Mohapatra D, Kumar S, Kotwaliwale N, Singh KK (2016) Strategies for control of fungal invasion in stored food grains. Pp. 37–43. In: Navarro S, Jayas DS, Alagusundaram K, (Eds.) Proceedings of the 10th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2016), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Strategies for control of fungal invasion in stored food grains

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ABSTRACT

Microorganisms, insects and other pests are the major impediments for safety of the grains and therefore, some precautionary measures are essential to safeguard the stored products. Invasion of various pests deteriorates the product quality. In case of fungi growth, poisonous mycotoxins are developed. Fungi growth causes both qualitative and quantitative loss of grains leading to economic loss. The future focus is on the saving each grain that is produced. In order to device appropriate strategies to combat fungal invasion in stored grains, the critical factors, which influence development and growth of fungi in the stored grains and existing practices as well as emerging technologies for their control have been reviewed in this paper. Grain m. c., initial infections, presence of other vector insects and mites, contaminated storage space, containers, and handling equipment are the factors that contribute to the growth of fungi during storage. Traditional practices like drying to safe moisture level, aeration, dry heating, hermetic storage (O_2< 5%; CO_2>10%) are practiced widely. The safe storage moisture level for cereal grains, legumes and oil seeds have been reported to about 12, 10 and 8%, respectively. Application of new age control measures such as microwave heating (at energy levels 2.45 to 9.7 GHz), application of gaseous ozone, corona discharge (application time 5 to 20 min), ionizing radiation (1 to 15 kGy), pulsed light (intensity of 1.08 $W cm^{-2}$), supercritical carbon dioxide co-solvent system, ultra-superheated steam (temperature of 300 –500°C) are being developed for controlling fungi growth in stored grains. Some of them are practiced sporadically, while some are still in the laboratory testing stage. Application of natural products like plant lecithin, essential oils and vegetable oil has shown to have good potential in controlling these fungi growth in different food grains, which needs nod from the regulating bodies for application in storage of food grains.

Key words: Fungi, Grains, Heating, Hermetic storage, Irradiation, Microwave heating, Ozonization, Pulsed light, Storage

The grains travel through different phases before reaching to the consumer's gut. In each of these, pre-harvest and post-harvest operations encounter various hurdles that affect the final quality of the grains. One of such factors that affects the quality of grains is microbial invasion. When microbes, specially fungi contaminate the grains, following deteriorations take place: (*i*) the grain lose its nutritional quality, (*ii*) its germination ability is severely damaged, (*iii*) free fatty acid content in the grain increases, (*iv*) non-reducing sugar content decreases, (*v*) off-odour develops, (*vi*) processing quality decreases, (*vii*) loss of weight, flavour and colour occur, (*viii*) hot spot generation resulting in grain charring and (*ix*) mycotoxins are produced (Accensi et al., 2004; Golumbic and Kulik, 2012).

Aspergillus, Penicillium and *Fusarium* are the most important mycotoxin producing fungi associated with food and feed (Jackson and Bullerman, 1999). Mycotoxins can cause diarrhoea, vomiting, pulmonary disease, gastro-intestinal inflammation, depressed feed efficiency, liver cancer, abnormal protein biosysnthesis, depressed immune response, carcinogenesis, and even death in animals in the severe case (Shephard, 2008).

In this review, causes of microbial growth in stored grains and the chemical-free preventive measures such as drying, hermetic storage, dielectric heating, cold plasma treatment, ozonation, irradiation, ultra-

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superheated steam treatment, application of vegetable oil, plant derivatives that can be taken up is discussed briefly.

FACTORS AFFECTING FUNGAL ATTACK IN STORED FOOD GRAINS

Major factors responsible for fungi growth on agricultural commodities depends on various factors such as m.c. of the grains, duration of storage, physical and physiological health of the grain going into storage, presence of insects and mites, storage temperature and humidity (Mills and Woods, 1994; Magan et al., 2010), type of storage structure and storage conditions. Various factors affecting the fungal growth in stored grains are discussed here.

