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Original Research Article

Occurrence of Aflatoxigenic *A. flavus* in Stored Rice and Rice Based Products of Coastal Odisha, India

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Keywords

A. flavus, aflatoxin, sclerotia, CZA and rice.

A B S T R A C T

Rice (*Oryza sativa* L.) in poor storage conditions usually get contaminated with aflatoxin producing fungi *Aspergillus flavus*. Aflatoxin is considered as Group IA carcinogen by IARC and regulated in many countries. Hence, a survey was carried out in rice growing areas of coastal Odisha regarding the occurrence of *A. flavus* in rice seeds, grains and rice based cattle feed. The farmers in those regions mainly used jute bags for storage besides traditional storage structures. Cultural, morphological and toxicological characterisations were carried out for 46 *A. flavus* isolates. Aflatoxin and sclerotia production was found to be independent of culture characters and growth patterns. Thirty seven percent of total isolates were found to be aflatoxin producing and higher aflatoxin producers (82%) were associated with parboiled grains irrespective of storage structures. Fifty percent *A. flavus* cultures isolated from parboiled husked grains and 55% *A. flavus* cultures from raw rice samples were toxigenic. Fifty eight percent *A. flavus* isolates were considered as aggressive strains which formed sclerotia (a resistive structure) in addition to aflatoxin production. Sclerotic isolates A153, A159 and A146 produced more than 25µg/ml of aflatoxin both in mycelia and culture filtrate. The current study shows higher contamination of rice and rice based commodities with *A. flavus* in this part of country.

Introduction

Paddy or rice (*Oryza sativa* L.) is the staple food for 65% of the Indian population and is a high calorific cereal grain. Due to improper conditions during harvesting and processing, this elementary important cereal remains susceptible to infection by many mycotoxigenic fungi including *Aspergillus flavus* which causes subsequent deterioration during storage and produce aflatoxins (Makun et al., 2007). Aflatoxins are highly carcinogenic secondary metabolites, produced mainly by the filamentous fungus *Aspergillus flavus*. Under improper storage conditions, *A. flavus* is capable of growing and forming aflatoxins in seeds both before and after harvest (Klich, 2007; Saini et al., 2013). Aflatoxin contamination can occur very widely and can be found in over a hundred kinds of agro-products and foods (Li et al., 2011). *Aspergillus flavus* and aflatoxin contamination of rice has been documented at international level in several reports (Tanaka et al., 2007) and also in many states of India (Siruguri et al., 2012; Gautam et al., 2012; Reddy et al., 2009; Sundaram et al., 1988; Prasad et al., 1986).

Fungi in the *A. flavus* group are ubiquitous, grow on practically any organic substrate (both living and dead plant tissues) and are superbly adapted to wide range of environmental conditions. *A. flavus* mainly produce aflatoxin B1 and B2 (AFB1 and AFB2), among which AFB1 is classified as Group 1A human carcinogen by the World Health Organisation-International Agency of Research on Cancer in 1993 (IARC, 1993) hence that is highly regulated in almost 77 countries (FAO, 2004). Some strains of *A. flavus* also produce sclerotia, structures resistant to harsh conditions and for propagation (Geiser et al., 2000; Cotty, 1988; Wicklow and Shotwell, 1983; Rollins and Dickman, 1998).

The state of Odisha in India has tropical climate, characterised by high temperature, high humidity, medium to high rainfall and short and mild winters. The normal average rainfall of the State is 1451.2 mm. Predominantly it is an agriculture based state where rice is the principal food crop occupying about 53% of gross cropped area. In the coastal regions, rivers like Mahanadi and their tributaries form a network having deltaic characteristics, the bank slopping inlands and consequently flood spilling over the bank flows away from the rivers side flooding vast stretches of lands (Khatua and Panigrahi, 2001). The geographical situation is very favourable and conducive for *A. flavus* growth on foodstuffs and subsequent toxin production.

