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Efficacy of antagonistic fungi for the control of *Colletotrichum lindemuthianum* in *in vitro* conditions

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Abstract

Antagonistic effect of *Aspergillus flavus, Cladosporium herbarum, Penicillium expansum* and *Trichoderma viride* against *Colletotrichum lindemuthianum* isolated from infected pods of eight French bean (*Phaseolus vulgaris* L.) varieties was studied in *in vitro* conditions by conducting dual culture and inverted plate techniques. In dual culture technique, *T. viride* and *A. flavus* revealed the highest percentage of inhibition on the mycelial growth of the pathogen, exhibiting 81% inhibition, and in inverted plate technique, 79% inhibition and 70% inhibition respectively. *P. expansum* showed an inhibitory percentage of 62% in dual culture and a low inhibition of 27% in inverted plate. The least effective antagonist was found to be *Cladosporium herbarum* which exhibited a percentage of inhibition of 38%in dual culture and 20% in inverted plate. *T. viride* and *A. flavus* were found to exhibit the maximum potentiality to suppress the mycelial growth of the pathogen and can be further exploited for controlling the disease at a commercial scale.

Keywords: *Antagonistic fungi, Colletotrichum lindemuthianum, French bean, In vitro conditions*

1. Introduction

French bean or common bean (*Phaseolus vulgaris* L.) is an important leguminous crop which among major food legumes, is third in importance, has broadest genetic base and is grown and consumed in almost every part of the world (Broughton *et al*., 2003). Annually, a large quantity of the crop is being lost or spoiled due to fungal infection both in terms of quality as well as quantity. Bean anthracnose is a major disease of French beans, caused by the fungus *Colletotrichum lindemuthianum*, which results in serious crop loss in many parts of the world. The infected seeds are the most important means of dissemination of this pathogen, hence its worldwide distribution (Mudawi *et al*., 2009). The crop is vulnerable to the attack of the pathogen at all the stages, from seedling to maturity, depending on the prevalence of favourable environmental conditions that are essential for the initiation and further development of the disease (Padder *et al.*, 2011). The high variability of *C. lindemuthianum* has resulted in continuous breakdown of resistance in commercial cultivars which has complicated the use of host resistance genes (Kelly *et al*., 1994; Melotto *et al*., 2000) and has made it difficult to develop or design effective anthracnose control strategies.

The use of microbial biocontrol agents have been considered a more natural and environmentally acceptable alternative approach to the existing chemical treatment methods for controlling fungal diseases in plants because it is ecofriendly in nature and safe to use in agricultural system to increase crop productivity since the action of such microbes is highly specific and cost effective. Several fungal species, such as species of *Alternaria, Aspergilluis, Cladosporium, Coniothyrium, Curvularia, Gilocladium, Fusarium, Metarhizium, Penicillium, Phoma, Phytophthora* and *Trichoderma* were found to be effective biocontrol agents (Feng, 2008). These biocontrol agents produce growth inhibitory substances called toxins or phytoxins or antibiotics as well as biologically active volatile substances which inhibit the growth of other fungi (Aktar *et al.*, 2014; Campbell, 1989).World's leading plant pathologists have been examining the use of antagonistic microorganisms and natural fungicides for plant protection that's safe, economical, and

effective (Chincholkar *et al*., 2004). In view of this, the present investigation is proposed to study the biological control of *Colletotrichum lindemuthianum*, the causal organism of anthracnose disease of French bean (*Phaseolus vulgaris* L.) and to generate important data for controlling the menace of fungal attack.

2. Materials and Methods

For the present investigation, infected pods of eight varieties of French bean namely, Phyrngop, Manipur, Naga Local, FB 19, FB 61, FB62, Director-1 and Director-3 were collected from Ri Bhoi District (ICAR, Umiam), Meghalaya. The collected pods were stored in storage bags under laboratory conditions. For isolation of the fungal pathogen (*C. lindemuthianum)* [Table 1] from the different varieties of French bean, potato dextrose agar medium was used and pure cultures of the isolates were maintained for further studies. The fungal species selected as antagonistic fungi against *C. lindemuthianum* are *Trichoderma viride*, *Aspergillus flavus*, *Penicilium expansum* and *Cladosporium herbarum.*

