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ANTAGONISTIC EFFECT OF THREE FUNGAL ISOLATES TO AFLATOXIN-PRODUCING^spergiY/HS/JavHS

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ABSTRACT

Aflatoxin contamination in preharvest peanuts can be controlled among others by using antagonistic fimgi to aflatoxin-producing fungi. *Aspergillus flavus* is one of the fungal species where certain strains can produce aflatoxin. Informations regarding the type of interactions between antagonistic fungi and toxigenic *A. flavus,* and the effects of culture filtrates of the test fungi on the growth and aflatoxin production of toxigenic *A. flavus* are necessary, before antagonistic fungi could be used as biocontrol agent. Three fungal isolates (nontoxigenic *A. flavus* BIO 2127, *A. niger* BIO 2129 and *Trichoderma harzianum* BIO 19130) were tested for their antagonistic properties against toxigenic *A. flavus* BIO 2132 using direct and indirect confrontation methods.

On direct confrontation method, four kinds of agar media were used, i.e PDA (Potato Dextrose Agar), MEA 1% (Malt Extract Agar 1%), SMKYA (Sucrose 200 g, $MgSO_47H_2O$ 0.5 g, KNO_3 3 g, yeast extract 7 g, and block agar AA 20 g), and the mixture of MEA 1 % + SMKYA (1:1). The results indicated that the type of interactions between toxigenic *A. flavus* either with nontoxigenic *A. flavus* or with *T. harzianum* was B type. In this type of interaction, the growth of both toxigenic *A. flavus* and the test fungi inhibited each other (mutual inhibition) with the zone of inhibition < 2 mm. Type of interaction between toxigenic *A. flavus* and *A. niger* depended on the kind of media. On SMKYA and MEA 1% + SMKYA media, the interaction was B type, while on PDA and MEA 1% media it was D type. In this D type of interaction, toxigenic *A. flavus* and *A. niger* inhibited each other (mutual inhibition) at a distance > 2 mm.

Culture filtrates derived from nontoxigenic *A. flavus* and *A. niger* grown on ME 1%, SMKY and ME 1% + SMKY inhibited the growth (based on dry weight) of toxigenic *A. flavus,* except culture filtrates derived from *T. harzianum* grown on SMKY and ME 1% + SMKY media stimulated the growth of toxigenic *A. flavus.*

Culture filtrates of nontoxigenic *A. flavus, A. niger* and *T. harzianum* inhibited aflatoxin *B*\ production of toxigenic *A. flavus.* Culture filtrates of *A. niger* and *T. harzianum* with conidial concentrations of $IxlO^6$, $2xl0^6$ and $3xl0^6$ per ml inhibited aflatoxin B, production up to 100%. The percentage of inhibition of aflatoxin Bi production increased with the increase of conidial concentrations of nontoxigenic *A. flavus.* The highest percentage of inhibition of aflatoxin BI production (62.5%) was obtained from conidial concentration of $3xl0^6$ per ml.

Aspergillus niger was the most potential fungus in inhibiting the growth of toxigenic *A. flavus,* either on agar media or on culture filtrates of test fungi. Culture filtrate of *A. niger* was also the most potential filtrate in inhibiting aflatoxin BI production of toxigenic *A. flavus.*

Keywords: Antagonistic effect / *Aspergillus flavus I Aspergillus niger I Trichoderma harzianum I* aflatoxin

INTRODUCTION

Aflatoxin contamination in peanuts occurs when kernels become infected by *Aspergillus flavus, A. parasiticus* and *A. nomius,* under drought stress before harvest, during the drying phase in the field, or under unsuitable storage conditions. Pitt *et al.* (1991) reported that *A. flavus* (and *A. parasiticus)* are able to grow as commensals in developing peanut plants, and from there the fungi can invade developing peanuts.

Levels of 1000 ppb of aflatoxin could cause acute liver damage in man and animals. Lower levels of aflatoxin in peanut products if consumed could cause liver cancer and premature death in humans, as well as reducing productivity of livestock (Pitt and Hocking 1996).

