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REVIEW

Botanicals in management of fungal and mycotoxin contamination of food commodities: retrospects and prospects

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Stored food items are highly prone towards fungal and mycotoxins contamination in tropical and sub tropical countries. Indiscriminate use of synthetic chemicals for food preservation has been cautioned due to their detrimental effects on human health and environment. In search of safer alternatives, different bioactive compounds of higher plants have been reported to have remarkable antifungal and antimycotoxigenic efficacy. Different EOs and their bioactive compounds have been recognized under categorized GRAS (Generally Recognized as Safe) in view of their long ethnomedicinal history. Recently, green nanoformulations have gained momentum of interest by food industries due to their excellent preservative potential, favorable safety profile, offering an eco-friendly and biorational approach for management of stored food biodeterioration by molds and associated mycotoxins. The present article deals with current knowledge regarding significant role of botanicals as a green preservatives and their future perspectives against food borne toxigenic fungi, their most probable antifungal and antimycotoxigenic mode of action and also the some major challenges associated with their large scale practical applicability.

Keywords: Aflatoxin, ergosterol, methylglyoxal, nanoencapsulation

INTRODUCTION

Agriculture and food industries are facing major challenge of crop losses and food safety worldwide due to fungal and mycotoxin contamination of stored food stuffs. Congenial environmental conditions of tropical and subtropical countries, unscientific prolonged storage of food commodities as well as their inappropriate post harvest processing facilitate proliferation of molds and their associated toxins in stored agri products making them unfit for human consumption. Molds and their associated toxins lead to cause approximately 25% annual post harvest crop loss worldwide (Adeyeye, 2020). A large number of food items including fruits, vegetables, nuts, cereals and spices have been reported to be heavily contaminated with different saprophytic molds *viz. Aspergillus, Fusarium, Penicillium* and *Alternaria* and their associated toxins *viz.* aflatoxin, ochratoxin, fumonisins zearalenone, deoxynivalenol and citrinin which are considered as the real threat to food safety (Ismaiel and Papenbrock, 2015). Exposure to these toxins imposes serious health threats to human beings and other organisms. Significant exposure to Fumonisins causes esophageal cancer and neural defects while Ochratoxin A exposure leads to cause kidney related disorders in organisms. Moreover, ochratoxin, citrinin and deoxynivalenol are also reported to have carcinogenic effects while zearalenone exerts oestrogenic effects in organisms through disturbing hormonal balance resulting in reproductive disorders. Among the all known 500 mycotoxins, aflatoxins are of great concern due to their serious health impacts. Aflatoxins are the difuran containing poylketides derived *Aspergillus* secondary metabolite which is one of the most common contaminant of stored food commodities. International Agency for Research on Cancer (IARC) has kept aflatoxins under group I category of carcinogen due to their high hepatocarcinogenic, teratogenic, mutagenic and immunosuppressive potential (IARC, 2012). On exposure to contaminated foods aflatoxins are easily absorbed through cell membrane due to lipolytic nature and in liver due presence of Cytochrome P450 get converted into highly

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reactive epoxide which leads to hepatocarcinogenesis through binding to DNA (Dohnal et al. 2014). Mycotoxins also lead to deteriorate nutritive value as well as shelf life of food items. Baking property of wheat is deteriorated due to storage fungi and their associated mycotoxins due to degradation of its sulfur rich nutritive protein *i.e.* gluten protein. In order to prevent fungal and mycotoxin contamination of stored food items, several synthetic preservatives are used but their detrimental effects towards human health and environment limit their applicability. At present some most commonly used synthetic preservatives such as butylated hydroxyanisole (BHA), butylated hydroxytaluene (BHT), benzoates, metabisulphites and sorbates are reported to cause health hazards effects *i.e.* hypersensitiveness and tumor formation (Anand and Sati, 2013). Most of the synthetic chemicals (grey chemicals) are also reported to impose residual toxicity, hormonal imbalance and induce pest resistance. Hence, there is an urgent need to develop safer and natural alternatives for management of post harvest losses of food commodities. The use of botanical formulations as food preservatives has been highly encouraged in the last few decades due to their eco-friendly, biodegradable and ephemeral nature in order to prevent qualitative and quantitative deterioration of food items due to mold and mycotoxin contamination. In recent period, different plant products are extensively used as efficacious antifungal and antimycotoxigenic agent by the food industries due to their biodegradable and ephemeral nature. Further, favorable safety profile of several EOs and their bioactive components such as clove, lemon grass, nutmeg, cinnamon, thyme, thymol, menthol and carvacrol towards mammalian system lead them to be Generally Recognized as Safe (GRAS) by US code of Federal Regulations (U.S. Federal Regulation, 2013). Thus, based on antifungal and antimycotoxigenic fumigant potential and favorable safety profile, different botanical formulations have been strongly recommended as safe and efficacious food preservatives. Based on the above background, the present article deals with cutting edge information on botanicals to be used in food preservation along with their possible mode of action against mold and mycotoxins. Furthermore, the article also gives brief description on practical limitations of botanicals and prospective of nanoencapsulation strategy in boosting their efficacy.