Table 1: *C. lindemuthianum* isolated from infected pods of eight varieties of French bean

Varieties	Pathogen strains
Phyrngop	DS 1
Manipur	DS 2
Naga Local	DS 3
FB 19	DS 4
FB 61	DS 5
FB 62	DS 6
Director 1	DS 7
Director 3	DS 8

2.1 Evaluation of antagonistic fungi against *C. lindemuthianum*

The colony interaction between the pathogen and the antagonistic fungi was evaluated by following dual culture technique by Skidmore and Dickinson (1976). The cultures were incubated at 23ºC for 7 days, after which, the linear growth of the pathogen and each antagonist from the centre of the disc towards the centre of the plate was recorded. Percentage of inhibition of antagonists on the growth of pathogen was calculated by using the formula of Vincent (1947).

$$\text{Percentage of inhibition (\%)} = \frac{R1 - R2}{R1} \times 100$$

where, R1= Radius of control plate, R2 = Radius of test plate

2.2 Evaluation of volatile metabolites produced by antagonistic fungi

The effect of volatile metabolites produced by antagonistic fungi on the growth of pathogen was evaluated by following the inverted plate technique by Dennis and Webster (1971a) [Plate 1]. Petriplates without antagonists served as control. For each treatment the colony size of the pathogen was recorded. Percentage of inhibition was calculated by using the above given formula.

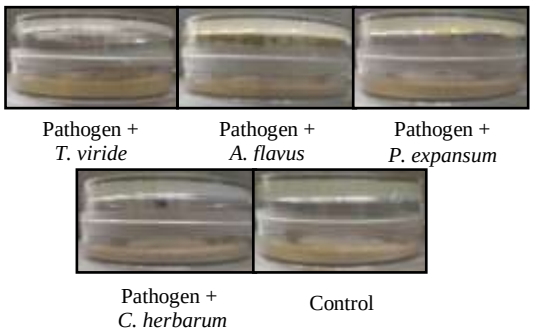

Plate 1: Inverted Plate Method

All experiments were performed in triplicate. To evaluate the significant variation ($p < 0.05$) between pathogen strains and between antagonistic fungi in both the techniques, ANOVA analysis was done with the SPSS statistics software.

3. Results

3.1 Effect of the antagonistic fungi on the radial growth of the pathogen

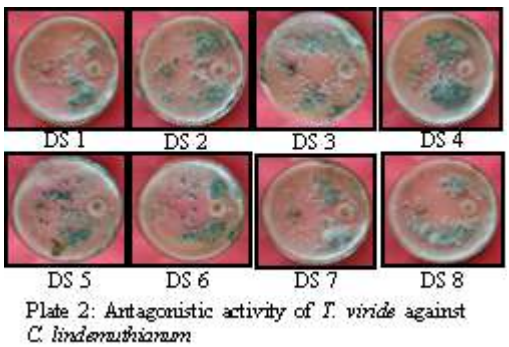

Plate 2: Antagonistic activity of *T. viride* against *C. lindemuthianum*

The colony interaction between *T. viride* and the pathogen (*C. lindemuthianum*) exhibited maximum percentage of inhibition on the mycelial growth of the pathogen [Table 2], [Fig.1]. Among the

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pathogen strains, the interaction between *T. viride* and DS 1 displayed the highest percentage of inhibition of 81% trailed by DS 3 (80%). The lowest percentage of inhibition was recorded in DS 5 (76%). The inhibition zone and intermingled zone were found to be absent in all the interactions [Plate 2].

Table 2: Effect of the antagonistic fungi on the radial growth of the pathogen (*C. lindemuthianum*) in dual culture method

