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An Overview of Plant-Derived Products on Control of Mycotoxigenic Fungi and Mycotoxins

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Abstract: Mycotoxin producing fungi may contaminate agricultural products in the field, during storage, or during processing. Mycotoxin contamination in foods poses serious health hazard to animals and humans. For lowering mycotoxin contamination of foods, several strategies have been investigated that can be divided into natural, biological, chemical and physical methods. Great success has been achieved to reduce mycotoxigenic fungi and mycotoxins in foods using plant products (plant extracts and plant essential oils). In this review, the potential of these plant-derived products to reduce mycotoxin contamination of foods with particular emphasis on aflatoxins, ochratoxins and fumonisins is discussed.

Key words: Plant extracts, plant essential oils, mycotoxigenic fungi, mycotoxins

INTRODUCTION

Mycotoxins occurring in food commodities are secondary metabolites of filamentous fungi, which can contaminate many types of food crops throughout the food chain (Reddy *et al*., 2010a). Although, hundreds of fungal toxins are known, a limited number of toxins are generally considered to play important roles in food safety (Shephard, 2008; Reddy *et al*., 2010a). Fungal toxins of most concern are produced by species within the genera of *Aspergillus*, *Fusarium* and *Penicillium* that frequently occur in major food crops in the field and continue to contaminate them during storage, including cereals and oilseeds. Among these mycotoxins, aflatoxin B1 (AFB1), fumonisin B1 (FB1) and ochratoxin A (OTA) are the most toxic to mammals, causing a variety of toxic effects including hepatotoxicity, teratogenicity and mutagenicity, resulting in diseases such as toxic hepatitis, hemorrhage, oedema, immunosuppression, hepatic carcinoma, equine leukoencephalomalacia (LEM), esophageal cancer and kidney failure (Donmez-Altunta *et al*., 2003; Santos *et al*., 2001). The AFB1 has been classified as a class I human carcinogen, while FB1 and OTA have been classified as class 2B (probable human) carcinogens by the International Agency for Research on Cancer (IARC, 1993). Several outbreaks of mycotoxicoses diseases in humans and animals caused by various mycotoxins have been reported after the consumption of mycotoxin-contaminated food and feed (Reddy and Raghavender, 2007).

Several strategies are used at controlling fungal growth and the mycotoxin biosynthesis in stored grains by chemical treatments with ammonia, acids and bases or with food preservatives by physical methods and by biological methods. These methods require sophisticated equipment and expensive chemicals or reagents. Use of natural plant extracts provides an opportunity to avoid chemical preservatives. Over the years, efforts have been devoted to search for new antifungal materials from natural sources for food preservation (Galvano *et al*., 2001; Juglal *et al*., 2002; Onyeagba *et al*., 2004; Boyraz and Ozcan, 2005; Haciseferogullari *et al*., 2005). Several edible botanical extracts have been reported to have antifungal activity (Reddy *et al*., 2009; Pradeep *et al*., 2003). The essential oils extracted from clove have been shown to possess significant antifungal properties (Reddy *et al*., 2007a). The inhibitory effects of neem plant extracts on mycotoxin biosynthesis have also been examined (Reddy *et al*., 2009; Bhatnagar *et al*., 1990; Zeringue and Bhatnagar, 1990). However, this study will review the developments in control of mycotoxigenic fungi and mycotoxins using plant extracts and plant oils to fill the existing gaps and to develop effective anti-mycotoxigenic natural products for reduction of mycotoxigenic fungi and mycotoxins in foods.

