Antagonistic potentiality of six wheat rhizosphere fungi against leaf blight pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker

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ABSTRACT: Six wheat rhizosphere fungi (*Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp., *Trichoderma harzianum* and *T. viride*) were evaluated for their antagonistic potentiality towards sixteen isolates of *Bipolaris sorokiniana* (Sacc.) Shoemaker under different cultural groups. Colony interaction using duel culture technique, inhibition owing to volatile and non-volatile substances in different concentrations were performed. Among them *Trichoderma harzianum* showed the best performance (colony interaction 61.69-79.02%, volatile substances 44.14-61.34% and non-volatile culture filtrate 47.28-68.14% at 20% concentration) to control *Bipolaris sorokiniana* followed by *Trichoderma viride*, *Aspergillus niger*, *Aspergillus flavus*, *A. fumigatus* and *Penicillium* sp..

KEYWORDS: Bipolaris leaf blight, antagonistic fungi, radial growth, wheat.

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Introduction

Bangladesh is an over populated country. The dietary habit of people of Bangladesh has changed due to rapid urbanization and industrialization of the country and the use of numerous bakery products. So, wheat is the second staple food crop next to rice. Among the different constraints to wheat production, diseases play a vital role. Bipolaris leaf blight has become the most destructive disease of wheat in the rice-wheat cropping system in Bangladesh. Disease symptoms appeared as numerous small, circular to oval and grey-brown eye-shaped spots on green leaves. The centers of the spot soon faded, becoming light grey to straw coloured with distinct dark brown margins[1]. In Bangladesh, the disease arises in almost all wheat growing areas with unpredictable degrees of severity, causing extensive loss in yield and seed quality. *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph, *Cochliobolus sativus*) is mainly a non-specialized foliar blight pathogen and it also causes germination failure or seed rot, root rot, seedling blight, head blight and black point in wheat[3]. Soil and seed borne inoculums, crop residues, soil moisture and environmental factors play dynamic role in disease development. Rice assists as a host for the leaf blight fungi and rice stubble plays its role as a substrate for the fungi after rice harvest[4].

To control diseases chemical fungicides are widely used. But negative impacts on human health and environment and the evolution of multi-resistant strains in pathogens, use of chemical fungicides is going to be regulated[5]. Biological control has now become one of the most exciting and developing areas in plant pathology because it has great prospective to solve many agricultural problems[6]. Recently, the biological control of plant diseases by means of antagonistic fungi has acknowledged increasing attention. The use of fungi and bacteria as biocontrol agents against seed-borne pathogens of wheat has been reported to be an alternative of great potential[7, 8]. The aim of the study to evaluate the potential of antagonistic fungi species against *B. sorokiniana*.

Materials and Methods

A total of 174 *B. sorokiniana* isolates were obtained from eight districts (Dhaka, Gazipur, Dinajpur, Joypurhat, Pabna, Sirajgonj, Kushtia and Chuadanga) of Bangladesh. BpLB infected leaf samples of 21 wheat varieties were collected at grain filling stage. The fungi were isolated from the samples following "Tissue planting method" on PDA (Potato Dextrose Agar) medium[9]. Detail morphological studies was done and cultural variation was characterized on the basis of colony colour and texture on PDA medium. They were – Black Mat (B-M), Black Fluffy (B-F), Ash-Mat (A-M), Brownish Ash Fluffy (Br.A-F), Blackish Ash Mat (Bl.A-M), Whitish Ash Mat (W.A-M), Greenish Ash Fluffy (G.A-F) and Pinkish White Mat (P.W-M).

Some soil fungi were isolated from the wheat field soil following serial dilution method[10]. From the isolated soil fungi, *Aspergillus flavus* Link., *Aspergillus fumigatus* Frese., *Aspergillus niger* Tiegh., *Penicillium* sp.,

Trichoderma harzianum Refai. and *Trichoderma viride* Pers. were selected randomly to study colony interactions with *B. sorokiniana* isolates. In dual culture, assessment of colony interactions grading was done based on intermingling and inhibition zone. Per cent inhibition of the growth of *B. sorokiniana* isolates due to the presence of antagonists were also calculated[11]. Effects of volatile and non-volatile metabolites of the selected soil fungi against *B. sorokiniana* isolates were also studied following the methods describe by Dennis and Webster (1971 a, b, c) and Bashar and Rai (1994), respectively.