Parameters	Biocontrol agents		Pathogen strains							
			DS 1	DS 2	DS 3	DS 4	DS 5	DS 6	DS 7	DS 8
Radial growth (cm)	*T. viride*	R1	3.4	3.5	3.3	3.4	3.35	3.6	3.3	3.4
		R2	0.7	0.8	0.7	0.8	0.7	0.8	0.8	0.7
	A. flavus	R1	3.4	3.5	3.3	3.4	3.35	3.6	3.3	3.4
		R2	1	0.7	1.1	0.9	1	1.3	0.7	1.3
	P. expansum	R1	3.4	3.5	3.3	3.4	3.35	3.6	3.3	3.4
		R2	2.3	2.1	1.9	2.6	1.7	2.3	1.3	1.4
	C. herbarum	R1	3.4	3.5	3.3	3.4	3.35	3.6	3.3	3.4
		R2	2.5	2.4	2.4	2.1	2.3	2.4	2.2	2.3
% of inhibition	*T. viride*		81	79	80	79	76	79	77	79
	A. flavus		71	81	68	72	63	75	80	62
	P. expansum		32	40	42	51	31	30	62	59
	C. herbarum		28	31	28	34	28	27	34	32
Inhibition zone (cm)	*T. viride*		–	–	–	–	–	–	–	–
	A. flavus		–	–	–	–	–	–	–	–
	P. expansum		0.6	0.5	0.4	0.4	0.8	0.3	0.5	0.5
	C. herbarum		0.4	0.2	0.2	0.2	0.2	0.1	0.3	0.4
Inter-mingled zone (cm)	*T. viride*		–	–	–	–	–	–	–	–
	A. flavus		2.5	2	–	–	1.5	–	–	1
	P. expansum		–	–	–	–	–	–	–	–
	C. herbarum		–	–	–	–	–	–	–	–

N.B.: '-' = absent

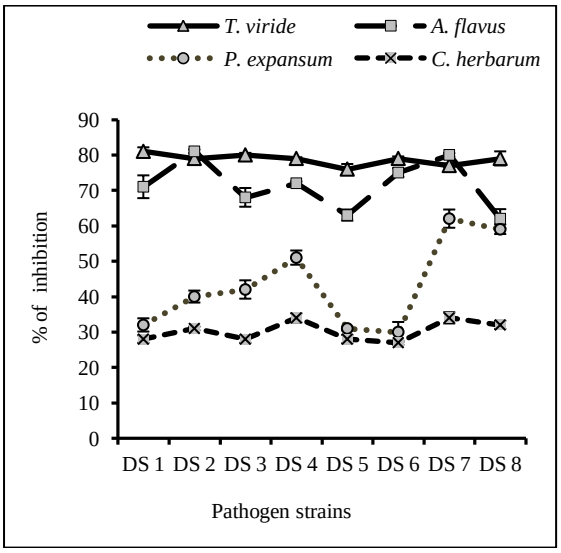

Fig. 1 Inhibitory effect (%) of the antagonistic fungi against the pathogen strains from the French bean varieties

The inhibitory effect of *A. flavus* on the pathogen came second to that of *T. viride*. The percentage of inhibition was found to be highest in DS 2 (81%) trailed by DS 7 (80%) and the lowest was observed in DS 8 (62%). The interaction between *A. flavus* and the pathogen did not indicate any inhibition zone in all the strains, however, the intermingled zone ranging from 1-2.5 cm was observed in some of the strains [Plate 3].

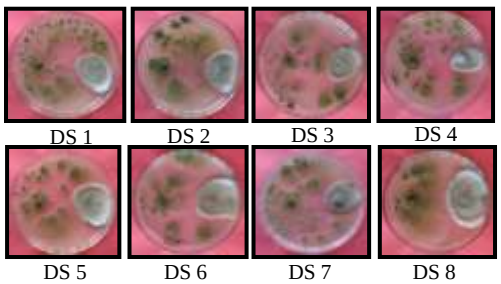

Plate 3: Antagonistic activity of *A. flavus* against *C. lindemuthianum*

The percentage of inhibition of *P. expansum* was found to be lower than that of *T. viride* and *A. flavus*. The interaction between *P. expansum* and the pathogen was observed to be the highest in DS 7 (62%) and lowest in DS 6 (30%). The interaction between *P. expansum* and the pathogen showed no intermingled zone, whereas, zone of inhibition

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ranging from 0.3-0.8 cm was observed in all the strains [Plate 4].