PLANT EXTRACTS ON CONTROL OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS

Control of aflatoxigenic fungi and aflatoxins: Aflatoxin contamination of crops is a worldwide food safety concern. Aflatoxins refer to a group of four mycotoxins (B1, B2, G1 and G2) produced primarily by two closely related fungi, *A. flavus* and *A. parasiticus*. Many strategies, including natural control, biological control,

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control of insect pest, development of resistant cultivar, have been investigated to manage aflatoxins in crops. Among them, natural control appears to be the most promising approach for control of aflatoxins in post-harvested crops. Health hazards from exposure to toxic chemicals and economic considerations make natural plant extracts ideal alternatives to protect food and feed from fungal contamination (Reddy *et al*., 2009). An inhibitory effect of neem extracts on biosynthesis of aflatoxins (groups B and G) in fungal mycelia was reported by Bhatnagar *et al*. (1990). In an another study aflatoxin production by *A. parasiticus* was suppressed depending on the concentration of the plant aqueous extract added to the culture media at the time of spore inoculation. Aflatoxin production in fungal mycelia grown for 96 h in culture media containing 50% neem leaf and seed extracts was inhibited by 90 and 65%, respectively (Razzaghi-Abyaneh *et al*., 2005).

More recently Mondali *et al*. (2009) studied the efficacy of different extracts of neem leaf on seed borne fungi, *A. flavus*. In this study the growth of fungi was inhibited significantly and controlled with both alcoholic and water extracts of all ages and of the concentrations used. Efficacy of various concentrations of four plant extracts prepared from garlic, neem leaf, ginger and onion bulb were studied on reduction of *A. flavus* on Mustard. They found that garlic extract is most effective followed by neem (Latif *et al*., 2006). Recently, Srichana *et al*. (2009) studied the efficacy of betel leaf extract on growth of *A. flavus* and it was found that the extract at 10,000 ppm completely inhibited the growth of these fungi. Hema *et al*. (2009) evaluated some of the South Indian spices and herbs against *A. flavus* and other fungi. They found that *Psidium guajava* is more effective on all tested fungi. In an another study by Satish *et al*. (2007) aqueous extracts of fifty-two plants from different families were tested for their antifungal potential against eight important species of *Aspergillus*. Among 52, twelve extracts have recorded significant antifungal activity against one or the other *Aspergillus* species tested. Similarly Pundir and Jain (2010) studied the efficacy of 22 plant extracts against food associated fungi and found clove and ginger are more effective than other plant extracts.

In our investigation, *Syzygium aromaticum* (clove) effectively inhibited the mycelial growth of *A. flavus* and aflatoxin production (Reddy *et al*., 2009). More than 280 plant species have been investigated for their inhibitory effect on toxigenic *Aspergilli* and nearly 100 of these plants had some activity on growth or toxin production by fungi (Montes and Carvajal, 1998). Clove completely inhibited the mycelial growth of *A. flavus* and aflatoxin formation (Mabrouk and Shayeb, 1980). Karapynar (1989) reported the inhibitory effect of crude extracts from mint, sage, bay, anise and ground red pepper on the growth of *A. parasiticus* NRRL 2999 and its aflatoxin production *in vitro*. Akgul and Kivanc (1988) studied antifungal activity of selected Turkish spices (black cumin, coriander, cumin, dill, laurel, oregano, parsley, spearmint, white mustard) on some food-borne fungi and found that ground oregano showed an inhibitory effect on *A. flavus* and *A. niger*. Saxena and Mathela (1996) found antifungal activity of new compounds from *Nepeta leucophylla* and *N. clarkei* against *Aspergillus* sp. Mathela (1981) screened 12 terpenoids against growth of *Aspergillus* species and found thymol and carvacrol to be more active than nystatin and talsutin.

Some traditionally useful plants have been shown to exhibit fungitoxic properties. Awuah (1996) reported that the following plants *Occimum gratissimum*, *Cymbopogon citratus*, *Xylopia aethiopica*, *Monodera myristica*, *Sizygium aromaticum*, *Cinnamomum verum* and *Piper nigrum* are effective in inhibiting formation of non sorbic acid, a precursor in aflatoxin synthesis pathway. Leave powder of *Occimum* has been successfully used in inhibiting mould development on stored soybean for 9 months (Awuah, 1996). The powder extracts of *Cymbopogon citratus* inhibited the growth of fungi including toxigenic species such as *A. flavus* and *A. fumigatus* (Adegoke and Odesola, 1996). Awuah and Ellis (2002) reported the effective use of powders of leaves of *O. grattisimum* and cloves (*S. aromaticum)* combination with some packaging materials to protect groundnut kernels artificially inoculated with *A. parasiticus*. Hell *et al*. (2000) found that the use of *Khaya senegalensis* bark to protect maize against insects increased the risk of aflatoxin development. There have been a number of reports citing the inhibitory effects of onion extracts on *A. flavus* growth, with an ether extract of onions, thio-propanol-S-oxide, being demonstrated to inhibit growth. In addition, Fan and Chen (1999) reported that welsh onion ethanol extracts depressed the mycelial growth and aflatoxin production of some strains of aflatoxin-producing fungi. Pepper extracts have been shown to reduce aflatoxin production in *A. parasiticus* IFO 30179 and *A. flavus* var *columnaris* S46 (Ito *et al*., 1994).

