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ANTAGONISTIC POTENTIAL OF SOIL FUNGI AGAINST POST-HARVEST PATHOGENIC FUNGI OF *MUSA SAPIENTUM* L.

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Abstract

The antagonistic potential of four soil fungi *viz., Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem and *Trichoderma viride* Pers. ex Gray against five pathogens of *Musa sapientum* L. were evaluated by "dual culture colony interaction" and volatile and non-volatile metabolites. In "dual culture colony interaction", out of four soil fungi, *T. viride* showed the highest growth inhibition on *Colletotrichum musae* (Berk. & Curt.) Arx (58.33%), *Curvularia brachyspora* Boedijn. (61.67%), *Fusarium semitectum* Berk. & Rav. (62.50%), *Fusarium* sp. Link (64.17%) and *Pestalotiopsis disseminata* (Thum) Steyaert (51.54%). The inhibition of radial growth of *C. musae* (45.41%), *C. brachyspora* (54.87%), *F. semitectum* (65.76%), *Fusarium* sp. (58.69%) and *P. disseminata* (75.56%) were observed in case of *T. viride* owing to the volatile metabolites. The inhibition of radial growth of *C. musae* (84.21%), *C. brachyspora* (73.33%), *F. semitectum* (85.71%) and *Fusarium* sp. (71.43%) were observed in case of *Aspergillus niger* and the maximum inhibition of radial growth of *P. disseminata* (77.37%) was found in case of *T. viride* owing to the effect of non-volatile metabolites. The present investigation suggests that *T. viride* and *A. niger* may be exploited commercially as a biocontrol agent against the pathogens of infected fruits of different varieties of *M. sapientum*.

Introduction

Banana (*Musa sapientum* L.) is one of the most popular fruits of the world belongs to Musaceae (Siddiqui *et al.* 2007). The United Nations Food and Agriculture Organization rank bananas as the world's fourth most important crop after the major cereals (Anon. 2004). In Bangladesh, total estimated production of banana was 801000 metric tons and cultivated area was 131 acres in 2010-2011. Various biotic and abiotic stresses are responsible for the lower production of banana. Among them fungal diseases play an important role. Over 25 to 30 per cent loss of banana caused by several fungi in post-harvest stage was estimated (Defosent 1933).

Over the past few decades, agricultural production has expanded and farmers have increasingly relied on chemical pesticides as a relatively dependable method of protecting plants against pathogens. However, the increasing use of chemical pesticides negatively affects the environment and human health. Biological control has been proposed as a replacement for chemical control of plant diseases (Compant *et al.* 2005, Gerhardson 2002, Harman 2000). It represents a natural and ecological approach that reduces chemical inputs and their effects on the environment. Biological control can be safer for humans, crops and the environment. It also has the potential to be more stable and longer-lasting than other control measures and is compatible with the concepts and goals of integrated pest management and sustainable agriculture.

Use of antagonist as biological control agent has now become one of the most exciting and rapidly developing areas in plant pathology. Presently, different species of *Trichoderma* are used to control different plant pathogens (Costa *et al.* 2005). In Bangladesh, research on biological control of fungal diseases of banana fruits is very limited. So, for the sake of economy we need

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more information on this regard. Considering the importance of this popular fruit the present investigation was undertaken to find out the biological efficacy of antagonistic fungi against the pathogens associated with postharvest diseases of banana varieties.

Materials and Methods

Three infected varieties (Sabri, Champa and Sagar Kola) of *Musa sapientum* L. having characteristic symptoms were collected from three different Upazila, namely Shibpur, Belabo and Polash of Narsingdi district during May to April, 2016. Samples were collected from local market in ripened stage in separate sterile polyethylene bags, labeled properly and then brought to the laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka for isolating associated fungi following "Tissue planting method" on PDA medium (Islam and Shamsi 2016). As surface sterilizer 10% chlorox solution was used.

Antagonistic fungi were isolated from rhizosphere soil of the host varieties following serial dilutionn method. Among the isolated soil fungi, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *Trichoderma viride* were selected to test their antagonistic potential 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). The results were statistically analyzed by "t" test following Steel and Torrie (1960).