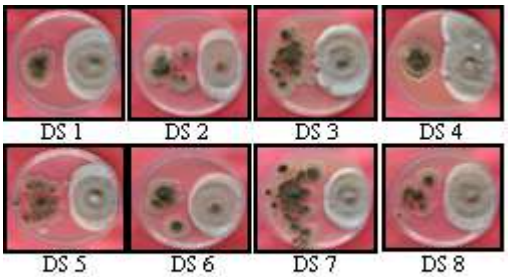

Plate 4: Antagonistic activity of *P. expansum* against *C. lindemuthianum*

C. herbarum exhibited the lowest percentage of inhibition on the pathogen. The highest percentage of inhibition was recorded in the interaction between *C. herbarum* and DS 4 as well as DS 7 (34%) and the lowest in DS 6 (27%). All of the interactions lacked the intermingled zone, whereas, the inhibition zone was observed in all the interactions ranging from 0.1-0.4cm [Plate 5].

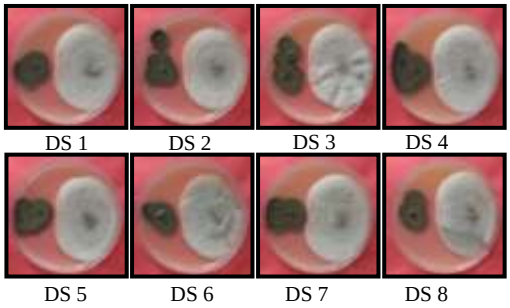

Plate 5: Antagonistic activity of *C. herbarum* against *C. lindemuthianum*

3.2 Effect of volatile metabolites produced by antagonistic fungi

The volatile metabolites produced by *T. viride* caused a significant inhibition on the mycelial growth of DS 8 (79.31%) and the percentage of inhibition was recorded to be the lowest in DS 6 (43.44%) [Fig.2], [Plates 6-13]. The highest mycelial growth inhibition of volatile metabolites produced by *A. flavus* was also observed in DS 8 (70.55%) and lowest in DS 7 (25.47%). In *P. expansum*, the highest growth inhibition was observed in DS 1 (26.61%) and the lowest was also recorded in DS 7 (11.59%). The volatile metabolites produced by *C. herbarum* exhibited the highest mycelial growth inhibition in DS 1 (19.48%), while, the lowest inhibitory effect was observed in DS 7 (10.28%).

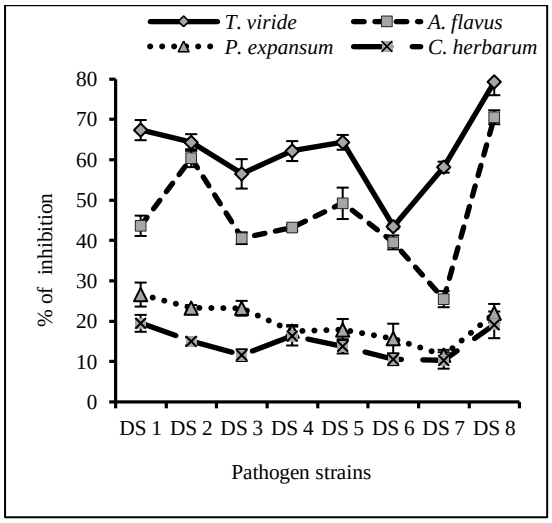

Fig. 2 Inhibitory effect (%) of the volatile metabolites against the pathogen strains from the French bean varieties

3.3 Statistical analysis

The two-way analysis of variance (ANOVA) of the colony interaction between the antagonistic fungi and the pathogen, showed a significant variation ($p<0.05$) of 4.21×10^{-5} between the pathogen strains and 1.83×10^{-37} between the antagonists [Table 3.]. In the ANOVA analysis of the effect of volatile metabolites produced by the antagonistic fungi, a significant variation ($p<0.05$) of 8.10×10^{-12} between the pathogen strains and a significant variation ($p<0.05$) of 2.22×10^{-38} between antagonists was observed.

Table 3. Two-way analysis of variance (ANOVA) of colony interaction and effect of volatile metabolites between pathogen strains and antagonistic fungi ($p<0.05$)

Parameters	Colony interaction		Effect of volatile metabolites	
	F-ratio	p level	F-ratio	p level
Pathogen strains	5.645	4.21×10^{-5}	15.736	8.10×10^{-12}
Antagonistic fungi	296.351	1.83×10^{-37}	317.994	2.22×10^{-38}
Pathogen strains x Antagonistic fungi	4.46	1.88×10^{-6}	3.44	7.33×10^{-5}

The Tukey's Post Hoc Test that was performed between the antagonists [Table 3.1], revealed significant variation ($p=<0.05$) between all the antagonists in both the techniques.