According to Pitt and Hocking (1996), 45% of 215 peanut samples collected from retailers in Bogor and Yogyakarta contained more than 50 ppb of aflatoxin, 33% more than 300 ppb, and 22% exceeding 1000 ppb. In foodstuff, the limit of aflatoxin content determined by U.S. Food and Drug Administration was 20 ppb (Park 1993). Codex Alimentarius Commision adopted the maximum level of total aflatoxin content in peanuts intended for further processing at 50 ppb (FAO and WHO 1999).

During the past 20 years, a number of approaches have been advocated and tested for reduction of aflatoxins in peanuts: resistant cultivars, thickened shells, waxy testa, improved farm management techniques and postharvest procedures involving drying and storage. Some of these approaches have merit, but despite the expenditure of large sums of research funding aflatoxin in peanuts remains a serious commercial problem. Other approaches such as using antagonistic fungi to aflatoxin-producing fungi are still needed (Pitt 1999).

One of the more promising method is the concept of biocontrol by competitive exclusion (Pitt 1999). This involves the use of competitive fungi to reduce the possibility of toxigenic fungi present in the soil entering developing peanuts and then producing aflatoxins in them. According to Dorner *et al.* (1992), nontoxigenic *A. parasiticus* can be used as biocompetitive agent to control aflatoxin contamination in peanuts before harvest. Dharmaputra *et al.* (2001) reported that among fungi isolated from the soil of peanut farms at Wonogiri regency (Central Java), nontoxigenic *A. flavus* BIO 2127, *A. niger* BIO 2129 and *Trichoderma harzianum* BIO 19130 were antagonistic to toxigenic *A. flavus* BIO 2128.

The objectives of this study were to get information on the type of interactions between each of the three fungal isolates (nontoxigenic *A. flavus, A. niger* and *T. harzianum)* and toxigenic *A. flavus;* and to investigate the effects of culture filtrates of the test fungi on the growth and aflatoxin production of toxigenic *A. flavus.*

MATERIALS AND METHODS

Fungal isolates

Fungal isolates used were toxigenic *A. flavus* BIO 2132, isolated from peanut farms in Pati regency, Central Java, in January 2002; nontoxigenic *A. flavus* BIO

2127, *A. niger* BIO 2129 and *T. harzianum* BIO 19130, isolated from soils of peanut farms at Wonogiri regency, Central Java, in May 2000 (Dharmaputra *et al* 2001). The four fungal isolates belong to the Plant Pathology Laboratory Culture Collection, SEAMEO BIOTROP. Each fungal isolate was subcultured on Potato Dextrose Agar (PDA) medium and incubated at room temperature (28 ± 2°C) for 7 days.

Test of antagonism between three fungal isolates and toxigenic *A. flavus*

1. Direct confrontation between fungal colonies

Nontoxigenic *A. flavus, A. niger* and *T. harzianum* isolates were tested for their antagonistic property against toxigenic *A. flavus* using direct opposition method (Dennis and Webster 1971) on four agar media, i.e. PDA, ME A 1% (Malt Extract Agar 1%), SMKYA (Sucrose 200 g, $MgSO_4$.7H O 0.5 g, KNO_3 3 g, yeast extract 7 g, block agar AA 20g) and the mixture of MEA 1% + SMKYA (1:1). Toxigenic *A. flavus* (4 mm in diam) was placed on the four media in a Petri dish (diam 9 cm). At the same time at a distance of 3 cm from toxigenic *A. flavus* inoculum, each test fungus was inoculated on the same dish. The plates were incubated at room temperature. Three replications were used for each treatment. Observations were carried out seven days after incubation on the inhibition of mycelial growth of toxigenic *A. flavus* by the test fungi using the formula of Fokkema (1973):

$$I = \frac{r_i - r_2}{R_1} \times 100\%$$

where I = percentage of inhibition
f] = radius of toxigenic *A. flavus* away from the test fungi
r_2 = radius of toxigenic *A. flavus* towards the test fungi