Moisture content

The respiration rate of grains and biological agents like insects and microorganisms present in the stored grains involve water. Thus m.c. of the stored grain plays an important role in the growth of fungi and other damage causing biological agents. Higher moisture level or water activity (a_w) above 0.85 in grains encourages mycotoxin producing fungi (Pardo et al., 2004) and other pest growth as well as metabolic activities of grains like respiration and subsequent heat generation (Dillahunty et al., 2000). When the available water is lower for such activity, grains automatically achieve biological safety as it reduces degradation through respiration, enzymatic activity and proliferation of microbes. Grains with higher m.c. should be dried before storage, so as to maintain seed viability, microbial stability and seed coat colour. Since at higher moisture content storage, life of the grain at any storage temperature is shortened (Karunakaran et al., 2001), grains should be stored at the safe moisture level or, which is recommended to be 12, 10 and 8% (wb) for cereal grains, legumes and oilseeds is about, respectively, for about 1 year of storage.

Temperature

Microbial growth is very eminent in tropical regions, where high temperature and humidity conditions prevail in most parts. Heat is generated due to grain respiration, and hot spots are generated due to differential heat content in the storage bin and environment. These conditions are favourable for the growth of fungi. Some of the species (*Aspergillus candidus* Link, *A. Terreus* Thom, *Dactylomyces crustaceus* Apinis & Chesters) have higher thermo tolerances and their growth increases even at higher temperatures. Since the grains are still live and respiring evolving CO_2 and heat, the heat of respiration increases the temperature of the grain causing the already present microflora to increase and invade the grain tissue (Mulinge and Apinis, 1969). Fungi are inactive if the grains are stored at temperatures below 20°C and above 40°C. The combined effect of m.c. and storage grain temperature was investigated by many researchers and the outcome has been temperature which has less effect on the growth of fungi if the grain m.c. is lower than the safe storage moisture level.

Invasion of insects and mites

Insect activity in a grain mass leads to an increase in both the temperature and m.c. of the grains surrounding the insect infestation. In these ‘hot spots’, conditions may be favourable for mold growth. Dunkel (1988) presented the hypothesis of insect–fungi relationship in stored grain damage. He stated that (*i*) some storage insect species are disseminators of storage fungi and some are exterminators; (*ii*) some storage fungi attract storage insects and promote their population increase and other repel and secrete toxins harmful to insects. Mites and most of the storage fungi are considered to be of the secondary importance as far as grain damage during storage is concerned. Mites, because of their microscopic size, are not easily visible but cause irreparable damages to the grain quality (Sinha et al., 1962). They can carry fungal spores on their bodies, thus introducing storage fungi into the grain mass. Hubert et al. (2003) postulated that due to their small size, mites are secondary vector for carrying fungus as compared to insects; they specially transmit fungal spores in heavily infected grains. Mites were also selective about the fungal spores that they feed on, carry and contaminate fresh grains during storage. Based on the outcomes and recommendations of these investigations, some of the fungi can be exploited as biocontrol agents for mites and insects.

Damaged grains

Abnormal and damaged seeds with crevices are most likely to carry pathogens and other fungi; hence are prone to fungi damage and source of infection for other healthy grains surrounding them. Fungi may attack if the grains are cracked and broken or contain large amount of foreign material; as broken or cracked kernels going into storage are more likely to be contaminated and invaded, once they are in storage as compared to the whole/sound/healthy kernels (Chulze, 2010).

Initial contamination from field

The fungal invasion can occur from the field itself. The grains should be free from fungi attack before storage. Grains that are moderately invaded by storage fungi develop damage at lower m.c. at a lower temperature and in a shorter time period than the grains free or almost free of storage fungi. The infected plant parts like leaves and stem came in contact with the grains during handling and processing eventually infecting them and the fungal growth in grains proliferates many times during storage (Lacey, 1989).

Storage containers or packaging material

Storage containers are good source of fungi in contaminating grains. Storage rooms, earthen pots, gunny bags, reed baskets, and other traditional storage structures are very good places of fungi infection. Grains stored in traditional masonary storage rooms were more susceptible to mold contamination (Sashidhar et al., 1992). Storing food grains in closed metal bins restricts air exchange, thereby reducing the oxygen, which causes lower production of aflatoxins; whereas, on storing the grains in gunny bags, mold growth and aflatoxins production would be much higher during the wet season (Ahmad, 1993).