Results and Discussion

The mechanisms by which antagonistic microorganisms affect pathogen populations are not always clear, but they are generally attributed to one of three effects: (1) direct parasitism or lysis and death of the pathogen, (2) direct toxic effects on the pathogen by antibiotic substances released by the antagonist, and (3) indirect toxic effects on the pathogen by volatile substances, released by the metabolic activities of the antagonist[12, 13, 14]. The result of the present investigation reveals the above mention mechanisms.

Colony interaction

Antagonistic potential of the selected six soil fungi against the sixteen isolates of *B. sorokiniana* are presented in table 1. In the study antagonistic relationship ranged from grade 2 to 4. However, grade 2 was found most commonly encountered type of confrontations as 59 interactions out of 96 were incorporated in this grade, followed by grade 3 (23 out of 96) and the grade 4 was recorded occasionally (14 out of 96). Among 6 soil fungi *Aspergillus niger*, *Trichoderma harzianum* and *T. viride* showed grade 4 interaction against some isolates. Prince *et al.* (2011) noticed grade 4 interaction between *T. harzianum* and *Colletotrichum falcatum*. P. Chowdhury (2020) noticed grade 4 interaction between *T. harzianum* and 8 pathogenic fungi of rice.

Table 1. Antagonistic potential of soil fungi against *Bipolaris sorokiniana* isolates

	Group	Name of fungi	Name of antagonist											
			Aspergillus flavus		*Aspergillus fumigatus*		*Aspergillus niger*		*Penicillium* sp.		*Trichoderma herzianum*		*Trichoderma viride*	
			Grades & Type	Per cent inhibition	Grades & Type	Per cent inhibition	Grades & Type	Per cent inhibition	Grades & Type	Per cent inhibition	Grades & Type	Per cent inhibition	Grades & Type	Per cent inhibition
1	Black Mat (B-M)	JSDSvL-01	2 Bii	52.12 efg	2 Bii	46.43 d	2 Bii	56.08 def	3 Bi	37.91 e	2 Bii	74.76 cd	4 C	67.77 b
		GJBEnL-01	2 Bii	51.97 efg	2 Bii	46.91 d	2 Bii	55.38 f	3 Bi	38.2 e	2 Bii	74.68 cd	4 C	67.6 b
2	Black Fluffy (B-F)	JSVPdL-08	2 Bii	54.48 b	2 Bii	47.77 bcd	2 Bii	60.87 b	3 Bi	41.22 cd	2 Bii	78.61 ab	2 Bii	67.73 b
		GJBKhL-01	2 Bii	54.27 bc	2 Bii	47.55 bcd	2 Bii	60.64 b	3 Bi	41.63 bcd	2 Bii	79.02 a	2 Bii	67.2 bc
3	Ash Mat (A-M)	JSDSvL-28	3 Bi	53.32 bcde	2 Bii	47.34 bcd	4 C	57.87 cd	3 Bi	41.35 cd	2 Bii	72.98 de	2 Bii	62.55 e
		GJBKnL-01	3 Bi	53.24 bcdef	2 Bii	47.24 cd	4 C	57.67 cde	3 Bi	41.5 bcd	2 Bii	72.98 de	2 Bii	62.98 de
4	Brownish Ash Fluffy (Br.A-F)	JSDBjL-03	3 Bi	56.87 a	3 Bi	51.74 a	2 Bii	65.48 a	2 Bii	46.68 a	4 C	76.44 bc	2 Bii	71.81 a
		DiWRBjL-01	3 Bi	56.92 a	2 Bii	52.09 a	2 Bii	65.63 a	2 Bii	46.73 a	4 C	76.83 abc	2 Bii	71.54 a
5	Blackish Ash Mat (BlA-M)	JSVPdL-10	2 Bii	51.6 fg	3 Bi	46.08 d	3 Bi	55.78 ef	2 Bii	41.03 d	2 Bii	61.78 g	2 Bii	66.07 bc
		PSVStL-02	2 Bii	51.49 g	3 Bi	46.44 d	3 Bi	56.14 def	2 Bii	41.21 cd	2 Bii	61.69 g	2 Bii	65.83 bc
6	Whitish Ash Fluffy (WA-F)	JSDSvL-26	2 Bii	52.45 defg	2 Bii	49.28 b	4 c	56.83 cdef	3 Bi	42.87 bcd	4 C	72.19 e	2 Bii	65.09 cd
		JSVPdL-04	2 Bii	52.73 cdefg	2 Bii	49.3 b	4 C	56.96 cdef	3 Bi	43.31 bc	4 C	72.88 de	2 Bii	67.02 bc
7	Greenish Ash Fluffy (GA-F)	JSDSvL-05	2 Bii	51.77 efg	2 Bii	47.21 cd	2 Bii	56.16 def	3 Bi	38.08 e	2 Bii	67.29 f	4 C	63.05 de
		GJBKnL-03	2 Bii	51.7 efg	2 Bii	47.06 cd	2 Bii	57.07 cdef	3 Bi	38.15 e	2 Bii	67.15 f	4 C	63.06 de
8	Pinkish White Mat (PW-M)	JSDSvL-09	3 BI	54.04 bcd	2 Bii	48.99 bc	4 C	58.21 c	3 Bi	43.27 bc	2 Bii	62.46 g	2 Bii	71.56 a
		JSDStL-01	3 Bi	54.22 bc	2 Bii	49 bc	4 C	58.24 c	3 Bi	43.59 b	2 Bii	62.33 g	2 Bii	72.32 a