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Table 3.1 Tukey's Post Hoc Test of colony interaction and effect of volatile metabolites between antagonistic fungi ($p<0.05$)

Biocontrol agents	p level	
	Colony interaction	Effect of volatile metabolites
T. viride* x *A. flavus	0.001	$1.53x10^{-11}$
T. viride* x *P. expansum	$5.82x10^{-13}$	$5.82x10^{-13}$
T. viride* x *C. herbarum	$5.82x10^{-3}$	$5.82x10^{-13}$
A. flavus* x *P. expansum	$5.82x10^{-13}$	$5.82x10^{-13}$
A. flavus* x *C. herbarum	$5.82x10^{-13}$	$5.82x10^{-13}$
P. expansum* x *C. herbarum	$8.54x10^{-7}$	0.024

The variation between the pathogen strains was also determined by Tukey's Post Hoc Test, for both the techniques. In the colony interaction, a significant variation ($p<0.05$) was observed between DS 4 and DS 5. DS 7 showed significant variaion ($p<0.05$) with DS 1, DS 3, DS 5 and DS 6 [Table 3.2]. In the effect of volatile metabolites, a significant variation ($p<0.05$) was observed between DS 1 and DS 6, DS 7, DS 8. DS 2 also showed a significant variation ($p<0.05$) with DS 6 and DS 7. A significant variation ($p<0.05$) was recorded between DS 3 and DS 8. DS 4 revealed a significant variation ($p<0.05$) with DS 7 and DS 8.