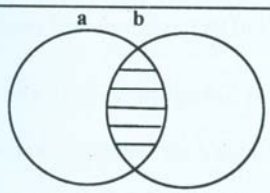
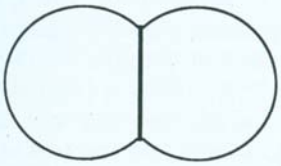
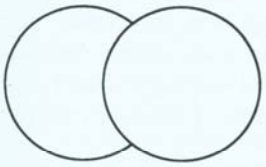
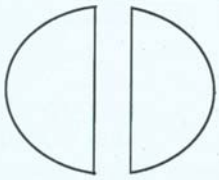
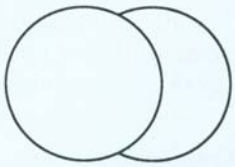
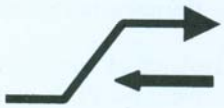
The type of interactions between toxigenic *A. flavus* and each of the test fungus was determined based on Wheeler and Hocking (1993), adapted from Magan and Lacey (1984), as presented in Table 1.

2. Indirect confrontation

2.1. Preparation of cultural filtrates of three test fungal isolates

Isolates of nontoxigenic *A. flavus, A. niger* and 7". *harzianum* were grown respectively on PDA medium in Petri dishes (9 cm in diam) and incubated at room temperature for 7 days. Fungal conidia were suspended in sterile distilled water. Two ml of each test fungal isolate suspension with three conidial concentrations ($IxlO^6$, $2xl0^6$ and $3xl0^6$ conidia/ml) were'grown on 100 ml ME 1%, 100 ml SMKY, and 100 ml of the mixture of ME 1% + SMKY (1:1) liquid media in 250 ml Erlenmeyer flask, respectively, and then incubated at room

Table 1. Type of interactions between two fungal colonies (Wheeler and Hocking 1993, adapted from Magan and Lacey 1984).

Interaction type	Classification description	
A	Mutual intermingling growth, where both fungi grew into each other without any macroscopic signs of interaction	
B	Mutual inhibition on contact or space between colonies small (< 2 mm)	
C	Inhibition of one species on contact, the inhibited species continued to grow at a significantly reduced rate, while the inhibitor species grew at a slightly reduced rate or unchanged	
D	Mutual inhibition at a distance (> 2 mm)	
E	Inhibition of one species on contact, the inhibitor species continuing to grow at a reduced rate through the inhibited colony	
F	Inhibition of one species on contact or at a distance, the inhibitor species then continuing to grow at an unchanged rate through or over the inhibited colony	

a = inhibitor species ; b = inhibited species

temperature for 14 days. The concentrations of conidia were determined by microscopically counting the number of conidia using a haemacytometer. After incubation, the culture filtrates of the fungi were separated from their colonies using sterile filter paper No. 1, followed by sterilization of the culture filtrates using Millipore (0.45 jam pore size).

2.2. Effect of culture filtrates of test fungi on the growth and aflatoxin production of toxigenic *A. flavus*

One ml of conidial suspension of toxigenic *A. flavus* (Ix1O^6 conidia/ml) was inoculated into 50 ml of sterilized culture filtrates derived from each treatment combination in a glass bottle volume 100 ml. They were then incubated at room temperature for 10 days. As control, toxigenic *A. flavus* was grown on ME 1%, SMKY and in the mixture of ME 1% + SMKY liquid media. Three replications were used for each treatment (including the control). Observations were made on the dry weight of mycelia of toxigenic *A. flavus* and aflatoxin production. The dry weight of mycelia was determined by drying the fungal colonies in an oven at 95°C until a constant weight was attained (Gourama and Bullerman 1995). Aflatoxins were analyzed using a Thin Layer Chromatography method (Bainton *et al.* 1980). In this case, aflatoxin in culture filtrates was extracted using chloroform and identified by two dimensional TLC using standard comparison. Aflatoxin content produced by the most potential test fungal isolate was confirmed using a High Performance Liquid Chromatography method (Rodriguez and Mahoney 1994).