BOTANICALS IN MANAGEMENT OF FOOD BORNE MOLDS AND MYCOTOXIN CONTAMINATION

Different botanicals mainly comprising plant secondary metabolites have currently been formulated by food industries as green preservatives to maintain health security through preventing mold and mycotoxin contamination to agri products. Figure.1 depicts significance of botanicals as green preservatives. Plant extracts, EOs and their bioactive components *viz.* flavonoids, isoflavonids, phenylpropannes, terpenes, coumarins and alkaloids due to their efficacy against storage fungi and mycotoxins, highly encouraged to be used as preservative. Individual plant extract of *Curcuma longa, Citrullus colocynthis, Pongamia pinnata, Cymbopogon nardus, Commiphora wightii* and *Azadirachta indica* is reported to exhibit significant *in-vitro* efficacy against *A.flavus* and aflatoxin production in stored *Madhuca indica* seeds although, combination of these plant extract were also reported to have enhanced bio-efficacy over individual plant extract due to syngergistic action between their bioactive compounds (Sidhu *et al.* 2009). Different plant products such as gallic acid, caffeine, glyceollin, kaempferol, luteolin, vanillic acid, vanillylacetone and phytic acid are also reported to have inhibitory potential against aflatoxin production. Carvone, a major terpene found in *Carum carvi* is reported as a potent natural preservative against aflatoxin B_1 and *Aspergillus flavus* contamination (Lasram et al., 2019). In addition, it has been reported that *Cymbopogon citratus*, *Zingiber officinale, Thymus vulgaris, Ocimum gratissimum* and *Monodora myristica* EOs are highly efficacious against mycotoxin producing food spoilage molds *viz. A. flavus, A. fumigatus* and *Fusarium moniliforme* and thus have strong preservative potential. Further, several other EOs such as *Zataria multiflora, Syzygium aromaticum* and *Gaultheria fragrantissima* are also investigated to have remarkable antifungal and antimycotoxigenic potency (Kumar *et al.* 2018; Moosavi Nasab *et al.* 2018). Besides, several essential components *i.e.* thymol, eucalyptol, carvone, eugenol and terpinen-4-ol have also been reported to have high potency against the toxigenic strains of *Aspergillus, Fusarium, Penicillium* and *Alternaria (*Morcia *et al.* 2012). *Satureja hortensis* EO and *Curcuma longa* EO have been reported to exhibit potent fungicidal action against *A. flavus* under both *in-vivo* and *in-vitro* condition and also suppress aflatoxin production encouraging their role as sustainable green preservative (Hu *et al.* 2017). Presently, several botanical based preservatives such as TALENT comprising carvone, DMC base natural having combination of sage, citrus and rosemary EO, Rosemary EO based EcoTrol and eugenol based EcoPCOR are commercially available and widely used as preservative in order to enhance self life of stored food products (Dwivedy et al. 2016). Efficacy of some EOs/bioactive compounds against different fungus and mycotoxins has been listed in Table1.

declined on fumigation with EOs. Several EOs such as *Cinnamomum zeylanicum, Origanum vulgare, Cymbopogon martini, Foeniculum vulgare* are reported to decline fungal ergosterol content subsequently with their increasing concentration (Perczak *et al.* 2019). Depletion in ergosterol content will disrupt structural and functional integrity of cell membrane which further leads to efflux of vital cellular materials such as proteins, nucleic acids and ions *i.e.* Mg^{+2}, K^{+} and Ca^{+2} from targeted fungal cells and ultimately resulting in cell death. Further, thymol and carvacrol, the common bioactive components of EOs, have also been reported to inhibit growth of fungus by declining the major sterol component (Ahmad *et al.* 2011).