Results and Discussion

A total of eleven fungal species were isolated from the infected fruits of three banana varieties *viz.*, Sabri, Champa and Sagar. The isolated fungi were *Aspergillus flavus, A. fumigatus, A. niger, Colletotrichum musae, Curvularia brachyspora, Fusarium semitectum, Fusarium* sp., *Penicillium* sp., *Pestalotiopsis disseminata*, *Rhizopus* sp. and *Trichoderma viride*. Among the isolated fungi *C. musae*, *Curvularia brachyspora*, *F. semitectum*, *Fusarium* sp. and *Pestalotiopsis disseminata* were selected as test pathogens owing to their higher percentage of occurrence in the diseased fruits.

The results of colony interactions have been summarized in Table 1 and Fig. 1. In this study, antagonistic relationships (Grade) among the soil fungi and test pathogens were 2 and 4. However, grade 2 was found to be the most commonly encountered type of colony interaction as 14 interactions were incorporated in this grade which was followed by grade 4 (6 out of 20) (Table 1).

Table 1. Grades of colony interaction between the test pathogens and antagonists.

Name of antagonists	Name of test pathogens				
	Colletotrichum musae	*Curvularia brachyspora*	*Fusarium semitectum*	*Fusarium* sp.	*Pestalotiopsis disseminata*
Aspergillus flavus	2	2	2	2	4
A. fumigatus	4	2	4	4	4
A. niger	2	2	2	4	2
Trichoderma viride	2	2	2	2	2

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

In "dual culture colony interaction" *T. viride* showed the highest (58.33%) growth inhibition on *C. musae* which was followed by *A. flavus* (48.50%), *A. fumigatus* (45.45%) and *A. niger* (44.44%). *Trichoderma viride* showed the highest growth (61.67%) inhibition on *C. brachyspora* which was followed by *A. niger* (60%), *A. flavus* (44.44%) and *A. fumigatus* (40%) (Fig. 2). *Trichoderma viride* showed the highest (64.17%) growth inhibition on *Fusarium* sp. which was followed by *A. flavus* (62.65%), *A. niger* (58.33%) and *A. fumigatus* (50%). *Trichoderma viride* showed the highest (62.50%) growth inhibition on *F. semitectum* which was followed by *Aspergillus niger* (54.65%), *A. fumigatus* (50%) and *A. flavus* (48.52%). *Trichoderma viride* showed the highest (51.54%) growth inhibition on *P. disseminata* which was followed by *A. fumigatus* (50.87%), *A. niger* (46.67%) and *A. flavus* (36.36%).

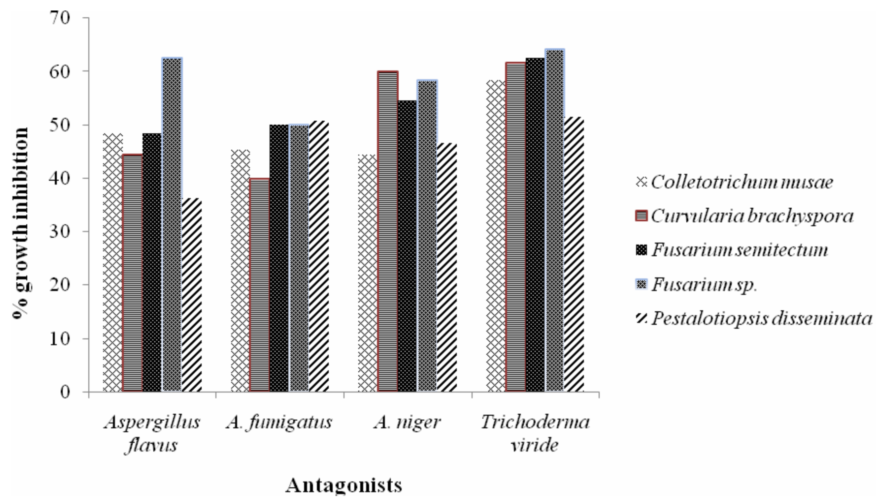

Fig. 1. Per cent inhibition of *Colletotrichum musae*, *Curvularia brachyspora, Fusarium semitectum*, *Fusarium* sp. and *Pestalotiopsis disseminata* owing to fungal antagonists.