Statistical analysis

The data were analyzed using a completely randomized factorial design with 2 factors. The first and the second factors were the isolates of test fungi (nontoxigenic *A. flavus, A. niger,* and *T. harzianum)* and conidial concentration of test fungi (1x10^6, 2x10^6 and 3xl0^6 conidia/ml), respectively.

RESULTS AND DISCUSSION

Type of interaction and percentage of growth inhibition between toxigenic *A. flavus* and test fungi on various agar media

Two types of interactions (B and D types) were found between each of the three test fungi and toxigenic *A. flavus* after 7 days of incubation (Table 2). On the type of interaction B, the test fungi and the toxigenic *A. flavus* inhibited each other's growth with a zone of inhibition < 2mm. This type of interaction was found on the interaction between the toxigenic and the nontoxigenic *A.flavus* on PDA, MEA 1%, SMKY A, and MEA 1% + SMKYA media; between the toxigenic *A. flavus* and

Table 2. Type of interactions between toxigenic *Aspergillus flavus* and test fungi on various media.

Media	Toxigenic *A. flavus* Vs test fungi	Type of interaction
PDA	Toxigenic *A. flavus* Vs nontoxigenic *A. flavus*	B
	Toxigenic *A. flavus* Vs *A. niger*	D
	Toxigenic *A. flavus* Vs *T. harzianum*	B
MEA 1%	Toxigenic *A. flavus* Vs nontoxigenic *A. flavus*	B
	Toxigenic *A. flavus* Vs *A. niger*	D
	Toxigenic *A. flavus* Vs *T. harzianum*	B
SMKYA	Toxigenic *A. flavus* Vs nontoxigenic *A. flavus*	B
	Toxigenic *A. flavus* Vs *A. niger*	B
	Toxigenic *A. flavus* Vs *T. harzianum*	B
MEA 1% + SMKYA	Toxigenic *A. flavus* Vs nontoxigenic *A. flavus*	B
	Toxigenic *A. flavus* Vs *A. niger*	B
	Toxigenic *A. flavus* Vs *T. harzianum*	B

PDA = Potato Dextrose Agar; MEA = Malt Extract Agar; SMKYA = Sucrose 200 g, $MgSO_4.7H_2O$ 0.5 g, KNO_3 3 g, Yeast extract 7 g, block agar AA 20 g

A.niger on SMKYA and MEA 1% + SMKYA media; between the toxigenic *A. flavus* and *T. harzianum* on PDA, MEA 1%, SMKYA, and MEA 1% + SMKYA media. On the D type of interaction, the test fungi and the toxigenic *A. flavus* inhibited each other's growth with a zone of inhibition > 2 mm. This type of interaction was found on the interaction between the toxigenic *A. flavus* and *A. niger* on PDA and MEA 1% media. On the two media a zone of inhibition was found between the colony of *A. niger* and toxigenic *A. flavus.* It was assumed that *A. niger* produced antibiotic. According to Jeffries and Young (1994), production of extracellular metabolites (such as antibiotics and lytic enzymes) was one of the mechanisms of antagonism between two fungal isolates. Dharmaputra *et al.* (2001) reported that the type of interaction between toxigenic *A. flavus* isolate 55_7 and *T. harzianum,* and between *A. flavus* isolate 10_2 and *T. harzianum* were A and B types, respectively; between toxigenic *A. flavus* isolate *23j* and nontoxigenic *A. flavus* isolate 18_t was B type; between toxigenic *A. flavus* isolate 10_2 and *A. niger* was D type.

The kind of media did not give significant differences on the percentage of growth inhibition (based on mycelial growth) of toxigenic *A. flavus* by the three test fungal isolates. Nevertheless, the highest percentage of growth inhibition was found on SMKYA medium (43.77%) (Table 3).

The test fungal isolates gave very significant differences on the percentage of growth inhibition of toxigenic *A. flavus* on SMKYA medium. *Trichoderma harzianum* caused the highest growth inhibition (49.09%) compared to toxigenic *A. flavus* on SMKYA medium (Table 4).

Table 3. The effect of various media on the percentage of growth inhibition of toxigenic *A. flavus* by test fungi.