Hence, a study on this fungus in this area is important from its prevention and control point of view. In the present investigation, a benchmark survey was carried out regarding *A. flavus* contamination in stored rice and rice based cattle feed in 29 villages of 9 coastal districts of Odisha. The diversity of cultural and morphological characters along with growth patterns and aflatoxin producing ability of forty six A. *flavus* isolates was studied.

Materials and Methods

The samples of rice based commodities i.e. rice seeds/ parboiled rice grains (husked and dehusked) or raw rice grains were collected from 29 villages of Cuttack, Jagatsinghpur, Puri, Kendrapara, Khurda, Balasore, Bhadrak, Dhenkanaal and Jajpur districts of Odisha (Figure 1). The sample size was 250g. Bhadrak and Balasore, both districts are flood prone, fertile coastal plains. Brahamgiri area of Puri district is low lying coastal area and in some villages paddy is not dried after threshing but directly packed in bags. Cuttack, Jagatsinghpur and Kendrapara are situated on the banks of river the Mahanadi and its tributaries (Figure 1).

Isolation of *A. flavus* cultures, study of cultural, morphological characters and aflatoxin production

Fungi associated with seed samples of popular rice varieties were isolated by blotter plate method (Agarwal & Sinclair 1996). The pure culture of associated micro-flora was maintained on PDA (250 gm Potato, 20 gm Dextrose, 15 gm Agar) slants. Aflatoxin was detected by thin layer chromatography (TLC) of cultural filtrates of fungi (Scott et al., 1970). Cultural characters were studied on CZA media (Sucrose 30g, Sodum Nitrate 2g, Di-Potasium Hydrogen Ortho Phosphate 1g, Magnesium Sulphate 0.5g, Potassium Chloride 0.5g, Ferrous Sulphate 0.01g, Agar agar 15g with pH 5.6).

Results and Discussion

Sample collection and pass port data

Benchmark survey in different rice growing areas of coastal Odisha regarding aflatoxin contamination was carried out. A total of 29 villages belonging to 9 coastal districts were explored for sample collection (Figure 1). About 74% of the samples were from jute bags which indicated that most of the farmers in those area stored rice or rice based commodity in jute bags but still others preferred traditional storage structures like Olia (made up of rice straw and rope), Amaar (wooden storage structure) and Doli (made up of bamboo and coated with clay cowdung straw). Olia was in use at some places of Jagatsinghpur and Dhenkanaal districts whereas Doli was used in some villages of Khurda, Puri and Bhadrak. Farmers of Kendrapara and Dhenkanaal districts preferred Amaar (Table 1 & 2). Forty six *A. flavus* cultures have been used in the present investigation of which 16 isolates were from rice seeds, 6 isolates from rice based cattle feed and 24 isolates from rice grains including raw rice (9 samples), dehusked parboiled(15 isolates) and parboiled husked (4 cultures).

Culture characters and colony growth of *A. flavus* isolates

The color of cultures on CZA media was observed and the shades were recorded as per the numbers of RHS color chart (Royal Horticultural Society). All the *A. flavus* colonies were of typical yellow green color (Koh and Tseng, 1975; Rodrigues et al., 2007) and as per the RHS color chart different shades of RHS numbers 152 or 153 were observed in the colony centers of most of the isolates (Table 3 & 4). Production of aflatoxin and sclerotia were independent of culture colors on Czapec dox agar media.

A. flavus cultures A120, A124, A126, A129, A131, A133, A137, A138 and A152 had very good growth on CZA media (Table 5 & 6). A148 was the slowest growing among the forty six isolates studied here.

Aflatoxin and Sclerotia production ability of *A. flavus* isolates

A. flavus isolates were tested for their ability to produce aflatoxin and sclerotia. Among the 46 *A. flavus* isolates used in the current investigation, 37% have been found to produce aflatoxin B1 in β-CDPD broth. When the sample type was considered, 82% isolates from Parboiled rice grains and 16% from cattle feed were aflatoxigenic. In our study, the parboiled rice stored in Olia at Jagatsinghpur and Dhenkanaal districts had aflatoxigenic isolates A124 & A145. Olia is made up of rice straw and if contaminated straw is used for making the rice rope then stored products may be contaminated. In large scale storage, where the grain may be exposed to unfavorable environments (particularly an increase in moisture content), the fungi grow and cause spoilage (Kalyanasundaram et al., 1997). *A. flavus* cultures from Parboiled samples stored in Doli and polythene bags were aflatoxigenic (Table 3). These observations confirm earlier report of Prasad *et al*. (1986) that out of 56 samples from stored rice, 12 were positive for aflatoxin.