CONTROL OF OCHRATOXIGENIC FUNGI AND OCHRATOXINS

Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin produced by certain species of *Aspergillus* and *Penicillium* (Reddy *et al*., 2010a). This

mycotoxin can contaminate agricultural products, including cereals, coffee, dried fruits, wine and pork (Reddy *et al*., 2010b). Various studies have been conducted to reduce the ochratoxigenic fungi and ochratoxins contamination using plant extracts. The effect of *Azadirachta indica* (neem) extracts on mycelial growth, sporulation, morphology and OTA production by *P. verrucosum* and *P. brevicompactum* were studied by Mossini *et al.* (2009). In this study they observed that inhibition mainly of fungal growth and not OTA production. The effects of four alkaloids on the biosynthesis of OTA and ochratoxin B (OTB) were examined on four OTA-producing Aspergilli: *A. auricomus*, *A. sclerotiorum* and two isolates of *A. alliaceus*. Piperine and piperlongumine, natural alkaloids of *Piper longum*, significantly inhibited OTA production at 0.001% (w/v) for all Aspergilli examined. Curcumin, a constituent of tumeric, completely inhibited mycelial growth of *A. alliaceus* isolate 791 at 0.1% (w/v) and decreased OTA production by 70% at 0.01% (w/v) (Lee *et al*., 2007). The antitoxigenic potential of the spices was tested against OTA-producing strain of *A. ochraceus* Wilhelm. Clove completely inhibited the micelial growth of the fungi *A. ochraceus*. Garlic and laurel completely inhibited the OTA production. Cinnamon and anis inhibited the synthesis of OTA starting from the concentration of 3% and mint starting from 4% (Pereira *et al*., 2006). Reddy *et al.* (2007b) reported the efficacy of certain plant extracts on mycelial growth of *A. ochraceus* and OTA biosynthesis.

CONTROL OF FUMONISIN PRODUCING FUNGI AND FUMONISINS

Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide (Reddy *et al*., 2010a). Several species from this genus are economically relevant because, apart from their ability to infect and cause tissue destruction on important crops such as corn, wheat and other small grains on the field, they produce mycotoxins on the crops in the field and in storage grains (Reddy *et al*., 2010a). Fumonisins are mycotoxins produced mainly by the fungi *F. verticillioides* and *F. proliferatum* (Dambolena *et al*., 2010). Fumonisin B1 (FB1) is generally the most abundant member of the family of mycotoxins and is known to cause various animal and human diseases (Reddy *et al*., 2008). Additionally, fumonisins are potent liver toxins in most animal species and are suspected human carcinogens (Bhat *et al*., 2010). Very few scattered reports are available on control of *Fusarium* sp. and their mycotoxins using plant extracts. The *in vitro* efficacy of different plant extracts viz., *Azardiachta indica*, *Artemessia annua*, *Eucalyptus globules*, *O. sanctum* and *Rheum emodi* were tested to control *F. solani*. All plant extracts showed significant reduction of pathogen (Joseph *et al*., 2008). Recently, Anjorin *et al.* (2008) reported the effect of neem extract on control of *F. verticillioides* in Maize. In an another study, Amin *et al.* (2009) reported the efficacy of garlic tablet against *Fusarium* sp., associated with cucumber and found that garlic tablet was effectively inhibited all the fungi tested. Still today there are no reports on effect of plant extracts on fumonisin biosynthesis.

