontrol agents. From the *in vitro* findings, it can be suggested that the antagonists such as *Aspergillus flavus, A.fumigatus, Trichoderma koningii, T.harzianum, T. pseudokoningii, T.viride, Penicillium citrinum, P. chrysogenum,* and *P.expansum* can be used as a bio-control agent against *Colletotrichum gloeosporioides*.

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