Table.1 Passport data of *A. flavus* cultures isolated from cattle feed/ rice grain samples from coastal Odisha

Sample type	Isolate ID	Rice variety	Place / Year of collection	stored in
Cattle feed	A126	Pateli	Ambiki, Jagatsinghpur-2005	jute bags
	A133	1060	Ramiaganj, Kendrapara-2005	jute bags
	A149	1018	Viswanathpur, Dhenkanaal-2006	jute bags
	A153	1009	Ramachandraour, Bhadrak-2006	jute bags
	A157	Swarna	Rampo, Balasore-2006	jute bags
	A160	Swarna	Rampo, Balasore-2006	jute bags
Parboiled Dehusked	A117	Gedi	Nayabazar, Cuttack-2005	Jute bags
	A124	Khandagiri	Chaulia, Jagatsinghpur-2005	Olia
	A128	1018	Juna,Kendrapara-2005	Jute bags
	A132	1060	Sanarajgada, Kendrapara-2005	Jute bags
	A142	Swarna+56	Chormuhan narsinghpur, Jajpur-2006	Polythene bags
	A145	Hero honda	Sorisiapada, Dhenkanaal-2006	Olia
	A150	Bankei	Nandapur, Puri-2006	Jute bags
	A154	Swarna	Rajpur, Bhadrak-2006	Jute bags
	A155	Swarna	Tihidi rajpur, Bhadrak-2006	Doli
	A158	Swarna	Rampo, Balasore-2006	Jute bags
	A159	Swarna	Rampo, Balasore-2006	Jute bags
Parboiled husked	A131	Gayatri	Juna,Kendrapara-2005	wooden Amaar not maintained
	A137	Gayatri	Patalda, Puri-Astarang-2005	Jute bags
	A144	Swarna	Chormuhan narsinghpur, Jajpur-2006	Jute bags
	A151	Mixed	Satpadagarh, Puri-2006	Doli
Raw Dehusked	A116	Mota	OMP square, Cuttack-2005	Jute bags
	A120	Kalashree	Kalara banka, Cuttack-2005	Jute bags
	A129	CR260-77	Juna,Kendrapara-2005	Jute bags
	A135	CR 898	Hiradeipur,Puri-Astarang-2005	Jute bags
	A138	1009	Timor, Puri-Astarang-2005	Jute bags
	A143	5656	Chormuhan narsinghpur, Jajpur-2006	Polythene bags
	A146	Swarna	Bania, Dhenkanaal-2006	Jute bags
	A152	Kartali	Satpadagarh, Puri-2006	Jute bags
	A161	Swarna	Pandu, Balasore-2006	Polythene bags

Table.2 Passport data of *A. flavus* cultures isolated from rice seeds collected from coastal Odisha

Isolate ID	Place / Year of collection	Rice variety	stored in
A118	Nanpur, Cuttack-2005	5656	Jute bags
A119	Nanpur, Cuttack-2005	Basmati Dhusura	Jute bags
A121	Bodara, Cuttack-2005	Mahandi	Jute bags
A122	Gothina, Jagatsinghpur-2005	1018	Jute bags
A123	Chaulia, Jagatsinghpur-2005	Langalmunda	Olia
A125	Ambiki, Jagatsinghpur-2005	Bhaluki	Jute bags
A127	Ambiki, Jagatsinghpur-2005	Pateli	Jute bags
A130	Juna,Kendrapara-2005	Gayatri	Wooden Amaar well maintained
A134	Hiradeipur,Puri-Astarang-2005	CR1009	Jute bags
A136	Patalda, Puri-Astarang-2005	Tulasi	Jute bags
A139	Timor, Puri-Astarang-2005	Mayurkantha	Jute bags
A140	Kolathala, Jajpur-2005	Khandagiri	Jute bags
A141	Chaandpur, Jajpur-2006	Swarna	Jute
A147	Bania, Dhenkanaal-2006	Kolia	Jute bags
A148	Deogaon, Dhenkanaal-2006	Kolia	Amaar
A156	Jagulaipatana, Khurda-2006	Pooja	Doli

