International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 11 (2014) pp. 116-121**
http://www.ijcmas.com

Original Research Article

Mycotoxigenicity of *Aspergillus, Penicillium* and *Fusarium* spp. isolated from stored rice

Archana Sawane* and Manish Sawane

Department of Botany, S.M. Mohota College of Science, Nagpur, India
**Corresponding author*

K e y w o r d s

Aspergillus, *Fusarium*, Mycotoxins, *Penicillium*, Rice

A B S T R A C T

Rice (*Oryza sativa* L.) is an important food crop worldwide. Presence of mycotoxins like aflatoxin, citrinin, sterigmatocystin and ochratoxin has been detected as natural contaminants of rice in one or the other region of the world. However, only scanty information is available pertaining to presence of mycotoxin producing fungi and their toxins in India. Seedborne storage fungi were evaluated by standard blotter test on the 36 rice samples collected from different "godowns" of traders in rainy season, winter and summer season. Out of 96 isolates belonging to 16 species of *Aspergillus*, *Penicillium* and *Fusarium*, 34 isolates produced one or more of the 8 known and 3 unknown secondary metabolites by thin layer chromatography.

Introduction

Rice (*Oryza sativa* L.) is an important food crop worldwide along with wheat and corn, and has been major food in several countries. As bulk of rice is grown in *kharif* or the rainy season, it is prone to natural mycotoxin contamination. Frequent and heavy rainfall and floods, particularly near harvest wet the crop and make panicles more prone to invasion by fungi and bacteria. Inadequate drying and faulty storage conditions may not adequately reduce the moisture content in grains leading to invasion by both field and storage fungi. High temperature and high humidity particularly in monsoon (rainy) season in India adds to vulnerability of the stored products for growth of mycotoxin-producing fungi (Goyal, 1989).

There are substantial studies on field fungi associated with rice in India; however, studies of storage fungi associated with rice particularly at the consumer end are conspicuous by their absence. One of the most important effects of post harvest decays of seed and feed deterioration by fungi is the production of mycotoxins and induction of mycotoxicoses in humans and animals. After ingestion, mycotoxins enter in human body and encounter various molecules. Mycotoxins interact with gastrointestinal microflora, epithelial cells of intestine, liver, bile, blood, kidney, reproductive and nervous systems, skin and lungs. The effects of mycotoxins may be carcinogenic, mutagenic, and teratogenic with the result of reduced immune response with low doses and acute disease syndrome with high doses (Wyllie and Morehouse, 1978). There is very meager research

studying mycotoxigenicity of storage fungi isolated from rice in Vidarbha and Central India. Mycotoxigenic potential of fungal species isolated from collected rice samples would give idea about potential danger associated with these storage fungi.

The aim of the present investigation, therefore, was to study prevalence of storage fungi associated with rice in samples collected from grain markets. One more aim of the study was to evaluate mycotoxin producing potential of storage fungal isolates obtained from rice samples.

Materials and Methods

Usually rice is harvested in November–December in India and polished rice is stored by the traders in gunny bags in their small godowns for up to one year. Thirty six rice samples were collected from different "godowns" of traders throughout the year, 12 each in rainy season, winter and summer. The evaluation of storage fungi on rice samples was done by standard blotter test (Mathur and Kongsdal, 2003). Sub-cultures were made from developing colonies and pure cultures of *Aspergillus, Fusarium* and *Penicillium* were obtained for subsequent studies by three point inoculation technique on Czapek agar (CA) for *Aspergillus*, potato sucrose agar (PSA) for *Fusarium*, Czapek yeast autolysate agar (CYA) for *Penicillium.* Cultures were incubated for 7 days at 25^0C without illumination in an upright position.

Identification of different species of storage fungi namely *Aspergillus, Penicillium* and *Fusarium* was done up to species level with the help of standard literature (Gilman 1945, Pitt, 1989; Singh *et al.* 1991, Leslie *et al.* 2006). The isolation frequencies (Fr) of different fungal species were calculated as

Isolation frequency = No. of samples infected by a species/Total No. of samples tested x 100

Small agar plugs were cut from the fungal colonies grown on these media using a cork borer with inner diameter 4 mm from the centre of the colony. The plugs were wetted by a drop of chloroform/methanol (2:1, v/v), and mycelia side of the plugs were immediately applied on to TLC plates (Silicagel 60, 20x20 cm, Merck) for few seconds, 2.5 cm from the bottom line. The diameter of the application spot was always less than 0.6 cm (Singh *et al.* 1991). As an external standard, griseofulvin solution was applied in the centre lane of the TLC plate. After the run in TEF (Toluene/ethylacetate/90% formic acid, 5:4:1, v/v/v) solvent, developed plates were allowed to dry and then viewed in daylight, under long wave (366 nm) and short wave (254 nm) ultraviolet (UV) light. Developed TLC plates were also treated with spraying agent 0.5% p-anisaldehyde in methanol/acetic acid/conc. sulphuric acid (17:2:1), heated for 8 minutes at 130^0C and again viewed in daylight, long and short wave UV light. Some mycotoxins appeared only on treatment with spraying agent. The retardation factors (Rfg values) were calculated relative to griseofulvin as:

Rfg = Distance travelled by a spot in mm / Distance travelled by Griseofulvin in mm.