In contrast to the present study, Akter *et al.* (2014) reported that in “dual culture colony interaction” *A. niger, T. viride, A. flavus* and *A. fumigatus* showed 68.66, 57.24, 54.19 and 50.25% growth inhibition on *Colletotrichum* sp., respectively. Again *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 75.87, 75.5, 51.78 and 45.52% growth inhibition on *C. lunata*, respectively. Further, *T. viride*, *A. niger, A. flavus* and *A. fumigatus* showed 56.52, 50.70, 47.36 and 46.15% growth inhibition on *F. semitectum*, respectively. Bashar and Chakma (2014) reported that in “dual culture colony interaction” *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* showed 65.21, 64.24, 57.14 and 34.78% growth inhibition on *F. oxysporum*, respectively. Tapwal *et al.* (2015) reported that in “dual culture colony interaction” *T. viride* showed 12.50% growth inhibition on *C. gloeosporioides*. The same antagonists also showed different effects on different fungi in the present investigation. This variation might be owing to selection of different test pathogens.

The results of volatile metabolites of antagonistic fungi against test pathogens are presented in Table 2. The maximum inhibition of radial growth of *C. musae* was observed in *T. viride* (45.41%) followed by *A. fumigatus* (29.63%), *A. flavus* (22.22%) and *A. niger* (20.40%) due to the volatile metabolites after 6 days of incubation at 25 ± 2˚C. The maximum inhibition of radial growth of *C. brachyspora* was observed in *T. viride* (54.87%) followed by *A. niger* (40%), *A. flavus* (36%) and *A. fumigatus* (10%) owing to the volatile metabolites after 6 days of incubation at 25 ± 2˚C. The maximum inhibition of radial growth of *F. semitectum* was also observed in *T.*

viride (58.69%) followed by *A. fumigatus* (48.24%), *A. niger* (37.93%) and *A. flavus* (34.48%) owing to the volatile metabolites after 6 days of incubation at 25±2˚C. The maximum inhibition of radial growth of *Fusarium* sp. was observed in *T. viride* (65.76%) which was followed by *A. niger* (57.14%), *A. fumigatus* (54.26%) and *A. flavus* (50%) owing to the volatile metabolites after 7 days of incubation at 25±2˚C. The maximum inhibition of radial growth of *P. disseminata* was also observed in case of *T. viride* (75.56%) which was followed by *A. flavus* (70.42%), *A. niger* (69.10%) and *A. fumigatus* (59.15%) owing to the volatile metabolites after 7 days of incubation at 25±2˚C.

Table 2. Per cent inhibition of radial growth of the test pathogens by volatile metabolites of antagonistic fungi.

Name of antagonists	% inhibition of radial growth of the test pathogens				
	Colletotrichum musae	*Curvularia brachyspora*	*Fusarium semitectum*	*Fusarium* sp.	*Pestalotiopsis disseminata*
Aspergillus flavus	22.22	36.00	50.00	34.48	70.42
A. fumigatus	29.63	10.00	54.28	48.28	59.15
A. niger	20.40	40.00	57.14	37.93	69.10
Trichoderma viride	45.41	54.87	65.76	58.69	75.56

In contrast to the present study, Aktar *et al*. (2014) reported that volatile metabolites produced by an isolate of *A. niger*, *A. flavus*, *A. fumigatus* and *T. viride* inhibited the mycelial growth of *Colletotrichum* sp. by 14.68, 11.78, 11 and 11% , respectively. Again the volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *Curvularia lunata* by 20.86, 14.85, 10.5 and 14.85%, respectively. Further the volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *F. semitectum* by 13.5, 9.5, 8 and 7.75%, respectively. 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. Thakur and Harsh (2014) reported that volatile metabolites produced from the culture of *A. niger* showed 42.43% inhibition of mycelial growth of *C. gloeosporioides*. Differences in per cent 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 *C. musae*, *C. brachyspora*, *F. semitectum*, *Fusarium* sp. and *P. disseminata*. The maximum inhibition of radial growth of *C. musae* was observed with the culture filtrates of *A. niger* (84.21%) which was followed by *A. fumigatus* (75%), *T. viride* (63.16%) and *A. flavus* (55.56%) at 20% concentration. The maximum inhibition of radial growth of *C. brachyspora* was observed with the culture filtrates of *A. flavus* and *A. niger* (73.33%) which was followed by *T. viride* (57.65%) and *A. fumigatus* (54.55%) at 20% concentration. The highest inhibition of radial growth of *Fusarium* sp. was observed with the culture filtrates of *A. niger* (71.43%) which was followed by *A. flavus* (61.90%), *T. viride* (57.72%) and *A. fumigatus* (52.17%) at 20% concentration. The maximum inhibition of radial growth of *F. semitectum* was observed with the culture filtrates of *A. niger* (85.71%) which was followed by *T. viride* (80%), *A. flavus* (44.83%) and *A. fumigatus* (44%) at 20% concentration. The maximum inhibition of radial growth of *P. disseminata* was observed with the culture filtrates of *T. viride* (77.37%) which was followed by *A. flavus* (76.06%), *A. niger* (68.52%) and *A.*