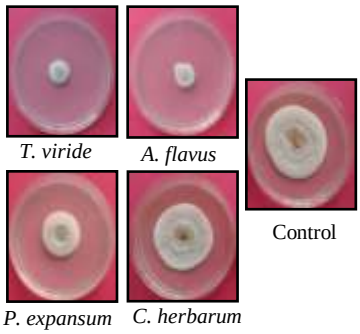

Plate 6: Effect of the volatile metabolites against the pathogen strain DS 1

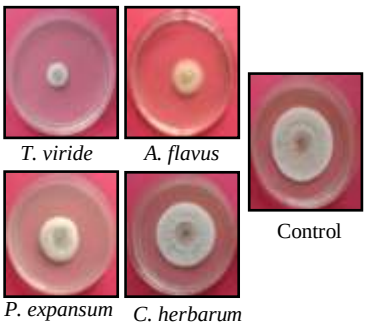

Plate 7: Effect of the volatile metabolites against the pathogen strain DS 2

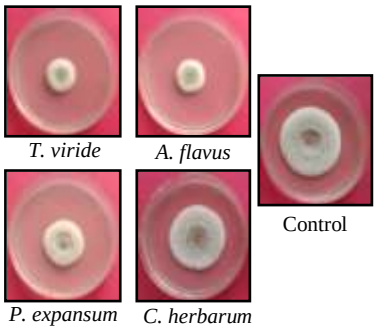

Plate 8: Effect of the volatile metabolites against the pathogen strain DS 3

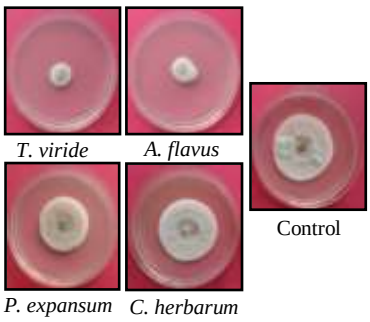

Plate 9: Effect of the volatile metabolites against the pathogen strain DS 4

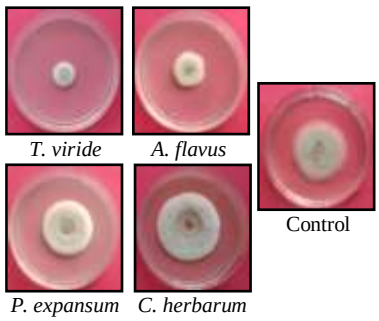

Plate 10: Effect of the volatile metabolites against the pathogen strain DS 5

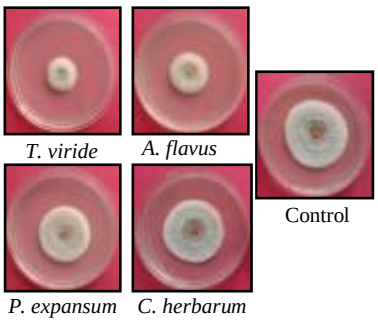

Plate 11: Effect of the volatile metabolites against the pathogen strain DS 6

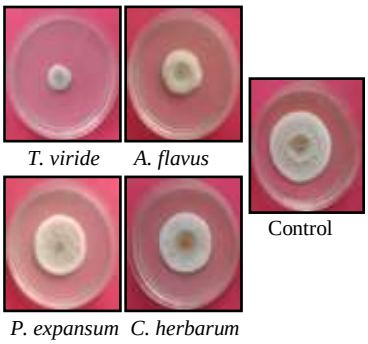

Plate 12: Effect of the volatile metabolites against the pathogen strain DS 7

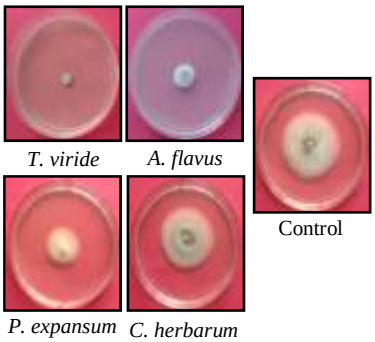

Plate 13: Effect of the volatile metabolites against the pathogen strain DS 8

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Table 3.2: Tukey's Post Hoc Test of colony interaction between pathogen strains ($p<0.05$)

Pathogen strains	DS 2	DS 3	DS 4	DS 5	DS 6	DS 7	DS 8
DS 1	NS	NS	NS	NS	NS	$9.23x10^{-4}$	NS
DS 2		NS	NS	NS	NS	NS	NS
DS 3			NS	NS	NS	0.038	NS
DS 4				0.025	NS	NS	NS
DS 5					NS	$1.73x10^{-5}$	NS
DS 6						0.002	NS
DS 7							NS

NS = Not significant

Table 3.3: Tukey's Post Hoc Test of effect of volatile metabolites between pathogen strains ($p<0.05$)

Pathogen strains	DS 2	DS 3	DS 4	DS 5	DS 6	DS 7	DS 8
DS 1	NS	NS	NS	NS	$3.01x10^{-4}$	$7.86x10^{-5}$	0.027
DS 2		NS	NS	NS	$3.55x10^{-5}$	$8.70x10^{-6}$	NS
DS 3			NS	NS	NS	NS	$4.60x10^{-6}$
DS 4				NS	NS	0.028	$7.53x10^{-5}$
DS 5					0.015	0.005	$6.21x10^{-4}$
DS 6						NS	$5.57x10^{-10}$
DS 7							$1.26x10^{-10}$

NS = Not significant

DS 5 showed a significant variation ($p<0.05$) with DS 6, DS 7 and DS 8. A significant variation ($p<0.05$) between DS 8 and DS 6, DS 7 was also observed [Table 3.3]. The variation between the other pathogen strains was found to be insignificant.

4. Discussion

The antagonistic effect of the selected antagonistic fungi viz, *T. viride*, *A.flavus*, *P. expansum* and *C. herbarum* against the pathogen under *in vitro* conditions was evident by the inhibiton of the mycelial growth of the pathogen. *Trichoderma* species produces antibiotics, such as trichodermin, trichodermol A and harzianolide which are responsible for the inhibition of most fungal phytopathogens (Nawar, 2007). *Trichoderma*, *Aspergillus* and *Penicillium* species are also known to secrete other inhibitory substances such as viridian, gliovirin, geodin, terricin, terric acid, aspergillic acid, dermadin etc. (Mondal *et al*., 2000; Vey *et al*., 2001; Yan *et al*., 2006). The species of the genus *Cladosporium* are known to produce various biologically active compounds and metabolites (Hosoe *et al*., 2001; Jadulco *et al*., 2002) which have antifungal properties.

