Fungal infection and mycotoxin contamination in mustard oilseeds during storage in India

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Abstract

To assess the quality of mustard seed during storage, an extensive survey was carried out in four mustard growing states of northern India i.e. Rajasthan, Haryana, Uttar Pradesh and Punjab. Mycoflora, surface characters and ultrastructure of freshly harvested and one year old stored seeds were investigated. Fungus induced damage in the seed coat was studied by scanning electron microscopy. Freshly harvested seeds were spherical and brown with reticulations on the surface. Growth of fungi on poorly stored seed leads to degradation of protective cuticular covering, exposing the small pores in seed coat. One year old stored seeds were often found to be heavily infected with diverse fungi. These seeds appeared to be unhealthy and discoloured due to fungal infection. Cells of the seed coat were shriveled and distorted. Transmission electron microscopy revealed degradation of oil and protein bodies in cotyledonary cells. Cytoplasm of the cells of fresh, mature seeds was packed with protein and lipid bodies, which constitute the nutrient reserves. Seed-borne mycoflora was isolated by plating the seed on Czapek's Dox agar medium. Thirty five fungal species were isolated from the seeds.

Key words: Aflatoxin, mustard, seed, mycoflora, storage, ultrastructure

Introduction

Mustard, *Brassica juncea* (Linn.) Czern. & Coss. is an important oilseed crop of India. Mustard crop is harvested in the month of March and April and is stored through hot summer and monsoon in conventional warehouses. Storage of seed under high humidity and temperature often leads to fungal infection and mycotoxin contamination. Maintenance of quality of seed during storage has been a problem in warm and moist conditions that permit invasion of seed by fungi. Fungal infection and aflatoxin contamination is greatly influenced by the type of storage structures used (Ranjan et al., 1992).

Aflatoxin contamination of mustard results from growth of toxigenic strains of the fungi, *Aspergillus flavus* and *Aspergillus parasiticus* (Diener et al., 1987). Fairly high relative humidity and temperature, unseasonal rainfall and floods prevail in different eco-regions of the India at or soon after harvesting of major crops (Bilgrami, 1991). The objective of the present study was to detect aflatoxin B_1 contamination in fresh and one year old stored mustards seed samples from the four major mustard growing states.

Material and Methods

Seeds of mustard, *Brassica juncea* (Linn.) Czern. & Coss. were collected from different places of Rajasthan, Haryana, Uttar Pradesh and Punjab (India). Seed mycoflora was isolated using Czapek-Dox agar medium, as recommended by International Seed Testing Association (1966). Surface characteristics of seed were studied with the help of scanning electron microscope. Seeds were washed, dried, and mounted on metal stubs. Seeds were coated with gold palladium (100 Å thickness) for 5 min under vacuum. JSM-84 scanning electron microscope was used and photographs were taken on ASA 125 black and white film. Anatomical studies were carried out in normal and fungal infected seeds. The seeds were fixed in formalin-acetic acid-alcholol (O'Brein et al. 1973). Embedding was carried out in paraffin wax. Sections were stained with aniline blue-black (Fisher, 1968). To study the sub-cellular changes in the fungal infected seeds, Transmission electron microscope (TEM) was used. Seeds up to 1 mm long were fixed in 4 % glutaraldehyde in 0.1M cacodylate buffer at pH 7.0 for 5h. The specimens were post-fixed in 1% OsO_4 in distilled water and dehydrated in ethylene-propylene oxide series. Spurr's resin was used for embedding (Spurr, 1969). Extraction of aflatoxin B_1 was carried out by the method given by Turksess et al. (1984). Determinations of afatoxin B_1 levels were carried out by HPLC using Hewlett Packard HPLC system (Hewlett Packard, Agilent 1100, Palo Alto, USA).

Results

All the samples collected from various places were visually examined. Fresh seeds were brown, spherical and did not show any discoloration. One year old seeds showed the presence of green, white and grey coloration. Seed samples (fresh and one year old) were analysed for percent incidence of fungal infection. Thirty five fungal species were isolated from the seeds. *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. proliferates, Alternaria alternata, Penicillium citrinum P. funiculosum, Fusarium moniliforme, Rhizopus nigricans* and *Cladosporium herbarum* were the dominant fungal species.

Anatomical studies revealed that in fresh samples, seed coat was intact with testa consisting of smooth epidermis, followed by palisade layer. Tegemen was represented by a pigmented layer of compressed cells. Aleurone layer along with a

few layer of endosperm parenchyma was filled with protein and oil bodies. In one year old seed the epidermis was degraded, palisade cells showed disintegration and large spaces were formed at the side of pigmented palisade layer.

In one year old seed the cytoplasm retracted from the cell walls and disorganization of cell organelles was observed. Degradation and coalescence of lipid bodies, hydrolysis of protein bodies and loss of integrity of the nucleus and the nucleolus were seen. Large vacuolar spaces were formed and plasma membrane was withdrawn from the cell wall. Intercellular spaces were filled with osmiophilic material, which is probably leached out from protein bodies. Forty three per cent of the samples tested positive for aflatoxin B_1. The concentrations ranged between 0.15µg/kg and 58.17µg/kg.

Discussion

In India most often the seeds are stored in warehouses in gunny bags. Before storage, the seeds are not subjected to any protective treatment. As moisture-testing facilities are not available in the fields, it is not feasible to optimize the storage conditions of seeds. This results in the poor quality of stored seeds and oil extracted is not of a high quality. Some of the hazards faced by the seeds during storage are moisture, high temperature, poor ventilation, pests and pathogens. When seeds are stored in warm and humid conditions the seeds absorb moisture. This increases the susceptibility to infection by storage fungi. Due to invasion of these fungal species, the seeds develop a tendency to clump together. More fungi begin to grow in pockets of moist seeds. Infection in the seed leads to an escalating process involving increase in respiration, water content and temperature. Seeds with a high rate of respiration, their own and of the fungi, attain high temperature and moisture. Present studies reveal that due to fungal infection palisade layer disorganizes in one year old seeds. Fungal hyphae have been observed in the endosperm and cotyledonary cells. The disintegration of plasmalemma can be ascribed to release of fungal toxins, which are reported to increase leaching of cellular constituents (Wheeller & Black, 1963). The membrane of the protein bodies may also disintegrate and then proteinaceous material leaches out through the disrupted membrane into the intercellular spaces. Membrane disintegration has also been reported by Roberts and Ellis (1982).

Aflatoxin contamination in mustard seeds may be ascribed to several environmental, agronomic and biological factors among which the temperature and humidity are most significant (Sinha et al., 1988). The study reveals that mustard seeds are prone to aflatoxin contamination during storage. Mustard seeds form good substrate for growth of toxigenic strains of *Aspergillus flavus.* Aflatoxin B_1 production was detected both in fresh and one year old seeds, but wide differences were found in the concentration of aflatoxin in fresh and old seeds. It was observed that the fresh as well as one year old mustard seed samples collected from Bikaner district of Rajasthan and Panipat, Riwari and Rohatak districts of Haryana were contaminated with aflatoxin B_1 above the WHO tolerance level in food. The permissible level of aflatoxin B_1 is 20µg/kg (World Health Organization, 1990).

Conclusions

Seed storage under hot and humid conditions leads to extensive fungal infection and loss of stored food reserves. Proper storage can help in enhancing oil yield and quality to a considerable extent. Much emphasis needs to be laid on improvement of storage environment to combat this problem through effective pre- and post-harvest operations.

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