CONTROL MEASURES FOR FUNGI

Several researchers have worked on the physical, chemical and biological methods of inhibiting microbial growth. Infected grains can be treated by either physical or chemical treatments, or a combination of both the methods. Since there is consumer awareness of the side effects of the fungicide residues in food grains; physical methods and fungicides based on plant derivatives which are accepted as safe are gaining foothold for food grain application. Some of the chemical free methods are discussed here.

Drying/aeration

The most effective method of treating mycotoxin problems is avoidance. This is possible by drying the grains to safe m.c. before storage, reducing physical damage to the grains during harvesting and storage and ensuring clean, dry insect-proof storage conditions. Non-aerated bins and drying using very less air flow favours the conditions for harbouring fungi growth and production of mycotoxins (Langseth et al., 1993). Therefore, the grains must be provided with adequate aeration for prevention of microbial growth and damage.

Hermetic storage

Hermetic storage refers to the storage of food grains in an airtight storage structure, which may be flexible or rigid, an over-ground or under-ground structure. The hermetic storage systems can be of three types: (*i*) organic hermetic storage, (*ii*) vacuum hermetic storage and (*iii*) modified atmospheric storage (Navarro, 2012). In the organic hermetic storage, there is a gradual depletion of oxygen and eventual rise in carbon dioxide content, which is attributed due to the natural respiration of biological agents like grains, fungi, insects and other pests. This condition helps in retarding the growth of insect, other pests and toxin-producing microorganisms. The second type is vacuum fumigation, in which partial air pressure is maintained by sucking out most of the air, so that an oxygen-deficit environment can be created that would prevent the growth of spoilage causing entities. In the third type, either carbon dioxide or nitrogen is flushed to generate controlled atmosphere so that the growth of insect, microorganisms can be retarded (Villers et al., 2006). During hermetic storage both insects and fungi consume oxygen, so that the oxygen level depletes rapidly causing an unfavourable environment for their own growth and survival. The major consumer of oxygen in grain storage are the insects, followed by fungi and then by the grain.

Microwave treatment

Dielectric heating such as radio waves and microwaves produce volumetric heating due to the vibration and collision of polar molecules like water. Microwaves have been used to disinfest insects (Mohapatra et al., 2014, 2015) and the same can be achieved for fungi treatment, as it works on the principle of dielectric heating which causes differential heating due to difference in composition of the materials. More et al. (1992) found that, for an exposure period of 60 sec. most of all fungi (*Eurotium* spp. *Aspergillus candidus*, *A. niger* Van Tieghem and *Penicillium* spp.) in sorghum [*Sorghum bicolor* (L.) Moench] were eliminated, where the temperature reached beyond 90°C during microwave (1.25 GHz) exposure. Fang et al. (2011) successfully decontaminated rice (*Oryza sativa* L.) sample from *Aspergillus parasiticus* Speare through microwave treatment (2.45 GHz) at 70°C, which was superior to conductive heating practice. The microwave heating caused increased electrolyte, Ca^{2+}, protein and DNA leakage from the fungi, which was responsible for the mortality of the fungi. Basaran and Akhan (2010) have successfully eliminated aflatoxin-producing *Aspergillus parasiticus* from hazelnuts through microwave irradiation. Simultaneous microwave drying-cum- disinfestations/ disinfection/ decontamination followed by suitable packaging of grains can prevent further infestation/infection during handling process. During microwave heating freshly harvested grains having higher initial m.c. loses moisture, at the same time the microbes and other damage causing biological agent will be eliminated through exposure to microwave energy. Further, proper packaging will ensure no further infection/infestation of grains by the damage causing biological agents.