*Values within the same column with a common letter (s) do not differ significantly at 5% level by LSD.

Grades from 1 (mutually intermingling growth) to 5 (mutual inhibition at a distance), as proposed by Skidmore and Dickinson (1976) are as follows: Grade 1 = Mutual intermingling without any microscopic sights of interaction. Grade 2 = Mutual intermingling growth where the growth of the fungus is ceased and being over growth by the opposed fungus. Grade 3 = Intermingling growth where the fungus under observation is growing into the opposed fungus either above (or) below. Grade 4 = Sight inhibition of both the interacting fungi with narrow demarcation line (1 - 2 mm). Grade 5 = Mutual inhibition of growth at a distance of > 2 mm.

In colony interaction all the antagonists reduced the growth of *B. sorokiniana* (37.91-79.02%), having *T. harzianum* the highest inhibition effect against isolate GJBKhL-01, followed by JSVPdL-08 (78.61%) under Black Fluffy (B-F) group. The range of per cent radial growth inhibition of 16 isolates of *B. sorokiniana* against *Aspergillus flavus* was 51.49-56.92%, *A. fumigatus* was 46.08-52.09%, *A. niger* was 55.38-65.63%, *Penicillium* sp. was 37.91-46.73%, *T. harzianum* was 61.69-79.02% and *T. viride* was 62.55-72.32% (Plate 1).