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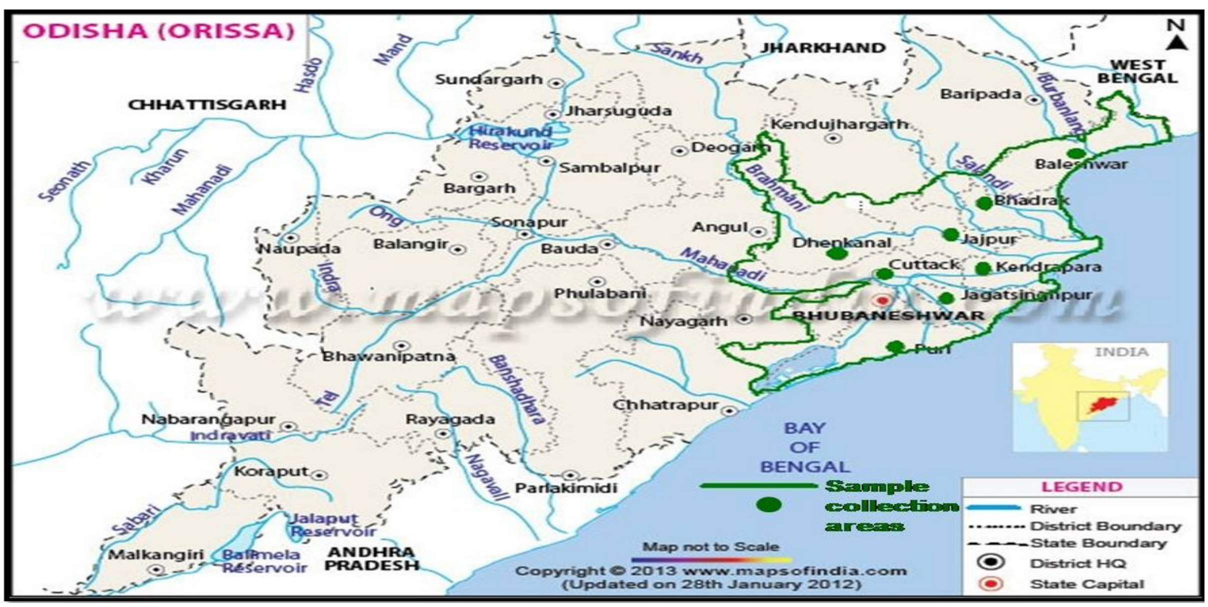

Figure.1 District and river map of Odisha state of India. The highlighted green coloured areas show the site of sample collection.
(Source of map; www.mapsofindia.com)

Table.3 Cultural characters and sclerotia producing ability on CZA and toxin production of *A. flavus* cultures isolated from cattle feed/ rice grain samples from coastal Odisha media