PLANT OILS ON CONTROL OF MYCOTOXIGENIC FUNGI AND MYCOTOXINS

Control of aflatoxigenic fungi and aflatoxins: Large-scale application of different higher plant products-azadirachtin from *Azadirachta indica*, eugenol from *Syzygium aromaticum*, carvone from *Carum carvi* and allyl isothiocyanate from mustard and horseradish oil have attracted the attention of microbiologists to other plant chemicals for use as antimicrobials (Reddy *et al*., 2007a; Singh *et al*., 2008). Such products from higher plants would most likely be biodegradable, renewable in nature and perhaps safer to human health (Varma and Dubey, 1999). Plant products, especially essential oils, are recognized as one of the most promising groups of natural compounds for the development of safer antifungal agents (Varma and Dubey, 2001). Many reports are available on use of neem oil to control toxigenic fungi and their toxins. Plant essential oils from *Azadirachta indica* and *Morinda lucida* were found to inhibit the growth of a toxigenic *A. flavus* and significantly reduced aflatoxin synthesis in inoculated maize grains (Bankole, 1997). Zeringue *et al.* (2001) observed the increase of 11-31% of dry mycelial mass along with a slight decrease (5-10%) in AFB1 production in 5-day-old aflatoxigenic *Aspergillus* sp., submerged cultures containing either 0.5 or 1.0 mL Clarified Neem Oil (CNO) in 0.1%. Recently, Sitara *et al.* (2008) extracted essential oils from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) were evaluated for their antifungal activity against seed borne fungi viz., *A. niger* and *A. flavus*. All oils extracted except mustard, showed fungicidal activity of varying degree against test species.

Clove oil and its major component, eugenol has been extensively used to control mycotoxigenic fungi and mycotoxins. On rice treated at 2.4 mg eugenol/g of grains, the inoculum of *A. flavus* failed to grow and thus AFB1 biosynthesis on rice was prevented (Reddy *et al*., 2007a).

Jham *et al.* (2005) reported antifungal activity of cinnamon bark oil against *A. flavus*. Juglal *et al.* (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxin-producing molds and noted that clove, cinnamon and oregano were able to prevent the growth of *A. parasiticus* while clove (ground and essential oil) markedly reduced the aflatoxin synthesis in infected grains. Eugenol has been extracted and purified from cloves (Hitokoto *et al.*, 1980) and from *O. gratissimum* (Faria *et al.*, 2006). Several reports are available on the inhibitory effect of clove oil on *A. flavus* and *A. parasiticus* as well as several other fungi (Bullerman *et al.*, 1977; Mabrouk and Shayeb, 1980). Hitokoto *et al.* (1980) reported the complete inhibition of mycelial growth of *A. flavus* and *A. versicolor* at 250 μg eugenol/ml in yeast-sucrose broth, but the biosynthesis of AFB1 *in vitro* was arrested only at 125 μg mL^{-1} of eugenol. The effects of cinnamon oil, clove oil, cinnamic aldehyde and eugenol on growth and aflatoxin production by *A. parasiticus* were studied earlier using YES media as the substrate. All four substances inhibited mold growth and subsequent toxin production (Bullerman *et al.*, 1977). More recently, Kumar *et al.* (2010) studied the efficacy of *O. sanctum* Essential Oil (EO) and its major component, eugenol against the fungi causing biodeterioration of food stuffs during storage. *O. sanctum* and eugenol were found efficacious in checking growth of *A. flavus* and also inhibited the AFB1 production completely at 0.2 and 0.1 μg mL^{-1}, respectively.

