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Mycotoxin production by *Aspergillus flavus* and *Penicillium citrinum* in competitive environment on mustard (*Brassica juncea*) var. NDR-8501 and Raurd-101 seeds

M.S. AHMAD

University Department of Botany, T.M. Bhagalpur University, Bhagalpur 812 007

dmsahmad@yahoo.co.in

ABSTRACT Mustard (*Brassica juncea* L.) is an important oilseed crop of Bihar state. This state also provides ideal environment for the association of various mycotoxin producing fungal species with mustard seeds. An attempt has been made, to record the influence of microbial interactions on mycotoxin production in liquid medium by the selected toxigenic strains of *Aspergillus flavus* and *Penicillium citrinum*, respectively. Based on these results five fungal species were selected for recording their influence on mycotoxin production in the seeds of two mustard varieties *viz*. NDR-8501 and Raurd-101 under laboratory conditions.

Maximum inhibition in aflatoxin B_1 (83.72%) and citrinin production (63.63%) in variety NDR-85501 was observed, when the seeds were co-cultured with *Rhizopus stolonifer*. In Raurd-101 variety maximum inhibition in aflatoxin B_1 production by *A. flavus* was observed with *F. moniliforme* (82.69%), whereas *Rhizopus stolonifer* inhibited citrinin production by *P. citrinum* (66.00%).

Key words: *Aspergillus flavus*, competitive environment, *Penicillium citrinum*, mycotoxin production, mustard seeds

Mycotoxin production, under laboratory conditions, when all the factors are favourable by the toxigenic strains of *A. flavus* and *P. citrinum* is a natural phenomenon. Earlier reports from different parts of the world have clearly established that a major fraction of *A. flavus* isolates occurring in nature are toxigenic [1, 2, 3]. These isolates produce appreciably high amount of toxins. However, in natural environment the toxigenic strains have to encounter a tough competition for the elaboration of toxins. The problem with regard to associating fungal organisms has not received much attention. There are conflicting reports about the competitive ability of *A. flavus* in relation to other fungi.

Alderman [4] found that *Penicillium rubrum* inhibits aflatoxin production in dual cultures of these organisims. There are also reports of increased/decreased mycotoxin production in association with some other fungi [5, 6].

Antimicrobial activity thus may not always be associated with fall in mycotoxin producing fungi by the use of some agricultural biocides and commonly inhibiting plant extracts [7, 8, 9 10]. Contemporary agricultural practices have resulted in the development of unique agro-ecosystem niches which are particularly suitable for microbes such as *A. flavus* and *A. parasiticus*. However, the idea of niche implies that species do not occur at random within a community but there is a definite pattern imposed by the interactions with the population of other species.

In the present investigation an attempt has been made to record the influence of microbial interactions on mycotoxin production by a toxigenic strain each of *A. flavus* and *P. citrinum* in

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liquid medium. Based on these results five fungal species under laboratory conditions were selected for recording their influences on mycotoxin production on the seeds of two mustard varieties *viz.* NDR-8501 and Raurd-101.

MATERIALS AND METHODS

Mode of interactions as well as mycotoxin production by a toxigenic strain of *A. flavus* and *P. citrinum* were studied in presence of ten fungal species belonging to the nine common genera (Tables 1&2) in SMKY liquid medium [11]. Methods of Schwenk *et al.* [12] were followed for the determination of citrinin production by *P. citrinum* isolates. 25 ml sterilized liquid medium was taken in 250 ml Erlenmeyer conical flask. The medium was inoculated with the test fungus separately. The flasks were incubated at $28^0 \pm 2^0$C for 11 days. All sets were maintained in triplicate. Aflatoxin and citrinin were extracted from the culture filterate with chloroform and subsequently estimated qualitatively and quantitatively on TLC plate [13, 14].

On the basis of above results five test fungi were selected for their efficacy against mycotoxin production by *A. flavus* and *P. citrinum* on two mustard varieties (NDR-8501 and Raurd-101) seeds, collected from Rajendra Agriculture College campus, Sabour, Bhagalpur.

50 g seeds were surface sterilized with 2% NaOCl solution for five min. The seeds were subsequently rinsed with distilled water. The moisture content of the seeds was also raised to 21% (by drying soaked seed samples at 60°C for 2-3 days). The seeds were taken in triplicate. The seeds were ground and aflatoxin and citrinin were extracted and estimated as per standard methods AOAC [15]. The results were subjected to one way analysis of variance (Tables 3&4).

