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Fungal Infection and Aflatoxin Contamination on Dried-Stored Spices

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Abstract. A review regarding fungal infection and aflatoxin contamination by *Aspergillus flavus* during pre and post-harvest handling on spices is presented. The results of research on spice storage management, physical and chemical treatments on dried spices before storage to prevent fungal infection are discussed

Keywords: *Aspergillus flavus*; aflatoxin; preharvest; postharvest; spices

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1. Introduction

Spices have been defined as aromatic natural compounds originated from dried plants or parts of plants such as seeds, fruits, flowers, roots, bulbs, leaves bark used in small quantities and added to preserve food, give taste, smell and flavor (Codex, 2017). Some spices such as ginger, turmeric, galangal, chili pepper, red onion and garlic used in fresh condition or stored several times after harvest. However, dried spices such as white and black pepper, star anise, candle nut, coriander, cardamom, nutmeg, cloves, etc. are processed very complex. Pre-harvest and post-harvest handling of the spices such as harvesting, drying and storing can span long periods of time before used (Codex, 1995). As with many agricultural products spices are susceptible infected by microbes. Among microbes, xerophilic storage fungi are the predominant contaminant of dried-stored spices (Kneifel and Berger, 1994; Dimic *et al*, 2000; Romagnoli *et al*, 2007). Hashem and Alamri (2010) reported spices obtained from supermarkets predominantly infected by *Aspergillus*, *Penicillium* and *Rhizopus*. Fungal infection on cinnamon and cloves was predominated by genus *Aspergillus chevalieri* (formerly *Eurotium*) (90 %), *Aspergillus* (80 %), *Penicillium* (75 %) and *Cladosporium* (45 %) (Dimic *et al*, 2008)

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Aflatoxins are mycotoxin produced as secondary metabolite by some strains of *Aspergillus* section *Flavi* that grow on improperly stored commodities. Among *Aspergillus* section *Flavi*, *Aspergillus flavus* is one of the aflatoxin producers that distribute world-wide especially in tropical regions (Tran-Dinh *et al*, 2009). *Aspergillus flavus* has no specific host and infect a wide variety of agricultural products including spices in the field, during storage, processing and distribution (Leger *et al*, 2000). However, not all *A. flavus* strains are toxigenic (aflatoxin producer) (Ehrlich, 2014) and the toxigenic strains produce different levels of aflatoxins. The presence of toxigenic *A. flavus* does not indicate aflatoxin contamination. Production of aflatoxins by toxigenic *A. flavus* strains was determined by genetic, substrate, geographic origin, climate change and plant culture technique (Horn *et al*, 1996; Vaamonde *et al*, 2003; Perrone *et al*, 2014). The fungal infection and aflatoxin contamination on spices occurred in the field during pre-harvest, harvesting and post-harvest handling (Dimić *et al*, 2008). Application spice management based on good agricultural practices and good handling practices by farmer, distributors and retailers are required to obtain high quality and healthy spices from farm to table.

Pre-harvest and harvesting contamination

Soil and air are the main inoculum source for causing contamination spices in field (Kneifel and Berger, 1994; Hussain, 2012). Some species of fungi grow as saprophytic fungi on plant debris or litter, therefore, fungal infection on plant can not be prevented. Herrera *et al* (2014) reported that infection of *A. flavus* in the field adapted to the aerial environment and infect upper parts of plant such as leaves, flowers and fruits. Their propagule such as conidia are easy to disperse by air movement, insect, water and their dormant sclerotia are able to stand in the soil yearly. Spices (seeds, fruits, roots) can contain *A. flavus* inoculum and aflatoxins at pre-harvest. Conidia of *A. flavus* can germinate on the stigma surface of flowering plants, infect and develop in seed embryo as endophytic fungi. Under drought stress for example drying process of spices, aflatoxin may be produced by the toxigenic strains. The influence of *A. flavus* infection and aflatoxin contamination on corn, peanut and cotton during pre-harvest such as plant water stress, root injury, insect damage, soil moisture was reported by Diener *et al* (1987). High moisture content at harvest leads to spoilage and occasionally the production of mycotoxin (Jayas and White, 2003). Good agricultural practice during harvesting of nutmeg was reported by Dharmaputra *et al* (2018) they reported that harvested nutmeg directly from the tree less aflatoxin and *A. flavus* infection than nutmeg that had been fallen on the ground.

Fungal infection and aflatoxin contamination are inevitable and still a big problem that has not been easy to solve. Most tropical spices originated from developing countries that pre-harvest and harvesting method were conducted conventionally. Efforts that can be done to

minimize *A. flavus* population and their secondary metabolites accumulation during pre-harvest and harvest by educate subsistence farmers including:

- Field hygiene and water used for irrigation at pre-harvest (Codex, 1995)
- Harvesting method suitable for the plant parts(Codex, 1995)
- Preventing spice commodities in contact to the soil
- Harvested at timely manner to reduce moisture content
- Separation fallen and picking spices (Dharmaputra *et al*, 2018)