Apart from neem and clove oils, various plant essential oils has been used for reduction of mycotoxins. Recently, Singh *et al.* (2008) extracted essential oils from different parts of 12 plants belonging to eight angiospermic families and tested for activity against two toxigenic strains of *A. flavus*. The oil of the spice plant *Amomum subulatum* Roxb. (Fam. Zingiberaceae) was found effective against two strains of *A. flavus*, completely inhibiting their mycelial growth at 750 μg mL^{-1} and AFB1 production at 500 μg mL^{-1}. Kumar *et al.* (2007) extracted oil from the leaves of *Chenopodium ambrosioides* Linn. (Chenopodiaceae) and tested against the aflatoxigenic strain of test fungus *A. flavus*. The oil completely inhibited the mycelial growth at 100 μg mL^{-1} and significant reduction of AFB1. In an another study, Jardim *et al.* (2008) reported antifungal activity of Essential Oil (EO) from the Brazilian epazote (*Chenopodium ambrosioides* L.) was evaluated by the poison food assay at concentrations of 0.3, 0.1 and 0.05% against postharvest deteriorating fungi (*A. flavus*, *A. glaucus*, *A. niger* and *A. ochraceous*). Growth of all fungi was completely inhibited at 0.3% concentration and by 90 to 100% at 0.1% concentration. From this plant extract they have identified 13 antifungal compounds. The effect of eucalyptus oil on growth and aflatoxin production by *A. flavus* was tested at three levels, viz., 0.05, 0.1 and 0.2 mL/50 mL SMKY medium. After 6 days of incubation on 0.05 and 0.1 mL supplemented SMKY medium, growth and toxin production were inhibited while at 0.2 mL concentration there was no growth. However, after 12 days of incubation toxin production was greater than the controls (Ansari and Shrivastava, 1991). Thanaboripat *et al.* (2007) studied the effects of 16 essential oils from aromatic plants against mycelia growth of *A. flavus* IMI 242684. The results showed that the essential oil of white wood (*Melaleuca cajeputi*) gave the highest inhibition followed by the essential oils of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*), respectively.

In addition lemon and orange oils while at concentrations of (0.05-2.0%) more than a 90% reduction in aflatoxin formation by *A. flavus* has been demonstrated (Hasan, 2000). Adegoke *et al.* (2000) found that the minimum inhibitory concentration of the essential oil of the spice *Aframomum danielli* for the aflatoxigenic mould *A. parasiticus*. Kumar *et al.* (2009) studied the efficacy of essential oil from *Mentha arvensis* L. to control storage moulds of Chickpea. The oil was effectively reduced mycelia growth of *A. flavus*. During screening of essential oils for their antifungal activity against *A. flavus*, the essential oil of *Cymbopogon citratus* was found to exhibit fungitoxicity. In another extensive study, Tamil-Selvi *et al.* (2003) demonstrated that *A. flavus* growth and AFB1 production were both inhibited by an essential oil, containing mainly garcinol; from the tropical shrub/tree *Garcinia indica* at 3000 ppm.

CONTROL OF OCHRATOXIGENIC FUNGI AND OCHRATOXINS

Very few scattered reports are available on effects of plant oils on growth of ochratoxigenic fungi and ochratoxin biosynthesis. With respect to OTA production, spice essential oils of oregano (*Origanum vulgare*), mint (*Menta arvensis*), basil (*Ocimum basilicum*), sage (*Salvia officinalis*) and coriander (*Coriandrum sativum*) have been shown to be effective against ochratoxin-producing fungi, with oregano and mint oils completely inhibiting the growth of *A. ochraceus* NRRL 3174 and OTA production after 21 days at the concentration of 1000 ppm (Basilico and Basilico, 1999). Recently Mossini *et al.* (2009) conducted *in vitro* trials to evaluate the effect of *Azadirachta indica* (neem) oil on mycelial growth, sporulation, morphology

and OTA production by *P. verrucosum* and *P. brevicompactum*. Oil extracts exhibited significant reduction of growth and sporulation of the fungi. No inhibition of OTA production was observed. Essential oils of 12 medicinal plants were tested for inhibitory activity against *A. ochraceus* and OTA production. The oils of thyme and cinnamon completely inhibit all the test fungi and OTA at 3000 ppm (Soliman and Badea, 2002).