RESULTS AND DISCUSSION

All ten types of fungal association with *A. flavus* and *P. citrinum* were found to inhibit aflatoxin B_1 and citrinin production (Tables 1&2) but the intensity was variable. In control sets (Inoculated only with *A. flavus* and *P. citrinum*), the mean values of aflatoxin B_1 and citrinin production were 1640±12.47 and 260±64.28 µg/kg. Inhibition in aflatoxin B_1 and citrinin production were more than 85% when *A. flavus* was co-inoculated with *A.*

Table 1. Inhibition in aflatoxin B_1 production by toxigenic isolate of *Aspergillus flavus* when co-inoculated with competing mycoflora in liquid medium

Test mycoflora	Aflatoxin B_1 production (µg/kg)±SE	% inhibition in aflatoxin B_1 production
Aspergillus flavus (alone)	1640±12.47	-
A. flavus + *A. niger*	86±16.70	94.75
A. flavus + *Alternaria brassicae*	1420±47.40	13.41
A. flavus + *A. alternata*	1220±18.20	25.60
A. flavus + *Curvularia lunata*	1040±16.36	36.58
A. flavus + *Helminthosporium sativum*	670±10.02	59.14
A. flavus + *Chaetomium* sp.	140±62.46	91.46
A. flavus + *Cladosporium herbarum*	144±09.37	91.12
A. flavus + *Fusarium moniliforme*	280±10.86	82.92
A. flavus + *Penicillium citrinum*	1280±12.62	21.95
A. flavus + *Rhizopus stolonifer*	170±14.39	89.63

Table 2. Inhibition in citrinin production by the toxigenic isolate of *Penicillium citrinum* when co-inoculated with competing mycoflora in liquid medium

Test mycoflora	Critinin production (µg/kg)±SE	% inhibition in critinin production
Penicillium citrinum (alone)	260±64.28	-
P. citrinum + *Aspergillus flavus*	180±14.22	30.76
P. citrinum + *A. niger*	150±22.26	42.30
P. citrinum + *Alternaria brassicae*	160±33.12	38.46
P. citrinum + *A. alternata*	140±17.90	46.15
P. citrinum + *Curvularia lunata*	120±44.60	53.84
P. citrinum + *Helminthosporium sativum*	80±70.26	69.23
P. citrinum + *Chaetomium* sp.	60±82.60	76.92
P. citrinum + *Cladosporium herbarum*	75±24.37	71.15
P. citrinum + *Fusarium moniliforme*	120±11.56	53.84
P. citrinum + *Rhizopus stolonifer*	40±79.33	84.61

niger (94.75%); *Cheatomium* sp. (91.46%); *Rhizopus stolonifer* (82.63%); and *Fusarium moniliforme* (82.92%). Whereas *P. citrinum* was co-inoculated with *Rhizopus stolonifer* (84.61%); *Cheatomium* sp. (76.92%); *Helminthosporium sativum* (69.23%) and *Fusarium moniliforme* (53.84%), respectively.

Lowest inhibition in aflatoxin B_1 and citirinin production was observed when *A. flavus* was inoculated in combination with *Alternaria brassicae* (13.41%), and *P. citrinum* was inoculated in combination with *A. flavus* (30.76%).

Maximum inhibition in aflatoxin production by *A. flavus* was observed on mustard seeds of NDR-8501 variety (Table 3) by *Rhizopus stolonifer* (83.72%) followed by *F. moniliforme* (81.62%), *C. herbarum* (72.09%), *P. citrinum* (68.60%) and *Chaetomium* sp. (59.30%).

In Raurd-101 variety, maximum inhibition in aflatoxin production by *A. flavus* was observed when the seeds were co-cultured with *P. citrinum* (74.51%), *R. stolonifer* (79.80%), *F. moniliforme* (74.51%), *C. herbarum* (60.57%) and *Chaetomium* sp. (47.11%).

Table 4 shows maximum inhibition in citrinin production on mustard seeds of NDR-8501 variety by *R. stolonifer* (63.63%) followed by *F. moniliforme* (59.09%), *C. herbarum* (50.00%), *Chaetomium* sp. (45.45%) and *H. sativum* (43.18%).