Table 1: EOs/Bioactive components antifungal/ antimycotoxingenic efficacy

S. NO.	EOs/Bioactives	Targeted fungus and mycotoxin	Antimycotoxigenic potency of botanicals	References
1	Monoterpenes (Thymol, p-cymene and carvacrol)	*Aspergillus Flavus,* Aflatoxin B_1	Aflatoxin B_1 biosynthesis is reduced by bioactive compounds through down regulation of *aflR, aflK, aflD* and *aflQ* genes expression.	Tian *et al.* (2018)
2	*Zingiber officinale*	*Fusarium verticilloides,* Fumonisin	EO reported to have significant inhibitory effect against both fumonisin B_1 and fumonisin B_2.	Yamamoto-Ribeiro *et al.* (2013)
3	*Origanum onites, Allium sativum, Salvia officinals and Mentha piperita*	*Penicillium verrucosum,* Ochratoxin A	Ochratoxin A production was effectively suppressed by *in-vitro* fumigation of EOs.	Ozcakmak *et al.* (2017)
4	*Curcuma longa*	*Fusarium graminearum,* Zearalenone	EO Fumigated maize sample depicted complete inhibition of Zearalenone production under both *in-vivo* and *in-vitro* condition by regulating mycotoxin metabolic pathway.	Kumar *et al.* (2016)
5	Eugenol, Cinnamaldehyde and citral	*Aspergillus flavus,* Aflatoxin B_1	Bioactive components suppress aflatoxin B_1 production through down regulating *aflM,aflP, aflT, aflR and aflD* genes expression.	Liang *et al.* (2015)
6	*Zingiber officinale*	*Fusarium graminearum,* Deoxynivalenol	*In-vitro* fumigation of EO inhibited growth of fungus ultimately leading to suppress Deoxynivalenol production.	Ferreira *et al.* (2018)
7	*Zataria multiflora*	*Penicillium citrinum,* Citrinin	Growth of fungus *P. citrinum* and *in-vitro* citrinin production is significantl y suppressed by EO fumigation	Mohajeri *et al.* (2018)
8	Decanal	*Penicillium expansum,* Patulin	Patulin production is inhibited due to decanal mediated alteration of regulatory and biosynthetic genes expression.	Zhou *et al.* (2018)

ANTIFUNGAL AND ANTI MYCOTOXIGENIC MODE OF ACTION OF BOTANICALS

Lipophilic nature of EOs imparts major role in their antifungal activity facilitating them to easily interact with membrane protein leading to disrupt membrane permeability and osmotic balance of cell. Ergosterol, a major sterol present in fungal cell membrane has also been reported to be Thus, plasma membrane is regarded to be the prime antifungal site for botanicals. Besides, EOs such as *Thymus eriocalyx* and *Thymus x-porlock* have been reported to exhibit antifungal action against *A. niger* through damaging its cell wall and mitochondrial membrane (Rasooli *et al.* 2006). In addition, EOs have also been reported to perform efficacious antimycotoxigenic effect through disrupting either fungal metabolic pathway or through down regulating biosynthetic or regulatory genes of secondary metabolite which ultimately prevent mycotoxins biosynthesis. EOs component *i.e.* phenolics have been reported to inhibit aflatoxin biosynthesis through disrupting mitochondrial electron transport chain which is the production site for aflatoxin precursor molecule *i.e.* acetyl COA. Recently, methylglyoxal, byproduct of several metabolic pathways has been reported as an inducer substance for aflatoxin production, through upregulating expression of its positive regulator gene *i.e. aflR*. Upadhyay *et al.* (2018) reported inhibition of methylglyoxal production as well as aflatoxin biosynthesis in *A. flavus* cells under *in-vitro* condition on fumigation with *Cistus ladanifer* EO depicting methylglyoxal inhibition as one of most probable antiaflatoxigenic action of EO. It has been reported that limonene and thymol, cyclic terpenes to suppress fumonisin B_1 biosynthesis by *F. verticillioides* due to their strong antioxidant efficacy (Dambolena *et al.* 2008). Cinnamaldehyde and citral based novel formulation is reported to have significant inhibitory potential against patulin production by *P. expansum* through down regulating expression of its biosynthetic genes (Wang *et al.* 2018). Further, EOs such as *Foeniculum vulgare*, *Elettaria cardamomum, Rosmarinus officinalis, Pimpinella anisum, Apium graveolens* and *Chamaemelum nobile* have been reported to suppress Ochratoxin A production by *A. carbonarius* through downregulating its regulatory genes *i.e. laeA* and *VeA* as well as biosynthetic genes *i.e. acOTAnrps, acOTApks and acpks* suggesting their efficacy as anti-ochratoxigenic agent during post harvest storage (El Khoury *et al.* 2016).