CONTROL OF FUMONISIN PRODUCING FUNGI AND FUMONISINS

Several reports are available on use of plant essential oils against fumonisin producing fungi and fumonisins biosynthesis. Recently Sitara *et al.* (2008) evaluated essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassica campestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) against seed borne fungi viz., *F. oxysporum*, *F. moniliforme*, *F. nivale*, *F. semitectum*. All the oils extracted except mustard, showed fungicidal activity of varying degree against test species. Kumar *et al.* (2007) extracted essential oil from the leaves of *Chenopodium ambrosioides* Linn. (Chenopodiaceae) and tested against the *F. oxysporum*. In another study, Jardim *et al.* (2008) reported antifungal activity of Essential Oil (EO) from the Brazilian epazote (*Chenopodium ambrosioides* L.) against postharvest deteriorating fungi *F. oxysporum* and *F. semitectum*. Growth of all fungi was completely inhibited at 0.3% concentration.

More recently Dambolena *et al.* (2010) investigated the constituents and the efficacy against *F. verticillioides* infection and fumonisin production of essential oils of *O. basilicum* L. and *O. gratissimum* L. from different locations in Kenya. All oils showed some inhibitory effects on growth of *F. verticillioides*. However, the extent of inhibition was widely dependent upon the composition and the concentration of oils. When maize was treated with *O. basilicum* oils, no effects were observed in the FB1 biosynthesis but *O. gratissimum* essential oils were found to induce a significant inhibitory effect on FB1 production with respect to control. Fadohan *et al.* (2004) showed that *O. basilicum* essential oil of Benin possess significant inhibitory effect on growth of *F. verticillioides* and FB1 production in corn. Juglal *et al.* (2002) reported spice oils of eugenol, cinnamon, oregano, mace, nutmeg, tumeric and aniseed displayed antifungal activity against *F. moniliforme* and 78% reductions in fumonisin B1 (FB1) formation by this fungus, when treated with 2 μL mL^{-1} clove oil.

The anti *F. oxysporum* f. sp., *cicer* (FOC) effects were evaluated for 75 different essential oils. The most active essential oils found were those of lemongrass, clove, cinnamon bark, cinnamon leaf, cassia, fennel, basil and evening primrose (Pawar and Thanker, 2007). The effect of cinnamon, clove, oregano, palmarosa and lemongrass oils on fumonisin B_1 (FB_1) accumulation by one isolate each of *F. verticillioides* and *F. proliferatum* in non-sterilised naturally contaminated maize grain at 0.995 and 0.950 a_w and at 20 and 30°C was evaluated. The concentration used was 500 mg kg^{-1} maize. Under these conditions it was shown that antimycotoxigenic ability only took place at the higher water availabilities and mostly at 20°C. Only cinnamon, lemongrass and palmarosa oils were somewhat effective. Moreover, it was suggested that competing mycoflora plays an important role in FB_1 accumulation. It was concluded that the efficacy of essential oils in real substrates, such as cereals, may be much lower than in synthetic media; different essential oils may be found to be useful and at different concentrations. Their effectiveness is highly dependent on both abiotic and biotic factors involved (Marín *et al.*, 2003).

CONCLUSIONS

In this review, we tried to give information on effects of plant extracts and plant essential oils in control of mycotoxigenic fungi and mycotoxins. Although the extensive use of various products, neem materials, such as leaves, seed or kernel powder and oil can be used economically to achieve acceptable levels of toxigenic fungi and mycotoxins control in developing countries, where neem is widespread. Also, as azadirachtin, the principal bioactive ingredient in neem, is heat sensitive and cold processing technology for neem seed would be needed. Neem oil obtained by cold processing of seed is light in color and can be rich in azadirachtin (Ramakrishna *et al.*, 1993). Neem materials, having fungal growth and mycotoxin inhibitory properties, offer a time-tested, novel approach to the management of storage fungi. This approach can be quite practical and preferable over other plant extracts and oils. A promising method for preserving stored products in villages and rural areas, which do not have access to modern storage facilities, will be through encouraging the use of neem-treated storage bags or bins. Oil, thus obtained, could be standardized for chemical properties and ingredients, biological activity and its efficacy stabilized and further enhanced by the addition of stabilizers, antioxidants and synergists. Also, improved methods of application e.g., mechanical mixers for uniform and bulk

coating of oil on grain, use of slow release dispensers/sachets which could be placed at different depths in storage structures, bins or bags, could be devised for ensuring and enhancing efficacy.

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