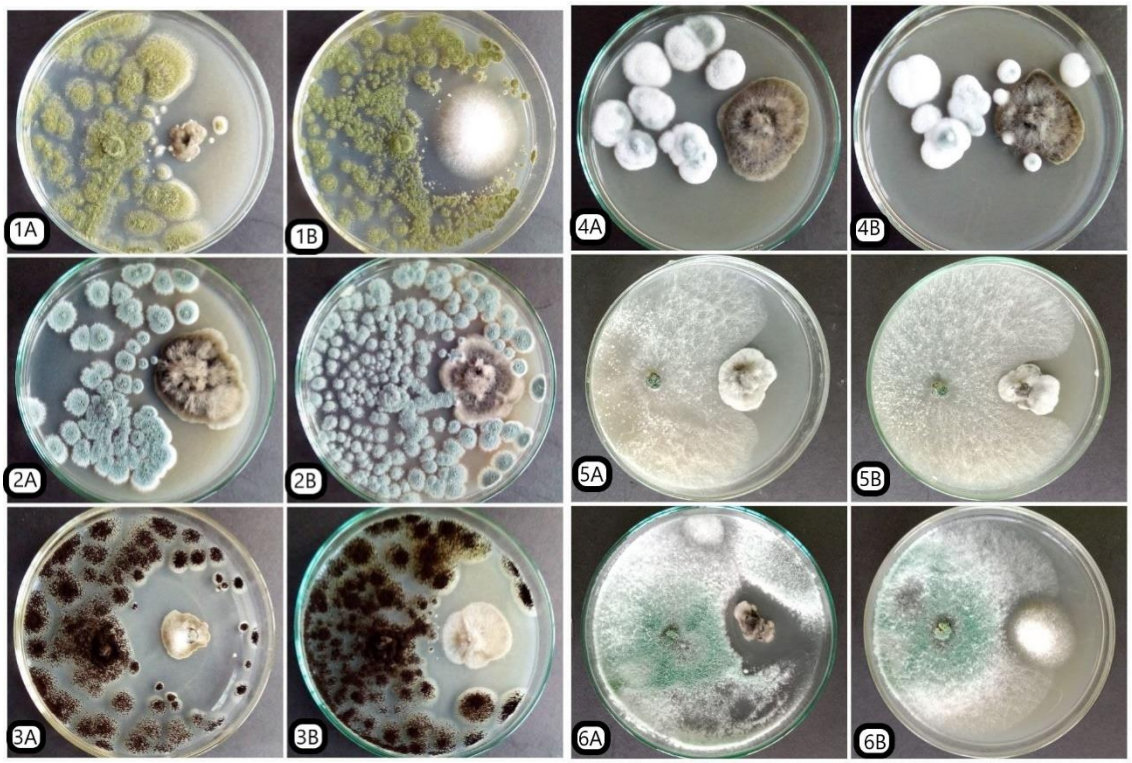


Plate 1. Colony interaction between randomly selected isolate of *B. sorokiniana* and 1A-B *Aspergillus flavus*, 2A-B *A. fumigatus*, 3A-B *A. niger* 4 A-B *Penicillium* sp. 5A-B *Trichoderma harzianum* and 6 A-B *T. viride*.

M. T. Yassin *et al.* 2022 reported *B. sorokiniana*, black point pathogen in duel culture 62.65% and 59.04% inhibition was done by *T. viride* and *T. harzianum*. Salehpour *et al.* 2005 tested biological control of common root rot of wheat. *T. harzianum* and *T. viride* were used towards two isolates of *B. sorokiniana*. In duel culture, inhibition varied from 29.56 to 69.82%, which is comparable to my investigation. *Trichoderma* spp. has shown inhibitory effect on the growth of *D. sorokiniana* in dual culture test[20].

The potent antifungal activity of *Trichoderma* spp. may be their fast growing nature, high colonization rate, rapid sporulation and production of different secondary metabolites resulting in suppression of the competitive microorganisms. *Trichoderma* spp. may show a suppressing role on the growth of *B. sorokiniana* as the hyphae of pathogens obviously collapsed between the interaction zones as clearly noticed by dual culture assay. The development of clear interaction zone of hypha disappearance of pathogenic fungi to the exploit of secondary metabolites as gliotoxin produced by *Trichoderma* spp which was supposed to play an important role in the antibiosis progression[14, 21].

Effect of volatile substances

The results of effects of volatile substances or metabolites of six antagonistic soil fungi are exhibited in Table 2. It is clear from the result that the volatile substances emanating from the culture of *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Penicillium* sp., *T. harzianum* and *T. viride* inhibited the radial growth of the isolates of *B. sorokiniana* to wide-ranging degrees (7.87-61.34%).

Table 2. Percent inhibition owing to volatile substances against *Bipolaris sorokiniana* isolates.