The Rfg values of the observed spots were compared with the Rfg values for different mycotoxins published by Singh *et al.* (1991).

Results and Discussion

Thirty six samples collected from "godowns" of traders were tested for the isolation of *Aspergillus, Penicillium* and *Fusarium.* Isolation frequency of different species from rice samples in three different seasons (rainy, winter and summer) are given in Table 1.

Mycotoxin potential of all the species of *Aspergillus, Penicillium* and *Fusarium* isolates obtained during this investigation are depicted in Table 2. As mycotoxins were identified by comparing Rfg values with the published Rfg values for different mycotoxins, evaluation of mycotoxins potential of different fungal species became preliminary in nature and requires confirmation by using different standards.

However, availability, cost and safety measures of standard mycotoxins justify the comparative method for preliminary study. Out of 96 isolates belonging to 16 species of *Aspergillus, Penicillium* and *Fusarium*, 34 isolates produced one or more of the 8 known and 3 unknown secondary metabolites. Aflatoxin B_1, ochratoxin A, citrinin, cyclopiazonic acid, sterigmatocystin, penicillic acid, moniliformin and fusarin C were produced by different isolates of storage fungi namely *Aspergillus, Penicillium* and *Fusarium* species.

Table.1 Prevalence of seed borne fungi on rice samples obtained during different seasons

S.N.	Fungus	Rainy		Winter		Summer	
		No. of samples infected (out of 12)	Isolation frequency	No. of samples infected (out of 12)	Isolation frequency	No. of samples infected (out of 12)	Isolation frequency
1.	*Aspergillus niger*	8	66.7	5	41.7	4	33.3
2.	*A. flavus*	3	25.0	5	41.7	4	33.3
3.	*A. versicolor*	2	16.7	6	50.0	3	25.0
4.	*A. ochraceus*	9	75.0	6	50.0	1	8.3
5.	*A. terreus*	2	16.7	4	33.3	-	0.0
6.	*A. fumigatus*	5	41.7	4	33.3	2	16.7
7.	*A. parasiticus*	4	33.3	3	25.0	2	16.7
8.	*Penicillium citrinum*	6	50.0	6	50.0	-	0.0
9.	*P. oxalicum*	4	33.3	8	66.7	1	8.3
10.	*P. purpurogenum*	3	25.0	6	50.0	3	25.0
11.	*P. chrysogenum*	4	33.3	7	58.3	3	25.0
12.	*P. frequentans*	5	41.7	9	75.0	3	25.0
13.	*Fusarium verticilloides*	7	58.3	4	33.3	3	25.0
14.	*F. semitectum*	6	50.0	5	41.7	-	0.0
15.	*F. proliferatum*	7	58.3	6	50.0	-	0.0
16.	*F. oxysporum*	6	50.0	6	50.0	2	16.7

Table.2 Secondary metabolites produced by some storage fungi isolates obtained from rice

S.N.	Species	Rfg values of spots on TLC	Secondary metabolites after comparison with published data	No. of Isolates tested	No. of Isolates producing sec. metabolites
1	*Aspergillus niger*	1.39.	Ochratoxin A	6	3
2	*A. flavus*	1.37	Cyclopiazonic acid	6	5
		0.56	Aflatoxin B_1		3
3	*A. versicolor*	1.75	Sterigmatocystin	6	3
4	*A. terreus*	1.75	Sterigmatocystin	6	2
5	*A. ochraceus*	1.39	Ochratoxin A	6	3
		1.03	Penicillic acid		2
6	*A. fumigatus*	0.99	Unknown	6	2
7	*A. parasiticus*	--	--	6	--
8	*Penicillium citrinum*	1.23	Citrinin	6	5
9	*P. oxalicum*	1.57	Unknown	6	2
10	*P. purpurogenum,*	--	--	6	--
11	*P.chrysogenum*	--	--	6	--
12	*P. frequentans*	0.95	Unknown	6	3
13	*Fusarium verticilloides*	0.58	Fusarin C	6	2
14	*F. semitectum*	--	--	6	--
15	*F. proliferatum*	0.1	Moniliformin	6	2
16	*F. oxysporum*	--	--	6	0

Similar potential hazard of mycotoxin production by rice associated fungi has been demonstrated in few studies in India. *Aspergillus flavus, A. niger, A. nidulans, A. terreus, A. parasiticus, Penicillium chrysogenum, P. citrinum, Fusarium* sp. were isolated from stored rice grains from samples collected from District Mandi, Himachal Pradesh in India with relative incidence of 41.6% for *Aspergillus*, 16.6% for *Penicillium* and 8.3% for *Fusarium* (Gautam *et al.* 2012). 72% samples tested showed presence of aflatoxins B1 and B2 in their study. However, aflatoxin levels in stored rice samples with damaged grains less than 5% did not exceed the tolerance limit of aflatoxin in India (Siruguri *et al.* 2011).