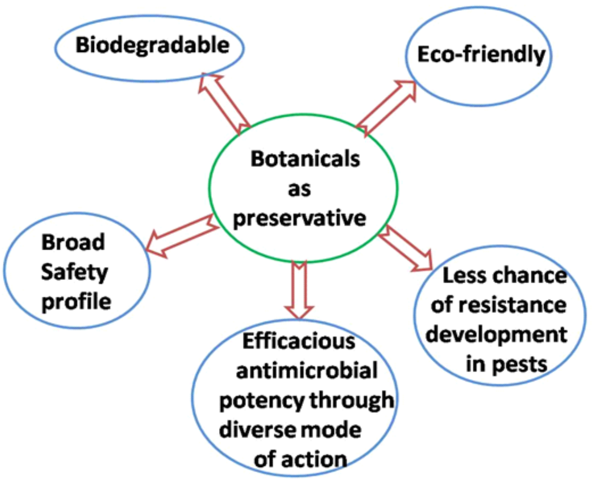

Fig. 1: Significance of botanicals as a preservative against mold and mycotoxins

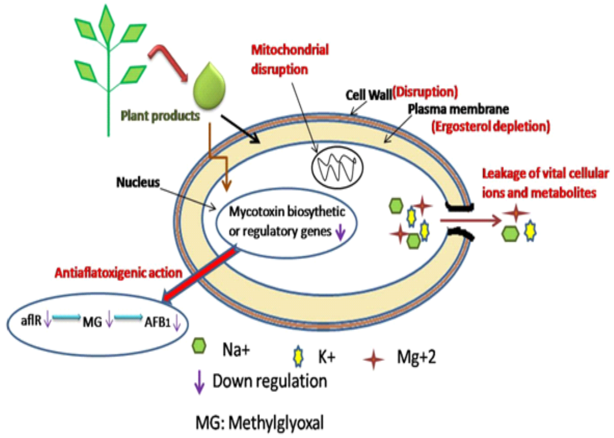

Fig. 2: Antifungal and antimycotoxigenic actions of EO

FUTURE PERSPECTIVES OF BOTANICALS AGAINST FUNGAL AND MYCOTOXIN CONTAMINATION OF FOOD ITEMS

Although plant products based preservatives are eco-friendly and safer approach in order to control storage mold and mycotoxin contamination. However, some limitations such as inadequacy of plant materials, hydrophobic nature of EOs and its less physical stability, reduce their prospects in commercial application as green preservative. Potential of botanicals are based on their bioactive principles which are reported to be vary depending on geographical and climatic conditions, age of plant, harvesting time, used plant parts as well as extraction methods which ultimately decrease their preservative efficacy. Hence, standardization of botanical products based on their chemical profile is required in order to avoid efficacy alteration caused due to chemotypic variation. Interaction of EOs and their components with foods components such as fat, protein and starch sometimes results in alteration of natural organoleptic properties of the in food items (Hyldgaard *et al.* 2012, Basak and Guha, 2018). Such hurdles associated with practical application of botanical preservatives can be solved through incorporation of nanoencapsulation technique. Nano encapsulation technique has been reported to enhance the preservative potential of EOs or their bioactive components through encapsulating them under polymeric matrix in a nano range *i.e.* 10-100 nm. Nano formulation of botanicals are reported to have improved solubility, high stability, ease of handling, controlled release of volatiles, their protection from degradation under adverse environmental as well as reduced organoleptic effects which may proved nanotechnology as a booster technology in field of food preservation (Donsi *et al.* 2014). Several encapsulated EOs such as *Syzygium aromaticum*, *Thymus capitatus* and *Cinnamomum zeylanicum* are reported to have enhanced potency against toxigenic fungal strains of *A. parasiticus* and *F. verticilloides* (Villegas-Rascón *et al.* 2018). In addition, cyclodextrin mediated encapsulation of bioactive components such as eugenol, estragole, trans-anethole, isoeugenol, ferulic acid, caffeic acid and p-coumaric acid are reported to have enhanced aqueous solubility, stability and antifungal efficacy suggesting nanoformulations as safe, efficacious and eco-friendly preservatives (Kfoury *et al.* 2016)

CONCLUSION

Different botanical formulations have currently proved their significance as green preservatives to manage post harvest mold and mycotoxin contamination of food commodities based on their favorable safety profile towards health and environment, and have become to be a prime interest of food industries. Incorporation of nanoencapsulation technique has emerged as a boon to food industries enhancing preservative potential of botanicals and boosting their bio-efficacy and bioavailability through overcoming challenges associated with their practical applicability. Different botanicals and their nanoformulations have promising future in eco-friendly preservation of food items from food borne molds and mycotoxin contamination and their bio-rational mode of action also supports their role in achieving goal of sustainable food security through increasing green consumerism.

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