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# Isolation and identification of pathogenic fungi from post-harvested stored grains in Jalgaon district of Maharashtra

Prakash S. Lohar and Sandhya M. Sonawane*

*PG Research Center, Department of Biotechnology, MGSM's A. S. C. College, Chopda 425107*
*DMV's Nutan Maratha College, Jalgaon India*

## ABSTRACT

Stored grain samples of cereals, pulses, rice and wheat were collected and separately analyzed to identify the presence of fungi. Pure cultures of mycoflora associated with post-harvested stored grains were prepared after first screening by spread plate method and colonies of fungi were grown using Sabouraud dextrose agar medium. Morphological features and DNA sequencing methods were adopted to identify and confirm the fungal species. The study area under investigation shown presence of *Aspergillus terreus*, *Aspergillus oryzae*, *Aspergillus glaucus*, and *Syncephalastrum racemosum* in the stored grains. It was found that the mycoflora of stored grains predominantly consisted of ubiquitous mould genera *Aspergillus* where as *Syncephalastrum racemosum* was found in lesser extent and infrequently.

**KEY WORDS:** STORED GRAINS, FUNGUS SPECIES, ISOLATION AND IDENTIFICATION

## INTRODUCTION

India is a world leader in the production of food grains and protection of grain in storage in bags or bulk is important to assure food security. Losses of stored grain worldwide are in the range of 5-10% or about 20 million tones a year with insects and molds, and can exceed to more than 50% if one has to include losses due to rodents and birds. Maintaining quality and integrity of food grains in storage is essential. Despite advances in science, protection losses do occur to this day.

The storage fungi damage the grains in several ways; they reduce the germinability, produce undesirable odor and kernel discoloration, decrease the food value and also produce toxins injurious to the health of consumers. The mycoflora of stored wheat grains predominantly consisted of ubiquitous mould genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Mucor*, *Rhizopus* and *Penicillium* possibly because of their omnipresence, capacity to grow on all possible substrates and a wide range of temperature and humidity. The grain losses found in quantity and quality; can be in the form of depletion in seed viability, hardness, color, size and shape, grain weight and various biochemical parameters viz., protein, carbohydrate and vitamins under post harvest storages. Fungal infestation is the major factor of stored grain spoilage. The post harvest loss of wheat grain has been found to be highest during storage. Stored grains can have losses in both quantity and quality. Losses occur when the grain is attacked by microorganisms and other organisms including insects, mites, rodents and birds (Magan *et al.*, 2003; Neetirajan *et al.*, 2007; Mathew *et al.*, 2010).

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**Corresponding Author*
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Mycotoxigenic moulds produce mycotoxins, which are secondary metabolites frequently, produced in grain crops, cereals, pulses, dried fruits, feeds, nuts and other commodities. Although a wide variety of moulds is known to produce mycotoxins, only a few genera, *Aspergillus, Penicillium, Fusarium, Alternaria* and *Clevicеps* are considered important in foods. Mycotoxins are metabolites from moulds that are toxic to humans and domestic animals associated with food, animal feeds including wild birds and raw materials.

## MATERIALS AND METHODS

### a) Collection of stored grain samples infected with fungal species:

The samples of stored grains (S1 to S5 ) including cereals, pulses, rice; wheat etc infected with fungal strains were collected from storage containers and godowns of Chopda, Amalner, Yawal, Bhusawal of Jalgaon district and in the city of Jalgaon (Figure 1). The collected samples were put into sterile polythene bags and seal properly. Samples were brought into laboratory for further processing as per the methods described by Fente *et al.* (2001) and Sekar *et al.* (2008).

### b) Processing of stored grains for isolation of fungal strain by dilution method:

The infected stored grains samples were brought in to laboratory under aseptic condition where they were screened (before washing and after washing with water) for their associated fungal flora. Ten grams of grain samples were aseptically weighed and transferred separately to 250 ml sterile flask containing 100 ml of sterilized water and 10 g of sterilized sand (a dispersing agent). The flasks were then shaken for thirty minutes on a rotary shaker. After the foam developed had subsided, serial dilutions were done for the original solution.