Aspergillus flavus, A. niger, A. terreus, and species of *Fusarium* and *Penicillium* were recorded with maximum percentage of frequency on paddy samples collected from Godavari belt region of Andhra Pradesh (Saini *et al.* 2013). Incidence of toxigenic strains was quite high in their

study with production of mycotoxins like aflatoxins, patulin, terreic acid, ochratoxin A, citrinin, zealarenone, nivalenon, deoxynivalenol and sterigmatocystin by one or other strains of the fungi isolated.

"In India, warm humid climate provides congenial atmosphere for the growth of fungi and production of toxins. Food grains are normally harvested at higher moisture content and then dried to bring down the moisture content up to safe level before storage. Delay in drying to safe moisture levels increases risks of mould growth and mycotoxin production. Natural calamities like floods or torrential unseasonal rains during pre, mid or post-harvest stages may render the crops vulnerable to microbial attack. Faulty storage conditions may also enhance the chances of microbial attack and production of mycotoxins. Thus, there are chances of microbial invasion/fungal attack at each and every stage starting from harvesting of the crop till the food or food products are consumed by the consumers." (Goyal, 1989).

"In developed countries, sophisticated equipment and procedures are used for post harvest storage, mycotoxins prevention and control; whereas in developing countries, substandard food grains may be consumed without any form of sorting or inspection. Mycotoxin ingestion remains far too high in many countries, especially in rural areas" (Pitt, 1989).

The production of mycotoxins by the storage fungi isolates obtained from rice samples in the present investigation has demonstrated the hazards associated with the presence of these species in stored rice.

In light of this report, further confirmatory investigations for different mycotoxins, particularly quantitative estimations directly in the grain samples collected in different seasons needs to be undertaken by the researchers, which can play an important role in formulating food safety guidelines.

References

Gautam, A.K., Gupta, H., Soni, Y. 2012. Screening of fungi and mycotoxins associated with stored rice grains in Himachal Pradesh. *Int. J. Theor. Appl. Sci.*, 4(2): 128–133.

Gilman, J.C. 1945. A manual of soil fungi, 2nd Indian edn. Biotech Books, Delhi, India.

Goyal, R.K. 1989. Prevention and control of mycotoxins in food grains in India. Section VI. In: Mycotoxin prevention and control in Food grains. Semple, R.L., Frio, A.S., Hicks, P.A., Lozare, J.V. (Eds). Food and Agriculture Organization of United Nations Corporate Document Repository, RAP publication, URL: http://www.fao.org/docrep/X5036E/X5036E00.htm

Leslie, J.F., Summerell, B.A., Bullock, S. 2006. The fusarium laboratory manual. Blackwell Publishing House, Oxford. 388 Pp.

Mathur, S.B., Kongsdal, O. 2003. Common laboratory seed health testing methods for detecting fungi, 1st edn. International Seed Testing Association (ISTA), Bassersdorf, Ch-Switzerland. 425 Pp.

Pitt, J.I. 1989. Section I: Toxigenic *Aspergillus* and *Penicillium* species. In: Semple, R.L., Frio, A.S., Hicks, P.A., Lozare, J.V. (Eds.), Mycotoxin prevention and control in Food grains. RAP publication Food and Agriculture Organisation of United Nations Corporate Document

Repository, URL: http://www.fao.or /docrep /X5036E/ X5036E00.htm.

Saini, K., Surekha, M., Reddy, S.R., Reddy, S.M. 2013. Incidence of toxigenic fungi on paddy of Godavari Belt region, A.P., India. *Asian J. Exp. Biol. Sci.*, 4(3): 426–430.

Singh, K., Frisvad, J.C., Thrane, U., Mathur, S.B. 1991. An illustrated manual on identification of some seed-borne *Aspergilli, Fusaria, Penicillia* and their mycotoxins. *Dan. Gov. Inst. Seed Pathol., Tech. Bull.,* Pp 133.

Siruguri, V., Kumar, P., Raghu, P., Rao, V.V.M., Sesikeran, B., Toteja, G.S., *et al.* 2012. Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India. *Ind. J Med. Res.*, 136(7): 89–97.

Wyllie, T.D., Morehouse, L.G. 1978. Mycotoxic fungi, mycotoxins, mycotoxicoses: An encyclopedic handbook. Vol. I, Marcel Dekker Inc., New York and Basel.