In Raurd-101 variety, maximum inhibition in citrinin production by *P. citrinum* was observed when the seeds were co-cultured with *F. moniliforme* (56.00%), *Chaetomium* sp. (46.00%), *C. herbarum* (44.00%) and *H. sativum* (30.40%).

Lowest inhibition in aflatoxin B_1 and citrinin production on mustard varieties NDR-8501 and Raurd-101 was observed when *A. flavus* was inoculated in combination with *Chaetomium* sp. (59.30 and 47.11%), whereas *P. citrinum* was inoculated in combination with *Helminthosporium sativum* (43.18 and 30.40%), respectively.

Incidence of mycoflora competing with *A. flavus* varied considerably. Individual seeds infected with more than one fungus have been reported [16, 17]. Variation in the pattern of association of competing mycoflora with *A. flavus* on mustard seeds were also reported [18, 19].

Table 3. Inhibition in aflatoxin B_1 production by the toxigenic isolate of *Aspergillus flavus* when co-inoculated with mycoflora on the seeds of two mustard varieties

Test mycoflora		Aflatoxin B_1 production (µg/kg)±SE		% inhibition in aflatoxin B_1 production	
		NDR-8501	Raurd-101	NDR-8501	Raurd-101
Aspergillus flavus (alone)		860±12.60	1040±18.32	-	-
A. flavus + *Chaetomium* sp.		350±08.44	560±20.46	59.30	47.11
A. flavus + *Cladosporium herbarum*		240±76.32	410±75.29	72.09	60.57
A. flavus + *Fusarium moniliforme*		158±50.39	265±66.10	81.62	74.51
A. flavus + *Penicillium citrinum*		270±61.73	180±35.38	68.60	82.69
A. flavus + *Rhizopus stolonifer*		140±77.80	210±30.24	83.72	79.80
	t	.320178	1.18747		
	r	.1817752	−.5654566		
	d.f.	3	3		

Table 4. Inhibition in citrinin production by the toxigenic isolate of *Penicillium citrinum* when co-inoculated with competing mycoflora on the seeds of two mustard varieties

Test mycoflora		Citrinin production (µg/kg)±SE		% inhibition in citrinin production	
		NDR-8501	Raurd-101	NDR-8501	Raurd-101
Penicillium citrinum (alone)		220±44.20	250±80.12	-	-
P. citrinum + *Helminthosporium sativum*		125±31.54	174±66.49	43.18	30.40
P. citrinum + *Chaetomium* sp.		120±19.32	135±34.27	45.45	46.00
P. citrinum + *Cladosporium herbarum*		140±77.55	140±27.20	50.00	44.00
P. citrinum + *Fusarium moniliforme*		90±24.37	110±27.20	59.09	56.00
P. citrinum + *Rhizopus stolonifer*		80±02.67	85±34.21	63.63	66.00
	t	.04375484	.3651446		
	r	.02525381	.2062822		
	d.f.	3	3		

Inhibition in aflatoxin production has been observed when *A. flavus* was co-inoculated with *A. niger* [20, 5, 21]. *A. niger* appeared to share or subdivide the resource so that each fungus sporulated in patchy sections over an equivalent area of kernel surface. Degradation of aflatoxin B_1 by *A. niger* and *Corynebacterium rubrum* has also been analysed by adding 14C-labeled aflatoxin B_1 to culture of these microorganisms [22]. One non-toxigenic strain of *A. flavus* has also been found to degrade the level of aflatoxin produced by the toxigenic strain on the same seed [23], whereas conversion of aflatoxin B_1 into other non-toxic form was recorded by different species of *Rhizopus* [24].

Higher inhibition of citrinin production has been observed when *P. citrinum* was co-inocluted with *Rhizopus stolonifer* than *Alternaria brassicae*.

Inhibition of mycotoxin production under a competitive environment is not necessarily applicable to natural condtion. In the laboratory experiment, the temperature was 28°±1°C, a very favourable condition. Under natural conditions, however, the various physical factors fluctuate. A biotic variable influence the incidence of competing fungi with *A. flavus* and *P. citrinum*. Depending on which species of competing fungal genera are present their abundance and ability to grow and compete under prevailing environmental conditions, mycotoxins may or may not be formed. Even if aflatoxins are formed, its microbial degradation can not be ruled out [25, 26, 27].

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