### c) Isolation of fungal strain by spreading method:

Triplicate plates of each dilution were made by pipetting out 1 ml solution from flask to sterilized petridishes containing 20 ml Sabouraud dextrose agar (SDA) medium and streptomycin (0.70 gm/liter) to inhibit bacterial growth. The dishes were gently swirled to disperse the spores uniformly and after the medium had solidified, the SDA plates (SDA 1 to 5) were incubated at 25 10C for 5 days. Each set of experiment had a control to differentiate the laboratory contaminants from the microflora actually associated with the sample.

### d) Isolation of fungal strain by streak method

After first screening by spread plate method, each colony of fungus was picked up and transferred to separate SDA plate to obtain pure culture (Figure 3).

### E) Identification of fungal strain by morphotaxo-nomy and DNA sequencing:

Isolates of five fungal cultures (SDA 1 to 5) isolates were identified by Mycology Division of Agharkar Research Institute, Pune based on the morphotaxonomy and DNA sequencing.

## RESULTS AND DISCUSSION

Morphology characterization of fungal species was done by microscopic observations and their genetic characterization was done by DNA sequencings and comparing with genetic database using BLASTN programme. Four different fungal species were identified from stored grains collected from containers and godowns of Chopda, Amalner, Yawal, Bhusawal of Jalgaon district and in the city of Jalgaon (Table1).

Mycoflora of the study area comprising *Aspergillus terreus, Aspergillus oryzae. Aspergillus glaucus*,and *Syncephalastrum racemosum* has shown distinct morphological features

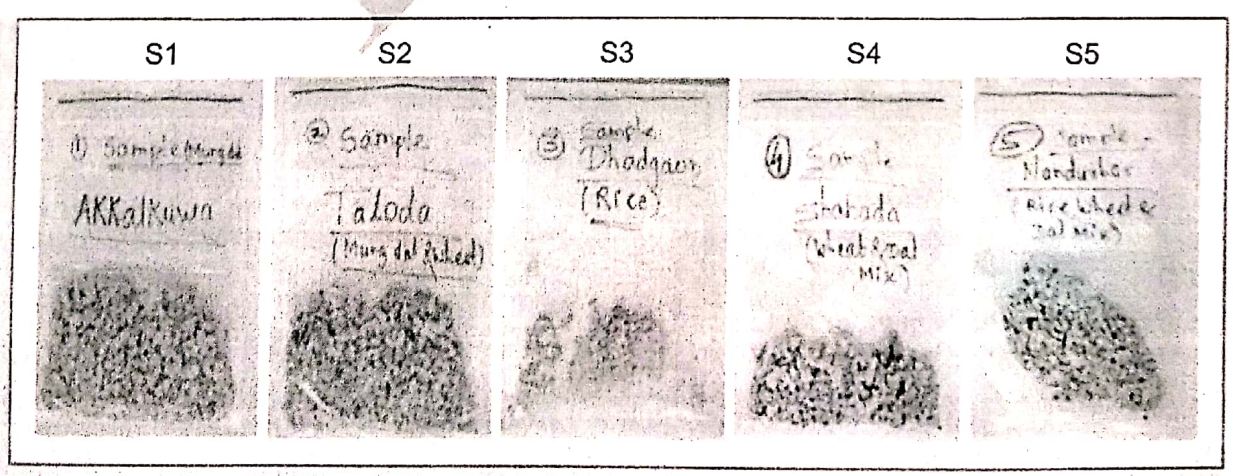


FIGURE 1: Infected stored grains samples collected from study area.

TABLE 1: Fungal species identified on the basis of morphological features and DNA sequencing.

| S. No. | Culture Code No. | Fungal species identified |
|---|---|---|
| 1 | SDA 1 | *Aspergillus terreus* Thom |
| 2 | SDA 2 | *Aspergillus oryzae* (Ahlburg.) Cohn. |
| 3 | SDA 3 | *Aspergillus glaucus* |
| 4 | SDA 4 | *Syncephalastrum racemosum* Cohn; Schröt |
| 5 | SDA 5 | *Aspergillus oryzae* (Ahlburg.) Cohn. |