Sample type	Isolate ID	*Culture Colour on CZA media			**aflatoxin		Mycelia weight (mg)
		On surface	Petridish bottom	Sclerotia	mycelia	filtrate	
Cattle feed	A126	146B, 152D	22D	-	-	-	280
	A133	146B, 152D	25D	-	-	-	335
	A149	146A, 152B	22A	+	-	-	355
	A153	146B, 152C	22B	+	++++	++++	230
	A157	146B, 152D	22A	+	-	-	305
	A160	146D, 152D	22B	+	-	-	435
Parboiled Dehusked	A117	146C, 152A	22C	-	-	-	370
	A124	146B, 153D	22A	+	-	++	455
	A128	146B, 153D	22B	-	++	+++	400
	A132	146A, 153D	22A	-	+	++	345
	A142	146A, 152C	22B	+	-	-	440
	A145	146A, 152D	22A	-	+	++	535
	A150	146A, 152C	22A	-	++++	++++	295
	A154	146A, 152C	22A	-	+++	++++	255
	A155	146A, 152A	22D	+	+++	++++	250
	A158	146B, 152D	22B	+	+++	++++	310
	A159	146D, 152D	22D	+	++++	++++	480
Parboiled husked	A131	146B, 153D	22B	+	+	++	335
	A137	146A, 152D	22D	-	-	-	390
	A144	146A, 152D	22B	-	-	-	505
	A151	146A, 152A	Colourless	+	++	++++	440
Raw Dehusked	A116	146C, 152B	22B	+	-	-	265
	A120	146C, 152A	22C	+	-	-	275
	A129	146B, 152C	22D	-	++	+++	370
	A135	146A, 152D	22D	-	-	-	345
	A138	146A, 152B	22D	-	-	-	405
	A143	146A, 152C	22C	+	-	-	425
	A146	146A, 152B	22D	+	++++	++++	435
	A152	146D, 152D	22A	+	++	+++	430
	A161	146D, 152A	22C	-	++++	++++	315

*Culture colour codes are according to RHS colour chart
**Quantity of aflatoxin: - No toxin; + Less than 2µg/ml; ++ 2µg/ml to 9µg/ml;

Table.4 Cultural characters and sclerotia producing ability on CZA and toxin production of *A. flavus* cultures isolated from rice seed samples from coastal Odisha

Isolate ID	*Culture Colour on CZA media			**aflatoxin (PD broth)		Mycelia weight (mg)
	On surface	Petridish bottom	Sclerotia	mycelia	filtrate	
A118	146C, 152A	22D	-	-	-	285
A119	146C, 152A	22D	+	-	-	320
A121	146B, 153D	34C	+	-	-	350
A122	146B, 153D	22B	+	-	+	455
A123	146B, 152D	32C	-	-	-	360
A125	146B, 153D	22B	-	-	-	390
A127	146B, 153D	22B	+	-	-	350
A130	146B, 152C	22D	-	-	-	255
A134	146A, 153D	22A	+	-	-	450
A136	146A, 152D	22D	-	-	-	280
A139	146A, 152D	22D	-	-	-	280
A140	146A, 152C	22B	+	-	-	335
A141	146A, 152C	22B	+	-	-	415
A147	146A, 152A	22D	+	-	-	425
A148	146A, 152D	22D	-	-	-	325
A156	146A, 152D	22B	-	-	-	290

*Culture colour codes are according to RHS colour chart

**Quantity of aflatoxin: - No toxin; + Less than 2µg/ml; ++ 2µg/ml to 9µg/ml; +++ 10µg/ml to 25µg/ml; ++++ More than 25µg/ml

Three of these came from containers of woven rice straw, 2 each from earthen pots, iron bins and gunny bags. The *A. flavus* cultures from paddy (viable rice seeds), except A122 (isolated from seeds stored in Olia in Jagatsinghpur), were non aflatoxigenic (Table 4). About 50% *A. flavus* cultures isolated from parboiled husked grains and 55% *A. flavus* cultures from raw rice samples were toxigenic (Table 3).

Bilgrami and Choudhary (1993) reported that the frequency of non-aflatoxigenic strains of *A. flavus* to be comparatively higher (ratio=1.07) than toxigenic strains. This ratio of toxigenic and nontoxigenic strains varies with the sources and location of their isolations and also culture media, growth conditions, age and purity of cultures (Saito and Machida, 1999; Koh and Tseng, 1975). The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage. Physiological stages of grains are important as well (Kacaniova, 2003).