Media	Growth inhibition (%)
SMKYA	43.77 a
MEA 1% + SMKYA	42.81 a
PDA	42.48 a
MEA 1%	39.35 a

Numbers followed by the same letter do not differ significantly according to Duncan Multiple Range Test at 95% confidence level.

Table 4. Percentage of growth inhibition (based on mycelial growth) of toxigenic *A. flavus* by test fungi on SMKY agar medium.

Test fungi	Growth inhibition (%)
Trichoderma harzianum	49.09 a
Aspergillus niger	44.01 b
Nontoxigenic *A. flavus*	38.21 c

Numbers followed by the same letter do not differ significantly according to Duncan Multiple Range Test at 95% confidence level.

The effect of culture filtrate of test fungi on the growth and aflatoxin production of toxigenic *A. flavus*

The effect of culture filtrates of nontoxigenic *A. flavus* and *A. niger* on the percentage of growth inhibition of toxigenic *A. flavus* (based on the dry weight of mycelia) was not significantly different. Nevertheless, the highest percentage of growth inhibition (69.44%) of toxigenic *A. flavus* caused by the culture filtrate of nontoxigenic *A. flavus* was on ME 1%, while that of the culture filtrate of *A. niger* (72.51%) was on SMKY (Table 5). It indicated that nontoxigenic *A. flavus* was more competitive to toxigenic *A. flavus* on ME 1% medium compared with those of SMKY and ME 1% + SMKY media, while *A. niger* was more competitive on SMKY medium compared with those on ME 1% and ME 1% + SMKY media.

Culture filtrate derived from *T. harzianum* grown only on ME 1% medium inhibited the growth of toxigenic *A. flavus* (63.64%), while on SMKY and ME 1% + SMKY media the filtrate stimulated the growth of toxigenic *A. flavus* (38.0 and 31.58%, respectively) (Table 6). It indicated that culture filtrate of *T. harzianum* was only effective in inhibiting the growth of toxigenic *A. flavus* on ME 1% medium.

Using TLC method, only aflatoxin B, was detected by toxigenic *A. flavus.* As aflatoxin was not produced on ME 1 % medium, consequently only SMKY and ME

Table 5. Percentage of growth inhibition (based on the dry weight of mycelia) of toxigenic *A. flavus* by culture filtrate derived from nontoxigenic *A. flavus* and *A. niger* grown on various media.

Media	Growth inhibition (%) by	
	Nontoxigenic *A. flavus*	*A. niger*
ME 1%	69.44 a	65.56 a
ME 1% + SMKY	63.55 a	68.68 a
SMKY	45.03 a	72.51 a

Numbers followed by the same letter on the same column do not differ significantly according to Duncan Multiple Range Test at 95% confidence level.

Table 6. Percentage of inhibition and stimulation of growth of toxigenic *A. flavus* by culture filtrate derived from *T. harzianum* grown on various media.

Media	Mycelial dry weight of toxigenic *A. flavus* (g)	Inhibition of growth (%)	Stimulation of growth (%)
Culture filtrate derived from *T. harzianum* grown on ME 1%	0.04	63.64	-
Control (ME 1%)	0.11		
Culture filtrate derived from *T. harzianum* grown on ME 1% + SMKY	0.46	-	31.58
Control (ME 1% + SMKY)	0.38		
Culture filtrate derived from *T. harzianum* grown on SMKY	0.69	-	38.00
Control (SMKY)	0.50		

1% + SMKY media were used to study their effects on the growth (based on dry weight of mycelia) and aflatoxin production of toxigenic *A. flavus.*

The kind of culture filtrates of test fungi on the growth of toxigenic *A. flavus* on SMKY media was significantly different. Conidial concentration of test fungi and the interaction between the kind of the culture filtrates and conidial concentrations were not significantly different. The percentage of growth inhibition of toxigenic *A. flavus* caused by culture filtrate of *A. niger* (72.51%) was higher than that of nontoxigenic *A. flavus* (45.62%). On ME 1% + SMKY medium, culture filtrates and conidial concentration of test fungi, and their interaction were not significantly different. Nevertheless, the percentage of growth inhibition of toxigenic *A. flavus* caused by culture filtrate of *A. niger* (68.68%) was higher than that of nontoxigenic *A. flavus* (64.0%).