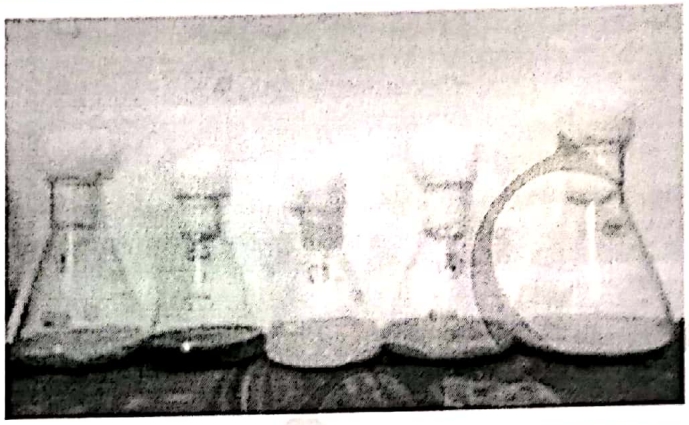

FIGURE 2: Flasks containing fungal strains to be identified.

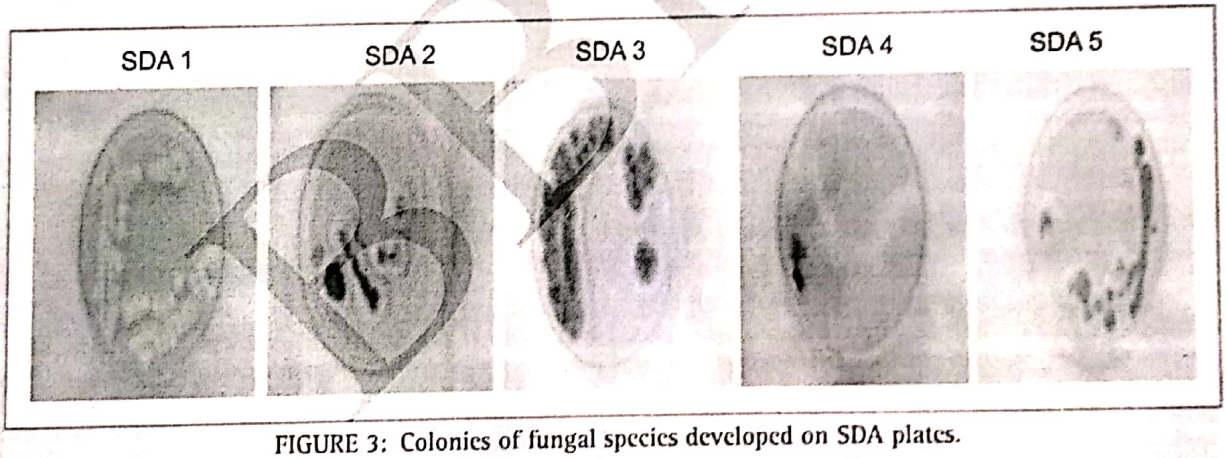


FIGURE 3: Colonies of fungal species developed on SDA plates.

(Figure 4). It was noted from the results that fungal species in SDA 2 and SDA 5 were identified as *Aspergillus oryzae* . It was also noted that the mycoflora of stored wheat grains sampled from study area predominantly consisted of ubiquitous mould genera *Aspergillus* where as *Syncephalastrum racemosum* was found in lesser extent and was infrequent.

Fungi are widely distributed in nature, grow over an extremely wide range of nutrients, temperature, pH, etc. and contaminate food products by many ways. Most of the fungi are toxigenic in nature, and those non-toxigenic species may impart a mouldy odour and taste during long storage. They are considered a major factor in the spoilage of foodstuffs, leading to great economic loss and a major public health hazard throughout the world by producing a wide variety of mycotoxins (U.S. Dept of Agriculture, 2003).