Sl. No.	Group	Name of fungi	Name of antagonist					
			Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Penicillium* sp.	*Trichoderma herzianum*	*Trichoderma viride*
			Per cent inhibition	Per cent inhibition	Per cent inhibition	Per cent inhibition	Per cent inhibition	Per cent inhibition
1	Black Mat (B-M)	JSDSvL-01	20.17 de	19.32 def	43.18 a	17.39 a	46.34 efg	45.6 b
		GJBEnL-01	35.63 a	8.04 h	30.29 d	16.54 a	61.34 a	41.85 bcd
2	Black Fluffy (B-F)	JSVPdL-08	29.08 b	23.97 bc	40.77 ab	17.39 a	51.16 bcde	53.98 a
		GJBKhL-01	22.27 cd	26.54 ab	39.26 abc	16.22 a	53.16 bcd	41.81 bcd
3	Ash Mat (A-M)	JSDSvL-28	26.61 bc	27.45 a	38.72 abc	16.97 a	50.86 cde	44.88 bc
		GJBKnL-01	19.99 de	19.24 def	43.6 a	17.28 a	46.41 efg	45.66 b
4	Brownish ash Fluffy (Br.A-F)	JSDBjL-03	17.36 e	12.3 g	43.5 a	9.65 b	56 b	46.28 b
		DIWRBjL-01	36 a	7.87 h	29.33 d	17.02 a	61.12 a	41.21 bcd
5	Blackish ash Mat (Bl.A-M)	JSVPdL-10	26.94 bc	27.34 a	38.8 abc	17 a	51.13 bcde	44.41 bcd
		PSVStL-02	17.57 e	12.68 g	43.56 a	9.29 b	55.54 bc	46.12 b
6	Whitish ash Fluffy (W.A-F)	JSDSvL-26	28.59 b	22.08 cd	34.13 bcd	13.75 ab	44.14 g	37.34 d
		JSVPdL-04	22.54 cd	26.97 ab	40.49 ab	17.62 a	53.66 bcd	41.58 bcd
7	Greenish ash Fluffy (Gr.A-F)	JSDSvL-05	29.11 b	21.77 cde	32.48 cd	13.99 ab	45.52 fg	38.3 cd
		GJBKnL-03	28.77 b	24 bc	40.38 ab	16.71 a	51.78 bcd	53.81 a
8	Pinkish White Mat (P.W-M)	JSDSvL-09	22.83 cd	18.68 f	39.02 abc	15.94 a	50.58 cde	43.9 bcd
		JSDStL-01	22.67 cd	18.88 ef	39.06 abc	16.27 a	50.34 def	44.05 bcd

*Values within the same column with a common letter (s) do not differ significantly at 5% level by LSD.

The maximum inhibition (61.34%) was found owing to volatile metabolites of *T. harzianum* against isolate GJBEnL-01 followed by DiWRBjL-01 (61.12%), JSDBjL-03 (56%), PSVStL-02 (55.54%) and JSVPdL-08 (53.98%) due to *T. viride*. *Aspergillus niger* inhibited the radial mycelial growth of 16 isolates of *B. sorokiniana* was 29.33-43.6%, in case of *A. flavus* inhibition range was 17.36-36%, due to *A. fumigatus* radial growth reduction was 7.87-27.45%, owing to *Penicillium* sp. inhibition range was 9.56-17.62%, the maximum inhibition record for *T. harzianum* was 44.14-61.34% and last of all *T. viride* inhibition range was 37.34-53.98% (Plate 2).

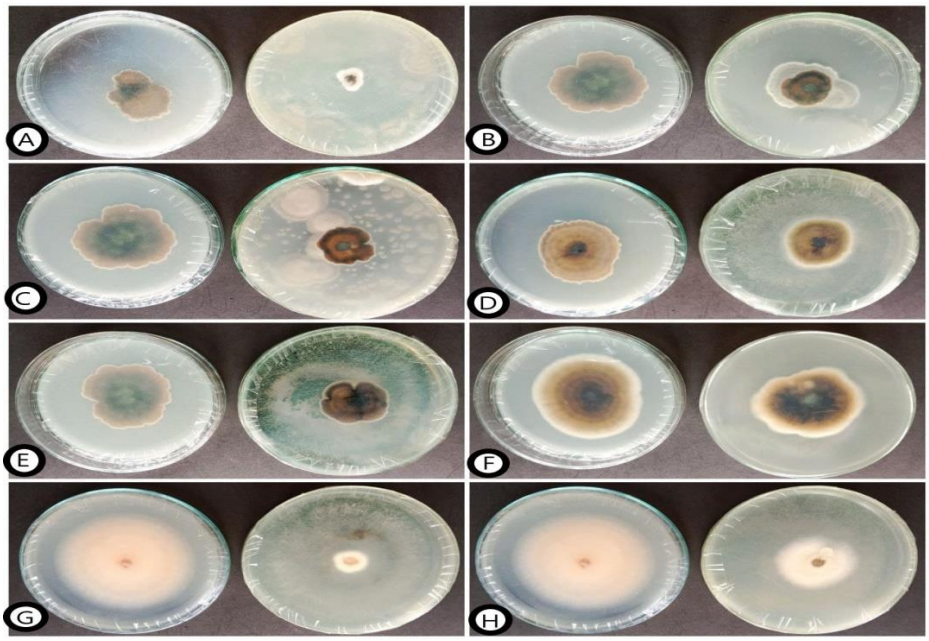


Plate 2. Per cent inhibition owing to volatile substances between randomly selected isolate of *B. sorokiniana* and different soil antagonists.