The inhibition percentage of aflatoxin B, production caused by culture filtrate derived from nontoxigenic *A, flavus* grown on SMKY medium (41.67%) was not significantly different with that of ME 1% + SMKY medium (58.33%).

Culture filtrate derived from *A. niger* either grown on SMKY or ME 1% + SMKY media completely inhibited aflatoxin production effectively (100%) (Table 7). Culture filtrate of *T. harzianum* grown on the two media also completely inhibited aflatoxin BI production up to 100% (Table 7), but also stimulated the growth of toxigenic *A. flavus* (Table 6).

Table 7. Percentage of inhibition of aflatoxin B_1 production caused by culture filtrate derived from test fungi grown on various media.

Test fungi	Media	Aflatoxin B_1 content (ppb)		Inhibition of aflatoxin B_1 production (%)
		Containing test fungi	Control (without test fungi)	
A. niger	SMKY	0	160	100
	ME 1% + SMKY	0	120	100
T. harzianum	SMKY	0	160	100
	ME 1% + SMKY	0	120	100

The kind of culture filtrates derived from test fungi and conidial concentration of test fungi and their interaction gave significantly different effects on the percent inhibition of aflatoxin production using SMKY medium. On ME 1% + SMKY medium, the kind of culture filtrates of the test fungi gave very significantly different effect on aflatoxin B) production, while conidial concentrations and the interaction between kind of culture filtrates and conidial concentrations were not significantly different.

Culture filtrates derived from *A. niger* with the concentrations of $1x10^6$, $2xl0^6$ and $3xl0^6$ conidia/ml grown on SMKY medium inibited aflatoxin B_t production up to 100% (Table 8). The percent inhibition of aflatoxin B, production increased with the increase of conidial concentrations of nontoxigenic *A. flavus.* The culture filtrate of nontoxigenic *A. flavus* with the concentration of $1x10^6$ conidia/ml was not significantly different with that of $2x10^6$ conidia/ml against the inhibition percentage of aflatoxin Bj production, while that of $3xl0^6$ conidia/ml was significantly different.

The culture filtrate derived from A *niger* grown on ME 1% + SMKY medium inhibited aflatoxin B] production higher (100.00%) than that of nontoxigenic *A. flavus* (56.25%).

Aflatoxins produced by the most potential test fungal isolate were confirmed by using High Performance Liquid Chromatography (HPLC). Two kinds of aflatoxins (Bi and G|) were found using this method.

Table 8. Effect of interaction between the kind of culture filtrates derived from test fungi grown on SMKY liquid medium, and the conidial concentration of test fungi on the inhibition percentage of aflatoxin B_1 production.

Kind of culture filtrate of test fungi	Conidial concentration per ml in the filtrate	Inhibition of aflatoxin B_1 production (%)
A. niger	1×10^6	100.0 a
	2×10^6	100.0 a
	3×10^6	100.0 a
Nontoxigenic *A. flavus*	3×10^6	62.5 b
	2×10^6	37.5 c
	1×10^6	25.0 c

Numbers followed by the same letter do not differ significantly according to Duncan Multiple Range Test at 95% confidence level.

The results of test of antagonism using direct confrontation showed that SMKYA medium was the best medium for antagonism study between toxigenic *A. flavus* either with nontoxigenic *A. flavus, A. niger* or *T. harzianum. This is* because the highest percentage of growth inhibition of toxigenic *'A. flavus* caused by the three test fungal isolates occurred on SMKYA medium (Table 3). On SMKYA medium the highest percentage of growth inhibition of toxigenic *A. flavus* was caused by *T. harzianum* followed by *A. niger,* while the lowest was caused by nontoxigenic *A. flavus* (Table 4). However, the results of test of antagonism study using indirect confrontation showed that the culture filtrate derived from *T. harzianum* grown on SMKY medium stimulated the growth of toxigenic *A. flavus* (Table 6). Therefore, *A. niger* was the most potential test fungus in inhibiting the growth and aflatoxin B, production of toxigenic *A. flavus,* either on agar media or on culture filtrates of the test fungi.