Mathew *et al.*, (2010) reported ten fungal species belonging to seven different genera viz., *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium* and *Penicillium* of the Phylum *Ascomycota*; *Mucor* and *Rhizopus* of the Phylum Zygomycota are associated with wheat grains. They also noted that frequency of *Aspergillus niger*, *A. fumigatus* and *Alternaria alternate* was higher (33.76 to 40.34%) than the other fungal species

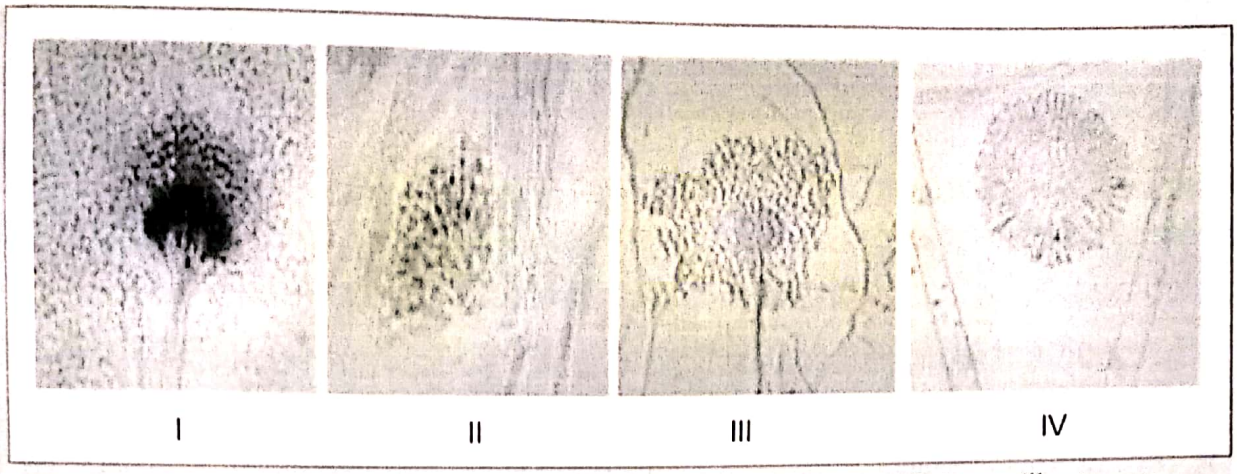


FIGURE 4: Microphotographs of fungal species isolated from stored grain : I. *Aspergillus terreus* II. *Aspergillus oryzae* III. *Aspergillus glaucus* & IV. *Syncephalastrum racemosum.*

identified. The frequency of *A. Nigerwas* was highest which is quite alarming because this strain can produce aflatoxins. In the present study, occurrence of *Aspergillus* species was found in higher stored grains indicating the resemblance with results of previous workers (Magan, *et al.*, 2003; Malaker *et al.*, 2008; Mathew *et al.*, 2010). Amongst the mycoflora associated with stored grains, *Aspergillus* is known to produce mycotoxins that deteriorate the quality of stored grains; it becomes quite essential to protect the stored grains from fungal infection by undertaking necessary steps to prevent qualitative and quantitative losses of stored grains.

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## REFERENCES

Fente C.A., J. Jaimez Ordaz, B.I. Vazques, C.M. Franco and A. Cepeda (2001): New addivive for culture media for rapid identification of Aflatoxin-producing *Aspergillus* strains, Applied and Environment Microbiology. 21: 4858-4862.

Hocking, A.D. (2003) Stored grain in Australia 2003. Proceedings of the Australian Postharvest Technical Conference, Canberra. CSIRO Stored Grain Research Laboratory, Canberra. pp. 55-58.

Magan, N., Hope, R., Cairns, V. and Aldred, D (2003): Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. European J. Plant Pathol. 109: 723-730.

Malaker, P.K., I.H.Mian, K.A.Bhuiyan, A.M.Akanda, and M.M.A.RezaL (2008) Effect of storage containers and time on seed quality of Wheat, Bangladesh J. Agril. 34 :469-477.

Mathew Shiju, George Thomas and Tufail Ahmad (2010): An evaluation on the Impact of Fungi on the Post-Harvested Stored Wheat Grains. International Journal of Biotech. and Biochem. ISSN 0973-2691, 6: 9951002.

Neethirajan, S., Karunakaran, S., Jayas, D.S. and White, N.D.G. (2007): Detection techniques for stored-product insects in grain. Int. Fd. Control 8: 157-162.

Sekar P, N. Yumnam, and K. Ponmurugan (2008) Screening and Characterization of mycotoxin producing fungi from dried fruits and grains. Advanced Biotech. 11:46-48

U.S. Department of Agriculture (2003). Annual World Production Summary, Grains, http://www.usda.gov/ Agriculture crop production.