Plate 3. Percent inhibition owing to non-volatile substances at 5, 10, 15 and 20% concentration A. Blackish Ash Mat isolate and B. Black Fluffy isolates of *B. sorokiniana* towards *Trichoderma harzianum*.

Salehpour *et al.* 2005 achieved greater per cent inhibition due to volatile metabolites of *T. harzianum* towards two isolates of *B. sorokiniana* 66.66 to 98.25%, where present investigation suggested the highest inhibition per cent was only 61.34% against 16 isolates. Inhibition of radial mycelial growth of different plant pathogenic fungi was achieved by *T. harzianum* owing to volatile metabolites was recorded by Akter *et al.* (2014), Yasmin and Shamsi (2019).

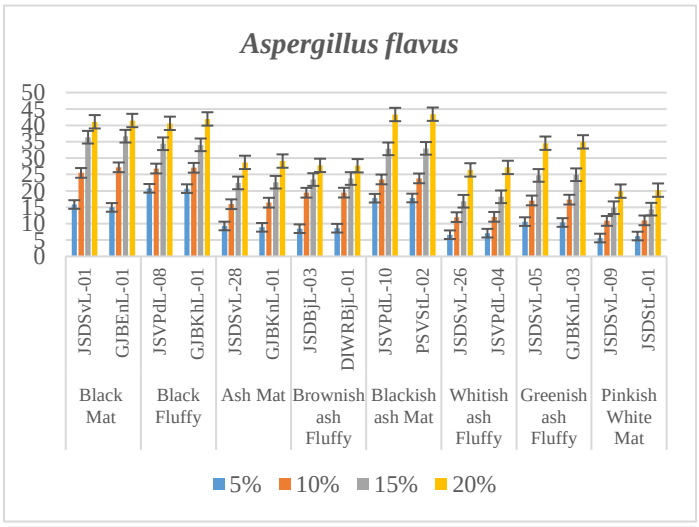
The reduction of mycelial growth of plant pathogenic fungi may be accredited by the presence of growth inhibitory substances in the metabolites of antagonistic fungi[24, 25].

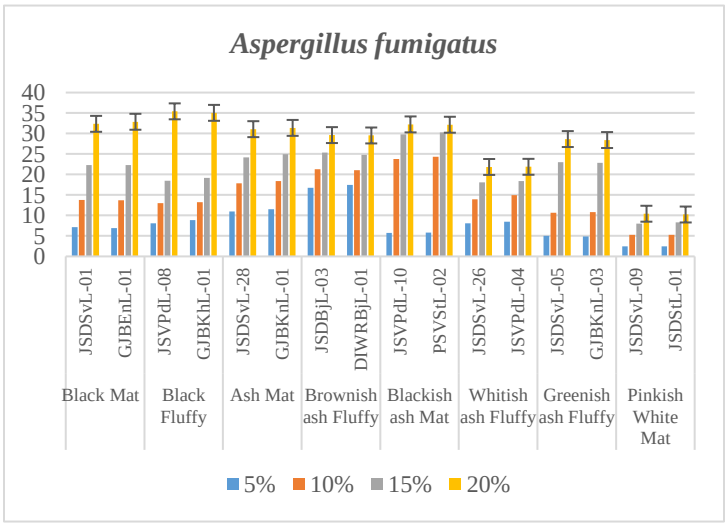
Biocontrol action of antagonist fungi and bacteria may partially be associated with production of antibiotic. *T. harzianum* acts as an antagonist by the production of isonitrin, homothallin II, melanoxadin[26, 27, 28]. Different isolates of *T. viride* produced trichodermin, ergokonin, viridin, viridiofungin A, B and C which acts in biological control[29,30,31,32]. The gross effect may also depend on the interaction between the volatile factors of two fungi as some sort of chemical reaction may occur there. However, in my experiment it is not possible to extract these substances from *T. harzianum* and *T. viride* used. The metabolites produced by *T. harzianum* and *T. viride* that provided biocontrol in this experiment is likely similar to metabolites produced by the other isolates tested by the other investigators mention earlier.