Irradiation

Stored products, especially grains, have been successfully decontaminated with ionizing radiation as it affects the internal structure (Hallman, 2013). Irradiation technology has been very effective in controlling the *Aspergillus*, *Penicilium*, *Rhizopus* and *Fusarium* infection in many grains and prolonging the shelf life over 6 months (Lima et al., 2011). The source of radiation is usually 60_{CO} and selenium. Maity et al. (2004) exposed food grains to gamma-rays up to 6 kGy (0.12 kGy/h). Significant depletion in fungal population on seeds was noted with irradiation dose at 1–2 kGy; however, for complete inhibition of the contaminating fungi, the dose was above 4 kGy, without significantly affecting the germination potential of the treated grains. In another study by Maity et al. (2008), complete depletion of *Aspergillus* spp. spores were found above 2.5 kGy dose, however, there was delayed germination in the chickpeas (*Cicer arietinum* L.) Their study recommended a dose of 2–4 kGy for food grains having m.c. about 10–11% (w.b.) for disinfecting aflatoxin-producing fungi. The efficacy of UV-C radiation depends on the method of radiation, exposure time and genera (Nemţanu et al., 2014), as each genus would respond to the radiation differently.

Pulse light application

Pulsed light (PL) is an emerging non–thermal technology, involving application of high intensity light for a very few seconds, in order of micro seconds, to decontaminate food. The high intensity light produces photothermal and/or photochemical reactions in the contaminating microbes, eliminating those (Gomez-Lopez et al., 2007). This is an alternative to continuous UV-C treatment. Its application to decontaminate stored grain is limited. The efficacy of PL treatment depends on the time of exposure, distance between electrode and voltage input (Maftei et al., 2014). During the PL treatment moisture loss was also noticed in paddy, apart from reduction in microbial count, when PL was applied with an intensity of $1.08\,W\,cm^{-2}$ for 21 s (Wang et al., 2016). This also reveals the thermal effect of PL treatment, which would have helped in moisture evaporation without affecting the milling quality of paddy.

Ultra super-heated steam technology

Super-heated steam has been employed to dry food materials, where saturated steam is heated under constant pressure to achieved degree of superheat. The energy is utilized in drying the material. Superheated steam (160–170°C) decontaminates naked oats (*Avena sativa* L.) from bacterial, mold growth without affecting the enzymatic activity (Chang et al., 2015). Recently, Ultra super-heated steam technology (USST) developed by FBI Co. Ltd. (Tokyo, Japan), employed high-frequency induction heating (IH) technology. The eddy current based induction heating can produce a temperature of 300-500°C so as to produce these highly energized radicals.

Super critical CO_2 co-solvent system

Carbon dioxide usually behaves like gas under normal temperature and pressure, but when the pressure and temperatures are increased above a critical point, it behaves like a liquid having greater solubility. This property has enabled SC-CO_2 to be used mainly in the extraction of bio compounds, which can also extract some compounds from microbial cell, causing their elimination. The exact mechanism of anti-microbial action of SC-CO_2 has been summarized as: (*i*) solubilization of pressurized CO_2 in the external liquid phase, (*ii*) modification of cell membrane, (*iii*) decrease in the intracellular *p*H, (*iv*) inactivation of key enzyme, (*v*) inhibitory effect of molecular CO_2 and HCO_3- on metabolism, (*vi*) intracellular electrolyte imbalance and (*vii*) removal of vital constituents from cells and cell membranes (Garcia-Gonzalez et al., 2007, 2009). Inactivation of microorganisms with the application of super critical carbon dioxide has been evaluated for different microorganisms and it was observed that sterilization was possible only on wet cells of microorganisms while it was ineffective against dry cells of microorganisms (Kamihira et al., 1987).

Cold plasma/corona discharge treatment

In order to have non-chemical treatment for stored products, various methods are being investigated. One of such non-toxic method for disinfestations of grains is cold plasma or corona discharge treatment. Plasma is highly energized fourth state of matter, composed of excited atoms and molecules, ionized gases, radicals, and free electrons. These highly active discharges such as ozone, atomic oxygen, hydroxyl, nitric oxide and super oxide radicals as well as other free radicals are antimicrobial in nature, as they interfere with the metabolism of the microbes (Selcuk et al., 2008). Therefore, sterilization by plasma may be an alternative to chemical disinfection method. The advantages of plasma application can be summarized: (*i*) these active discharges can be applied uniformly over the surface of grains; (*ii*) they can perform activity at low temperature, (*iii*) they have good diffusivity even into complicated structures, (*iv*) short processing times, (*v*) non-destructive in nature, (*vi*) do not leave toxic residues; hence harmless for operators and consumers.