Confirmation of aflatoxin contents produced by toxigenic *A. flavus* grown on culture filtrate of *A. niger* cultivated on SMKY and ME 1% + SMKY is presented in Table 9. The results showed that there were differences in aflatoxin BI contents analyzed using TLC and HPLC methods, because different methods could have different results. Nevertheless, based on the two methods used, culture filtrates derived from *A. niger* cultivated either on SMKY or ME 1% + SMKY liquid media with different conidial concentrations completely inhibited aflatoxin B] production of toxigenic *A. flavus.*

Table 9. Aflatoxin Bj contents produced by toxigenic *A. flavus* grown on culture filtrate derived from *A. niger* cultivated on SMKY and ME 1% + SMKY liquid media with various conidial concentrations.

Conidial concentration of *A. niger* (conidia/ml)	Media	Aflatoxin Bl content (ppb)	
		TLC method	HPLC method
1×10^6	Culture filtrate (using SMKY)	0	0
	Control (SMKY)	160	97.57
	Culture filtrate (using ME 1% + SMKY	0	0
	Control (ME 1% + SMKY)	120	60.79
2x10"	Culture filtrate (using SMKY)	0	0
	Control (SMKY)	160	98.89
	Culture filtrate (using ME 1% + SMKY	0	0
	Control (ME 1% + SMKY)	120	69.37
3x 10'	Culture filtrate (using SMKY)	0	0
	Control (SMKY)	160	103.58
	Culture filtrate (using ME 1 % + SMKY)	0	0
	Control (ME 1% + SMKY)	120	73.68

TLC = Thin Layer Chromatography; HPLC = High Performance Liquid Chromatography

CONCLUSIONS

The type of interactions between toxigenic *A. flavus* either with nontoxigenic *A. flavus* or with *T. harzianum* was B type, respectively. In this type of interaction, mutual inhibition on contact or space between the test fungi and toxigenic *A. flavus* was small (< 2 mm). The type of interactions between toxigenic *A. flavus* and *A. niger* depended on the kind of media. On SMKYA and MEA 1% + SMKYA media, the interactions were B type, while on PDA and MEA 1% media, it was D type. In this D type of interaction, toxigenic *A. flavus* and *A. niger* inhibited each other (mutual inhibition) at a distance > 2mm.

Culture filtrates derived from nontoxigenic *A. flavus* and *A. niger* grown on ME 1%, SMKY and ME 1% + SMKY inhibited mycelial growth (based on dry weight) of toxigenic *A. flavus,* except those derived from *T. harzianum* grown on SMKY and ME 1% + SMKY media which stimulated the growth of toxigenic *A. flavus.*

The culture filtrates of nontoxigenic *A. flavus, A. niger* and *T. harzianum* inhibited aflatoxin BI production of toxigenic *A. flavus.* The culture filtrates of *A. niger* and *T. harzianum* with the conidial concentrations of $1x10^6$, $2xl0^6$ and $3xl0^6$ per ml could inhibit aflatoxin B] production up to 100%. The percent inhibition of aflatoxin B, production increased with an increase of conidial concentrations of nontoxigenic *A. flavus.* The highest percent inhibition of aflatoxin *B*\ production (62.5%) was obtained from the conidial concentration of $3x10^6$ per ml.

Aspergillus niger was the most potential fungus in inhibiting the growth of toxigenic *A. flavus,* either on agar media or on culture filtrates of the test fungi. The culture filtrate of *A. niger* was also the most potential filtrate in inhibiting aflatoxin BI production of toxigenic *A. flavus.*

The results of this study gave important informations on antagonistic effect of three fungal isolates (nontoxigenic *A. flavus, A. niger* and *T. harzianum)* before they could be used to control aflatoxin-producing *A. flavus* in peanuts grown under green-house and field conditions.

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