Postharvest contamination

Tropical spices commonly produced and harvested traditionally by subsistence farming . They are dried in open air on the ground and stored insufficiently controlled with respect to food hygiene (Sádecká, 2010). Lack knowledge of handling methods and equipment of farmers, subsequently the dried stored spices are susceptible infested by storage fungi (Dharmaputra *et al*, 2015). Post-harvest practices such as drying process, transportation and storage cause additional contamination. The percentage of damaged kernels, aflatoxin B₁ and total aflatoxins of Indonesian nutmeg collected from farmers, collectors and exporters were relatively high (Dharmaputra *et al*, 2015).. Dried-stored spices are higroscopic and and tend to absorb water favor from its environment leading to increase spice moisture content that results in accelerate deterioration. Most spices deterioration are caused by molds that survived during drying and storage (Stankovic *et al*, 2006; Toma and Abdulla, 2013). The increase of spice moisture content caused by infection of xerophilic fungi or physical damaged by insect infestation during storage can accelerate the other fungal species that are able to grow at higher level water activity and produce aflatoxin. Physical and chemical treatments on dried spices before storage were required to prevent deterioration. Fungal infection at post-harvest handling can be minimized by:

- Quick and hygiene drying process
- Sortation and separation of physical damage/injury with intact seeds/kernels
- Keep dried stored-spices in sanitized container or polyethylene bag

Physical treatments

Dried stored-spices below safe moisture content is limiting factor for fungal growth. Maintaining stable moisture content during storage are required to prevent deterioration and mycotoxin contamination. Fungal population on nutmeg kernels at various water activities (a_w) during storage was reported by Nurtjahja *et al* (2017) the authors reported that low a_w level reduce population of xerophilic fungi. Preventing mechanical damage of seeds or kernels at harvest, drying process and insect infestation can reduce fungal infection. Dried stored-spices

packed in impermeable bag prevent absorbing water vapour from the atmosphere. However, relationships cause-effect some factors such as pre and post-harvest methods, temperature during storage, chemical compound of spices, initial level of fungal population and toxigenic strains have yet to be investigated.

Modified atmosphere packaging on spices have been used only for dried spices. Most filamentous fungi are obligate aerob. Magan *et al* (1984) and Taniwaki *et al* (2001) found that to inhibit microbial growth during storage, oxygen level must be lower than 0.14% and carbondioxide level should more than 50%.

Ionizing radiation is another physical treatment to decontaminate microbes of food and feedstuffs. Spices are the most commonly preserved by gamma irradiation where the packaged spices is exposed by gamma photon emitted by Cobalt-60 (^{60}Co) or infrequently Cesium 137 (^{137}Cs) to kill insects and to eliminate microbes. Gamma irradiation dose is different for each kinds of food and feedstuffs. The maximum dose for dry food including spices up to 10 kGy has been considered safe and effective (Farkas, 1998).. Irradiation with maximum dose 10 kGy not radioactive on spice (FAO/IAEA/WHO,1981; Farkas and Mohácsi-Farkas, 2011). and every species of molds has different sensitivity and resistance on gamma irradiation as shown in Table 1.

Table 1. Gamma irradiation doses and fungal sensitivity on various spices

Spices	Gamma Irradiation doses	Target	References
Black pepper, red chili	5-10 kGy	<i>Aspergillus niger</i> , <i>Cladosporium</i> spp., <i>Penicillium</i> spp.	Legnani <i>et al</i> (2001)
Black cumin	6 kGy	Reduce fungal population	Zeinab <i>et al</i> (2001)
Nutmeg	10 kGy 5 kGy	<i>Aspergillus flavus</i> <i>A. chevalieri</i>	Nurtjahja et al(2018)
Red chillies	6 kGy	Reduce aflatoxin	Abrar <i>et al</i> (2009)

The effectivity of irradiation depend on irradiation dose, moisture content (Aquino, 2012) fungal population (Calado, 2014), melanin pigment (Dadachova and Casadevall, 2008) and the presence of oxygen during irradiation. Fungal with multicell spores such as *Alternaria* and *Fusarium* more resistant to irradiation than that of a single spore. Fungal mycelia or spores with pigment (melanin) are less sensitive to irradiation than non-pigmented fungi was reported by Dadachova and Casadevall (2008). Reducing initial microbes and fungal infection by properly pre and posthandling are prerequisite before irradiation is conducted.

Chemical treatments

The use fumigant on spices such as methyl bromide (CH₃Br) and ethylene oxide (EtO) is effective to eliminate molds. however, both of the chemicals are highly toxic compounds, carcinogen when inhaled and has been prohibited in many countries such as Japan and European countries because of the residues it leaves in spices (Chmielewski and Migdal, 2005). In addition, methyl bromide is capable depleting atmosphere ozone layer. The effect of phosphine (PH₃), fumigant, used as a treatment to replace methyl bromide for insect control also inhibit *A. flavus* and aflatoxin production (Dharmaputra *et al*, 1991), they reported that phosphine concentration 0.5 mg/L commenced inhibit mycelial growth of the *A. flavus*.

2. Conclusion

Pre-harvest and post-harvest handling management of spices are essential particularly in tropical countries where spices were cultivated, dried and stored conventionally. Fungal population in spice distribution chain is determined by farmer level as primary producers. Integrated spice management at farmers level, collectors, distributors and retailers is necessary to minimize fungal infection on each level. Physical treatments by keep dried-stored spices at low water activities during storage and ionizing irradiation (gamma irradiation) are effective to reduce population of storage fungi and aflatoxin contamination.

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