Table.5 Growth *A. flavus* cultures isolated from cattle feed and rice grain on CZA media

Isolate ID	Colony Diameter On Different Days (mm)							
	day2	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**
Cattle feed								
A126	14.5	22.3	32.5	42.8	53.5	67.8	80.8	89.5
A133	11.3	21.5	31.3	41.3	51.5	61.5	79.0	84.5
A149	11.8	22.0	30.3	38.8	47.8	52.8	63.5	73.8
A153	14.5	22.3	30.5	36.3	44.3	50.5	72.0	83.8
A157	13.5	20.5	28.5	32.5	39.8	48.5	61.5	67.8
A160	12.3	22.5	31.3	36.5	45.3	56.8	66.3	71.3
Parboiled Dehusked								
A117	12.0	20.5	28.5	37.5	46.0	54.0	64.0	67.3
A124	14.8	25.0	34.3	44.5	54.5	72.5	79.3	87.8
A128	12.3	18.5	27.3	38.5	49.0	54.3	70.5	78.5
A132	14.5	20.5	29.0	38.8	49.3	56.8	60.5	69.8
A142	14.5	23.0	28.5	35.8	44.8	51.0	65.5	78.8
A145	15.3	24.3	30.8	37.3	44.0	48.5	63.5	73.3
A150	16.0	25.3	37.3	40.0	43.3	59.8	74.3	80.3
A154	14.8	23.8	34.3	39.5	47.3	59.8	78.5	90.0
A155	14.0	22.5	31.3	40.5	51.8	58.5	71.0	79.5
A158	14.0	21.8	31.0	37.5	45.5	52.5	67.0	77.0
A159	11.0	17.5	24.3	30.0	37.8	46.0	52.3	56.8
Parboiled Husked								
A131	11.8	20.5	31.0	41.3	52.8	71.3	82.5	88.3
A137	12.3	21.5	32.8	44.8	57.5	68.5	79.5	83.8
A144	14.5	22.3	29.5	37.0	46.0	51.0	64.5	75.3
A151	15.0	22.0	30.0	37.3	46.5	53.5	69.3	77.5
Raw Dehusked								
A116	11.8	20.3	27.5	34.8	43.8	52.3	58.3	65.0
A120	15.5	28.5	38.8	48.8	58.5	81.0	89.0	90.0
A129	11.0	21.8	30.5	40.3	48.8	63.3	80.8	87.3
A135	13.0	22.8	31.5	40.5	50.0	66.0	75.3	81.8
A138	11.5	21.5	31.8	42.3	53.3	68.8	85.8	90.0
A143	15.5	22.3	29.0	36.3	43.5	52.0	63.8	73.5
A146	14.5	23.0	29.3	34.3	40.3	47.5	64.8	75.5
A152	15.3	25.5	31.8	39.0	48.3	61.3	79.5	90.0
A161	15.5	23.0	30.3	36.8	46.0	57.0	69.0	76.0

LSD at $p<0.05$ is 1.42 LSD at $p<0.01$ is 1.87

Table.6 Growth *A. flavus* cultures isolated from rice seed grain on CZA media

Isolate ID	Colony Diameter On Different Days (mm)							
	day2	**day 3**	**day 4**	**day 5**	**day 6**	**day 7**	**day 8**	**day 9**
A118	12.3	21.8	30.5	39.5	49.0	61.0	67.0	77.0
A119	15.3	27.0	37.5	47.5	59.0	77.5	89.0	90.0
A121	14.8	22.5	31.5	41.5	51.5	55.5	63.8	71.5
A122	14.3	21.0	30.3	38.8	49.0	59.0	65.0	73.8
A123	14.8	26.0	38.3	49.8	62.8	75.0	81.8	90.0
A125	11.8	19.3	28.3	36.8	46.0	64.3	70.5	78.3
A127	12.3	18.5	27.8	36.8	46.8	56.5	63.3	72.8
A130	13.0	19.8	28.0	36.3	44.5	53.0	58.3	66.0
A134	12.3	20.8	29.3	38.3	47.3	59.8	63.3	74.8
A136	12.3	21.3	31.0	40.8	51.0	65.8	74.8	83.8
A139	13.5	21.8	31.8	43.0	55.3	68.8	84.0	90.0
A140	17.0	24.5	30.5	36.8	42.3	52.5	65.0	75.0
A141	17.0	24.0	29.0	35.5	43.3	51.8	64.8	74.5
A147	14.0	23.3	31.0	37.0	44.0	52.3	69.5	80.0
A148	11.3	17.3	26.0	30.8	37.0	42.3	51.5	57.5
A156	12.0	19.5	26.5	30.5	36.5	46.3	58.0	68.0