Among the four antagonists, *T. viride* exhibited the strongest inhibition on the mycelial growth of the pathogen followed by *A.flavus*. The maximum potentiality of *T. viride* to suppress the growth of the pathogen maybe attributed to the capability of *T. viride* in producing strong antibiotics. In inverted plate technique, *T. viride* also showed the highest mycelial growth inhibition. This result is in agreement with Mortuza (1997) who reported *T. viride* as a better antagonist in producing harmful toxic metabolites that caused a significant reduction in mycelial growth of *C. musea*. Kaur *et al*. (2006) reported that volatile metabolites from *T. viride* caused significant reduction in mycelial growth of *C. capsici*. *A. flavus* exhibited an inhibitory effect of 81% and 70.55% in dual culture and inverted plate respectively. This is in conformity with the findings of Bosah and co-workers (2010) who reported that *Aspergillus* species inhibited the growth of pathogenic fungi *Sclerotium rolfsii* with inhibition of 73.12 to 88.35%. Soltani and Hosseyni (2015) reported *Aspergillus* species as endophytes with antifungal activity and produce several metabolites such as phenolic and bioactive flavonoid compounds that inhibit the growth of other pathogenic fungi.

P. expansum and C. herbarum exhibited a comparatively weaker inhibitory effect. The degree of effectiveness varies according to the nature, quality, and quantity of antibiotics or inhibitory substances secreted by the antagonists (Kubicek *et al*., 2001; Singh, 2006). *Penicillium* species are well known for producing mycotoxins (Palumbo *et al*., 2008) and production of these toxins might have enabled the antagonist fungi to inhibit the growth of the pathogen. Hossain *et al*. (2007) reported that a number of *Penicillium* species are antagonistic to plant pathogens with a mechanism of action based on the induction of resistance and the establishment of mycoparasitic interactions (Sempere and Santamarina, 2008).

5. Conclusions

The present study revealed that, in both the techniques conducted for the *in vitro* biocontrol of *C. lindemuthianum*, *T. viride* and *A. flavus* had the strongest inhibitory effect on the growth of the pathogen, while, *P. expansum* and *C. herbarum* showed a weaker inhibitory effect on the pathogen. This suggests that *T. viride* and *A. flavus* produce and contain volatile compounds, antibiotics and inhibitory substances causing drastic reduction on the mycelial growth of the pathogen which can therefore be helpful in disease reduction by checking the survival and spread of the pathogen.

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References

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

[2] Bosah, O., Igeleke, C.A. and Omorusi, V.I. In vitro microbial control of pathogenic Sclerotium rolfsii. Int. J. Agr. Bio., 12: 474-476, (2010).

[3] Broughton, W.I, Hernandez, G., Blain, M., Beebe, S., Gepts, P. and Vanderleyden, J. Beans (Phaseolus spp.) model food legumes. Plant Soil, 252: 55 – 128, (2003).

[4] Campbell, R. Biological control of microbial plant pathogen, Cambridge University Press, Cambridge 8-24, (1989).

[5] Chincholka,r S. B. and Mukerji, K. G. Biological control of plants diseases. Haworth Food & Agricultural Products Press,Science. pp. 426, (2004).

[6] Dennis, C., and Webster, J. Antagonism properties of species groups of Trichoderma, III. Hyphal interaction. T. Brit. Mycol. Soc., 57: 363-369, (1971).

[7] Feng, S. J., Yang, M. and Zhou, D.B. Fungal and bacterial species isolated from Sclerotia of Rhizoctonia solani and their effects on the growth of R. solani. Acta Phytopathol. Sin. 38: 557-560, (2008).

[8] Hosoe, T., Okamoto, S., Nozawa, K., Kawai, K., Okada. K., Takaki, G.M., Fukushima, K. and Miyaji, M. New pentanorlanostane derivatives, cladosporide B-D, as characteristic antifungal agents against Aspergillus fumigatus, isolated from Cladosporium sp. J. Antibiot. (Tokyo) 54: 747–750, (2001).

[9] Hossain, M.M., Sultana, F., Kubota, M., Koyama, H. and Hyakumachi, M. The plant growth-promoting fungus Penicillium simplicissimum GP17-2 induces resistance in Arabidopsis thaliana by activation of multiple defense signals. Plant Cell Physiol. 48(12):1724-36, (2007).