Ozone application

Gaseous ozone is formed when free oxygen radicals bonds with diatomic oxygen to form triatomic oxygen molecules. On decomposition, it converts back to oxygen molecules and leaves no residues. Breakage of O-O bond can also be accomplished through by chemical, thermal, chemonuclear and electrolytic methods (Tiwari et al., 2010). Zotti et al. (2008) observed that, ozonation retarded the growth of *Aspergillus flavous* and eliminated *A. niger*, it also had bleaching effect on the pigments presented in the fungal colonies. The insecticidal and antimicrobial effect of ozone in food material has been a mixed success (Kim et al., 1999).

Application of botanical derivatives

In different parts of the world, plant parts like bark, leaf, flower, seed powder, and derivatives like ash, vegetable oil, spice oil have been in used to control insect and microbial growth since ages. Several researches have also highlighted the advantages of using the botanical derivatives as chemical fumigants and contact pesticides. Some of these plant products that have lower phytotoxicity are easily biodegradable and has traditionally been used in some parts of the world (Dubey et al., 2008); their application for pest control seems to have promising future for the bulk storage of grains.

Vegetable oil application

Several household in India apply vegetable oil on their stored grains for preventing damage caused by insects and fungus. Oil layer on the grains reduces the fungus to grow, as it limits the oxygen supply and works as a thin barrier for the moisture to seep into the grain. Many studies indicated the effectiveness of vegetable oil in controlling both fungal and insect growth in grains, which can be a low-priced and effective method for safe grain storage (Hall and Harman, 1991a,b).

Application of plant derivatives

Natural products (flavonoids, isoflavonoids, terpenoids and their derivatives) have potent fungicidal effects. Therefore, instead of going for chemicals which have bio safety issues, these plant-derived products can be used against storage fungi. Plant proteins like albumins and vicilins (Gomes et al., 1997; Santos et al., 2008; Uchôa et al., 2009) have antifungal characteristics and have potential to limit the growth of the specified fungi in the stored seeds. Plant essential oils (EO) are as effective as fungicide in small quantities which can be the alternative to chemical fungicides (Isman, 2000). Plants oils and essential oils from eucalyptus, neem seed oil have been used traditionally to control insects and fungi (Boeke et al., 2004; Batish et al., 2008).

Through several reports have claimed the efficacy of plant essential oil to prevent fungal growth; commercialization of these plant-derived products in grain storage system needs clearance from the regulatory bodies. Most of the studies are limited to laboratory study only; large scale application of these oils as anti-fungal agents is still not established.

CONCLUSION

Little can be done to prevent or reduce the invasion of crops in the field by fungi; however, the following recommendations should help in preventing storage fungi problems or minimizing damage from storage fungi in stored food grains. Harvest time is closely related to grain m.c. and care should be taken to harvest the food grains at the optimum moisture level and drying them immediately to bring it to safe moisture level, so that microorganism cannot grow. Insect infestation should be avoided and proper hygienic practices should be maintained throughout the supply chain. Harvest, handling equipment that could cause damage should be suitably designed, so that minimum damage can occur to the grains, thereby reducing the possibility of microbial invasion. Technologies such as microwave drying-cum-disinfestation/ decontamination of freshly harvested grains followed by suitable packaging, practices have good potential in reducing grain damage both qualitatively and quantitatively. However, their effect on the cooking, baking and seed viability, limits its use to a large extent, though this technology has a potential to be used for grains which are not for seed purpose. Gamma radiation has been practiced as quarantine and disinfection measures and generally regarded as safe by many countries, can be another technological alternative. However, some countries in EU have not accepted irradiated foods; hence the food grains meant for export to those countries needs alternative solution, which can be accomplished through ozonation or corona discharge technology. Some emerging technologies like USST needs elaborate quality studies before being implemented and adopted commercially. Nevertheless, the uses of natural product as oil or plant derivative such as lectins, vicilins have potential to act as fungicide and can be the alternative to chemicals as green technologies. These plant-derived products nod from the regulatory bodies for wider application.

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