fumigatus (58.76%) at 20% concentration. The inhibition of the pathogen increases with the increase of the concentration of the culture filtrates in culture medium.

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

Name of fungi	Concen-tration (%)	% inhibition of radial growth of test pathogens by non-volatile metabolites owing to different antagonists			
		A. flavus	*A. fumigatus*	*A. niger*	*Trichoderma viride*
Colletotrichum musae	5	22.22[b]	53.57[b]	52.63[b]	17.89[NS]
	10	29.63[b]	60.71[a]	63.16[b]	36.84[c]
	15	40.74[a]	71.43[a]	71.05[a]	60.53[b]
	20	55.56[a]	75.00[a]	84.21[a]	63.16[c]
Curvularia brachyspora	5	44.44[b]	22.73[c]	53.33[b]	17.65[NS]
	10	62.22[a]	40.91[b]	63.33[a]	43.53[b]
	15	68.89[a]	75.60[a]	86.66[a]	51.21[b]
	20	73.33[a]	54.55[a]	73.33[a]	57.65[a]
Fusarium semitectum	5	20.69[c]	12.00[NS]	54.29[b]	22.85[c]
	10	31.03[b]	28.00[c]	65.71[b]	40.00[b]
	15	37.93[b]	36.00[b]	71.43[b]	54.9[b]
	20	44.83[b]	44.00[a]	85.71[a]	80.00[a]
Fusarium sp.	5	50.00[a]	17.39[NS]	47.62[b]	36.21[b]
	10	52.38[a]	39.13[c]	57.14[a]	39.66[b]
	15	57.14[a]	47.83[b]	61.90[a]	44.83[b]
	20	61.90[a]	52.17[b]	71.43[a]	57.72[a]
Pestalotiopsis disseminata	5	47.89[a]	45.95[b]	29.63[c]	33.33[b]
	10	52.11[a]	49.68[b]	51.85[b]	51.85[b]
	15	69.01[a]	54.05[a]	59.26[b]	61.11[a]
	20	76.06[a]	58.76[a]	68.52[a]	77.37[a]

a, b and c indicate significance of ‘t’ value at p = 0.001, 0.01 and 0.05, respectively. In a row, figures with same letter do not differ significantly, whereas figures with dissimilar letter differ significantly. NS = Not significant.

In contrast to the present study, Aktar *et al.* (2014) reported that non-volatile metabolites produced by an isolate of *A. niger*, *T. viride*, *A. flavus* and *A. fumigatus* inhibited mycelial growth of *Colletotrichum* sp. by 52.56, 44.72, 40 and 37.2%, respectively. Again the non-volatile metabolites produced by an isolate of *Trichoderma viride*, *Aspergillus niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *Curvularia lunata* by 60.07, 52.5, 40.32 and 28.5%, respectively. Further the non-volatile metabolites produced by an isolate of *T. viride*, *A. niger*, *A. flavus* and *A. fumigatus* inhibited the mycelial growth of *F. semitectum* by 50, 45, 8 and 7.75%, respectively. Differences in per cent inhibition with the present study might be owing to the difference in organism strains involved in the interaction. 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 owing to non-volatile metabolites.

The present investigation suggests that *Trichoderma viride* and *Aspergillus niger* may be exploited commercially as a biocontrol agent to protect postharvest decay of banana fruit varieties, namely Sabri, Champa and Sagor belong to *Musa sapientum*.

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