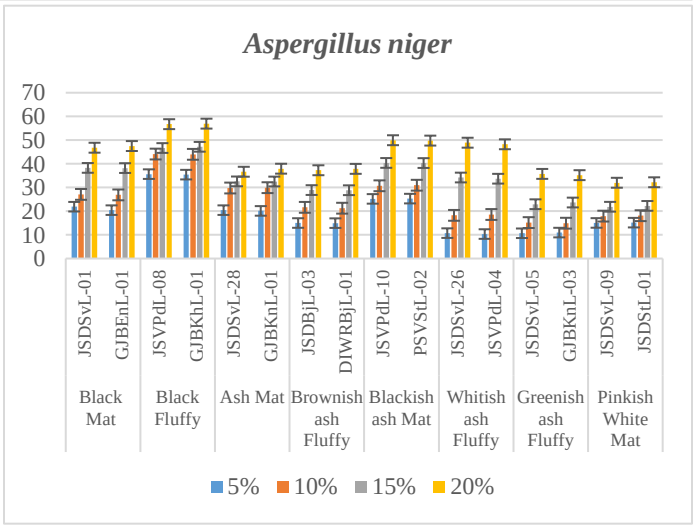
To identify the metabolites and their functions further research is needed. The present investigation suggests that there were qualitative and quantitative differences in per cent inhibition due to the amount of volatile substances produced by various soil fungi or might be due to the difference in organism involved in the interaction. Dennis and Webster (1971 b) noted that certain *Trichoderma* spp. produced volatile antibiotics for the growth inhibition of *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium oxysporum*. No lethality to any of the test fungi was reported by these authors and comprehensive chemical analysis of the volatile components of fungal cultures were not performed, although acetaldehyde was suggested one of the volatiles.

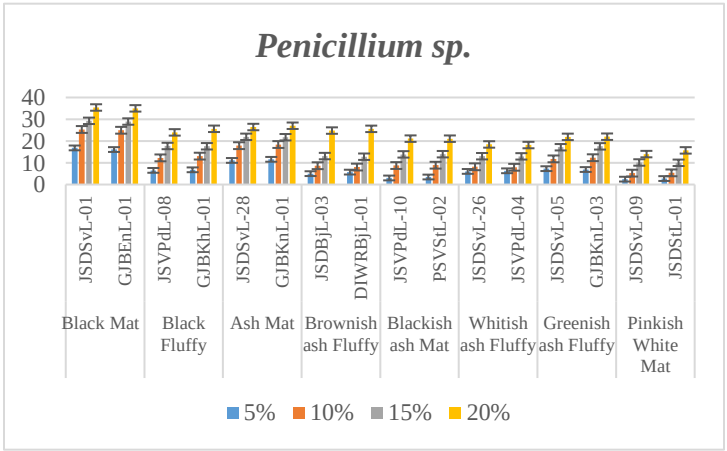
Effect of non-volatile substances

The effects of non-volatile substances or metabolites of six antagonistic soil fungi towards 16 isolates of *B. sorokiniana* are demonstrated in Fig 1,2,3,4,5 and 6. At 20% concentration, all the antagonistic fungi inhibited radial growth of *B. sorokiniana* isolates from 13.99 to 68.14%. The best inhibition was achieved due to culture filtrate at 20% concentration by *T. harzianum* towards isolate JSDSvL-09, followed by JSDSvL-01 (67.83%), JSDStL-01 (67.5%). The lowest per cent inhibition owing to *T. harzianum* was shown against isolate JSVPdL-04 (47.28%) at 20% concentration.