[10] Jadulco, R., Brauers, G., Edrada, R.A., Ebe, R., Wray, V., Sudarsono, S. and Proksch, P. New metabolites from sponge-derived fungi Curvularia lunata and Cladosporium herbarum. J Nat Prod 65: 730–733, (2002).

[11] Kaur, M., Sharma, O.P. and Sharma, P.N. In vitro effect of Trichoderma species on Colletotrichum capsici causing fruit rot of chilli (Capsicum annuum L.). Indian Phytopathol. 59:243–245, (2006).

[12] Kelly, J.D., Afanador, L. and Cameron, L.S. New races of Colletotrichum lindemuthianum in Michigan and implications in dry bean resistance breeding. Plant Dis. 78: 892 – 894, (1994).

[13] Kubicek, C.P., Mach, R.L., Peterbauer, C.K. and Lorito, M. Trichoderma: From genes to biocontrol. J. Plant Pathol. 83:11-23, (2001).

[14] Melott,o A., Balardin, R.S. and Kelly, J.D. Host–pathogen interaction and variability of Colletotrichum lindemuthianum. In: Prusky D, Freeman S and Dickman MB (eds) Colletotrichum; Host Specificity, Pathology, and Host–Pathogen Interaction; APS Press, St. Paul Minnesota. pp 346 – 361, (2000).

[15] Mondal, G., Dureja, P. and Sen, B. Fungal metabolites from Aspergillus niger AN27 related to plant growth promotion. Indian J. Exp. Bio. 38:84-7, (2000).

[16] Mortuza, M.G. Effects of antagonists, alum and ultraviolet irradiation on major fruit rots of banana [Musa sapientum (L.) Kuntze]. Philippines: Languna College. p. 138, (1997).

[17] Mudawi, H.I., Idris, M.O. and Mustafa, M.A. E. Balla, U. of K. J. Agric. Sci. 17: 118 -130, (2009).

[18] Nawar, L. S. Pathological and rhizospherical studies on rootrot disease of squash in Saudi Arabia and its control. Afric. J. of Biotech. 6: 219-226, (2007).
[19] Palumbo , P. and O'keeffe, T. L. Microbial interactions with mycotoxigenic fungi and mycotoxins. Toxin Rev. 27: 261–285, (2008).
[20] Padder, B.A., Sharma, P.N., Sharma ,O.P. and Kapoor, V. Genetic diversity and gene flow estimates among five populations of Colletotrichum lindemuthianum across Himachal Pradesh – a north-western state of India. Physiol. Mol. Plant. Pathol. 70: 1 – 8, (2007).
[21] Sempere, F. and Santamarina, M.P. Suppression of Nigrospora oryzae (Berk. & Broome) Petch by an aggressive mycoparasite and competitor, Penicillium oxalicum Currie & Thom. Int. J. Food Microbiol**.** 122(1–2)**:** 35-43**,** (2008).
[22] Skidmore, A. M. and Dickinson, C. M. Colony interactions and hyphal interference between Sepatoria nodorum and phylloplane fungi. Trans. Br. Mycol. Soc. 66: 57 -64, (1976).
[23] Singh, H.B. Trichoderma: A boon for biopesticides industry. J. Mycol. Pl. Pathol., 36:373-384, (2006).
[24] Soltani, J. and Hosseyni Moghaddam, M.S. Fungal endophyte diversity and bioactivity in the Mediterranean cypress Cupressus sempervirens. Curr Microbiol. 70(4):580-6, (2015).
[25] Vey, A., Hoagland, R.E. and Butt, T.M. Toxic metabolites of fungal biocontrol agents. Progress, problems and potential. CAB international, Brisol. pp. 311-346, (2001).
[26] Vincent, J.H. Distortion of fungal hyphae in the presence of certain inhibitors. Nature.15:850, (1947).
[27] Yan, X.S., Quing-Tao, S., Shu-Tao, X., Xiu-Lan, C,, Cai-Yun, S. and Yu-Zhong, Z. Broad spectrum antimicrobial activity and high stability of trichokonins from Trichoderma koningii SMF2 against plant pathogens. FEMS Microbial Lett., 260: 119-125, (2006)