LSD at $p<0.05$ is 1.91 LSD at $p<0.01$ is 2.509

Frequent and heavy rainfall along with flash floods, particularly during harvest, in coastal areas in eastern (such as Odisha), southern, and western regions of the country provide ideal substratum for the growth of moulds which become more prone to invasion by filamentous fungi and bacteria (Khatua and Panigrahi, 2001; Makun et al., 2007; Reddy et al., 2009; Saini et al., 2013). Farmers in this region usually keep the contaminated rice grains in poor storage conditions which lead to the growth and proliferation of storage fungi like *A. flavus* which include toxic and nontoxic strains along with sclerotia producing and nonproducing strains under favorable environmental conditions (Kacaniova, 2003).

Aflatoxin is also water soluble hence quantity of aflatoxin retained in mycelia or filtrate was estimated. Though in all toxigenic isolates, aflatoxin B1 was detected in both mycelia and culture filtrates (except for A124 and A122 in which aflatoxin B1 was present in culture filtrates only), higher amount was always detected in the later (Table 3 & 4). The moderate aflatoxin producers A131, A132 and A145 had aflatoxin in 1:2 ratio in mycelia and cultural filtrate. *A. flavus* isolates with very good aflatoxin producing ability (i.e. having more than 25μg of aflatoxin/ml) retained toxin in 1:1 or 3:4 ratio in mycelia: filtrate. The paddy may be exposed to rain due to cyclone at the time of harvesting in coastal areas of

Odisha and these findings indicate that major portion of produced toxin may be dissolved in rain water and may contaminate paddy or straw. The growth of some of the aflatoxigenic isolates was slow and some were fast growing (Table 5 & 6). These observations concur the findings of Schindler et al (1967) that the aflatoxin production was not related to growth rate of *A. flavus*. Priyadarshini and Tulpule (1978) also found no correlation between growth and aflatoxin production of *A. flavus* among varieties of maize and groundnut.

This strictly mitotic fungus, besides producing conidia asexually also produced sclerotia which are the structures resistant to harsh conditions and meant for fungal propagation Dawar and Ghaffar (2002) reported that the sclerotial strains of *Aspergillus flavus* showed greater aflatoxin production as compared to non-sclerotial strains. This differs from the findings of Chang et al (2001) who found an inverse relationship between aflatoxin and sclerotial production. It also differs from the results of a survey of 70 isolates from Arizona where all isolates produced sclerotia and S-strain isolates produced about ten times more aflatoxin, in vitro, than the L-strain isolates (Cotty, 1989).

In present investigation fifty eight percent of the toxigenic isolates produced both sclerotia and aflatoxin, hence considered as aggressive strains. Among them A153, A159 and A146 were very aggressive strains as in addition to sclerotia, more than 25µg/ml of aflatoxin both in mycelia and culture filtrate was produced by these isolates (Table 3 & 4). These sclerotia were like tiny toxin balls which adhered to the surface of rice seeds, thus contaminated those and also might have crossed geographical boundaries.

It was found that rice and rice based agricultural commodities in coastal Odisha were contaminated with aflatoxin and sclerotia producing strains of *A. flavus*. Although all the *A. flavus* were not toxigenic but most of the isolates associated with parboiled rice were aflatoxigenic. The highly variable pattern in colony, growth, morphological and toxicological characters indicated high degree of diversity among the isolates included here. The sclerotia producing aflatoxigenic strains are a matter of concern. Hence, quick identification and management of these aflatoxigenic strains in this region is essential in public interest.

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