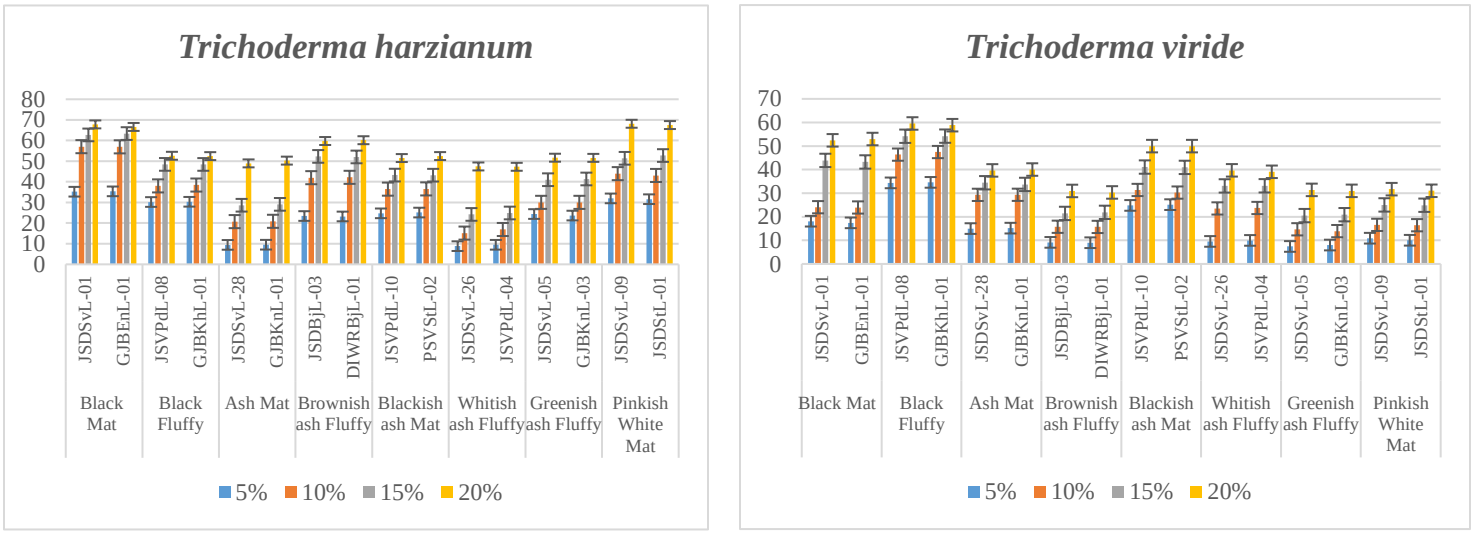


Figure. Percent inhibition owing to non-volatile substances of six soil antagonists at 5, 10, 15, 20% concentrations on radial growth of 16 *Bipolaris sorokiniana* isolates.

The inhibition ranges owing to non-volatile metabolites at 20% concentration of *A. flavus* was 19.93 to 43.41%, *A. fumigatus* was 10.23 to 35.42%, *A. niger* was 31.99 to 56.95%, *Penicillium* sp. was 13.99 to 35.4% and second highest *T. viride* was 30.32 to 59.57%.

From my investigation, per cent inhibition owing to non-volatile metabolites was higher than volatile substances. This may be due to production of antibiotic or toxic substances in the culture filtrates, impoverishment of nutrient and alteration of pH of the culture medium resulting from staling growth products[33, 34, 12, 11] attribute the inhibition of radial growth of plant pathogen. Antibiotic or inhibitory substances production varies in nature, quality and quantity depending on the comparing organisms.

Although no antibiotic/mycolytic enzymes were extracted, it is possible that the antagonists and their culture filtrates contain some kind of antibiotic or enzymes inhibitiory to suppression of mycelial growth of *B. sorokiniana*. *Trichoderma* spp. produced trichodermin which completely controlled *Helminthosporium* and *Fusarium* rots in wheat[35]. *Trichoderma* spp. exhibited a wide range of secondary metabolites (volatile, non-volatile, diffusable) responsible for the protection of plants from harmful pests, nutrient support, mineral solubilisation and pharmacological activities. *Trichoderma* showed mycoparasitism, antibiosis and competition mechanisms to combat major agricultural pests.

The future of the biocontrol of plant pathogenic fungi absolutely lies in the use of integrated control approaches. The current study confirmed the potent activity of *T. harzianum* and *T. viride* against *B. sorokiniana* causing leaf blight disease of wheat. The proved antagonistic efficacies of *T. viride* and *T. harzianum* against *B. sorokiniana* were coincident with the report by Yassin *et al.* 2022.

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