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Control of Toxigenic Fungi and Mycotoxins with Phytochemicals: Potentials and Challenges

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1. Introduction

Mycotoxin-producing fungi are significant contaminant and destroyers of agricultural products and seeds in the field, during storage, during processing and in the markets, and reduce their nutritive value (Jimoh and Kolapo, 2008). Mycotoxin contamination in foods and feedstuffs poses serious health hazard to animals and humans (Mokhles *et al.*, 2007; Iheshiulor *et al.*, 2011). Mycotoxins are commonly produced by species of *Aspergillus*, *Penicillium* and *Fusarium* (Chandra and Sarbhoy, 1997; Masheshwar *et al.*, 2009). Several strategies are used at controlling fungal growth and the mycotoxin biosynthesis in seeds, grains and feedstuff by chemical treatments, and food preservatives, by physical and biological methods. These methods often require sophisticated equipment and expensive chemicals or reagents. Chemical control of fungi and mycotoxins also result in environmental pollution, health hazard and affects the natural ecological balance Yassin *et al.*, 2011). Use of plant products inform of plant extracts and essential oils provides an opportunity to avoiding synthetic chemical preservatives and fungicide risks (Mohammed *et al.*, 2012).

Phytochemicals, a term given to naturally occurring, non-nutritive biologically active chemical compounds of plant origin, have some protective or disease-preventive properties. Some phytochemicals are injurious to fungi and could be used to protect crops, animals, humans, food and feeds against toxigenic fungi and mycotoxin (OMAF, 2004). Phytofungicides could be prepared or formulated from the leaves, seeds, stem bark or roots of plants of pesticidal significance and could be applied inform of extract, powders and cakes or as plant exudates (Owino and Wando, 1992; Anjorin and Salako, 2009). Phytochemicals vary in plants depending on their growing conditions, varietal differences, age at harvest, extraction methods, storage conditions and age of sample. The use of plant derivatives for fungal control is common in developing countries before the advent of

synthetic fungicides and due to relatively cost implication of imported fungicides (Galvano *et al.*, 2001). Over the years, efforts have been devoted to the search for new antifungal materials from natural sources for food preservation (Juglal *et al.*, 2002; Onyeagba *et al.*, 2004; Boyraz and Ozcan, 2005). Several edible botanical extracts have been reported to have antifungal activity (Ferhout *et al.*, 1999; Pradeep *et al.*, 2003). The essential oils extracted from clove have been shown to possess significant antifungal properties. Afzal *et al.*, (2010) reported that *A. sativum* has a wide antifungal spectrum, reached about 60-82% inhibition in the growth of seed borne *Aspergillus* and *Penicillium* fungi. This was attributed to phytochemical properties of garlic plant, allicin which could decompose into several effective antimicrobial compounds such as diallyl sulphide, diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide, dithiins and ajoene (Salim 2011; Tagoe, 2011).

In recent years, the need to develop fungal disease control measures using phytochemicals as alternative to synthetic chemicals has become a priority of scientists worldwide (Reddy *et al.*, 2007). Therefore, it is important to find a practical, cost effective and non-toxic method to prevent fungal contamination and mycotoxins load in stored farm produce. Use of natural plant extracts and biocontrol agents provides an opportunity to avoid chemical preservatives. A multitude of fungitoxic plant compounds (often of unreliable purity) is readily available in the fields. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market (Pal and Gardener, 2006). However, in order to protect food quality and the environment, low persistent synthetic fungicides are still relevant at present to prevent diseases of food crops. The search for an alternative or a complement to synthetic fungicide is justified. This paper reviewed the potential of botanicals in the control of toxigenic fungi and mycotoxin, constraints in their formulation and usage or in their proper formulation. Ascertaining the quality of fungitoxic phytochemicals during the production, registration, marketing and their usage is very important.

2. Why the use of phytofungicides?

Vast fields in developing countries are blessed with abundant plants with fungicidal potential with preparation and application attracting lower capital investment than synthetic fungicides (Anjorin and Salako, 2009). Rotimi and Moens (2003) reported that botanical pesticides are locally renewable, user-friendly and environmentally safe. The knowledge and technology involved in using botanicals is embedded in folklores and tradition of the farmers (Anjorin and Salako, 2009). Among several control strategies, natural control appears to be the most promising approach for the control of mycotoxins such as aflatoxin in post-harvested crops. Though synthetic fungicides improve plant protection but most of them are hazardous to man. 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.*, 2007). Hence antimicrobial properties of some plant constituents are being exploited in protecting food, feed and seeds from storage moulds (Centeno *et al.*, 2010).

Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Further, these are safe and effective in view of their systemic action and lack residual effect, easily biodegradable and exhibit stimulating effect on plant metabolism. Also, large number of earlier workers has reported antifungal properties of several plant species (Naganawa *et al.*, 1996; Kubo *et al.*, 1995). Efficacy of some plant phytochemicals against mycotoxin producing fungi suggests its possible use in minimizing the risk of mycotoxins as well as fungicides exposure. Varieties of secondary metabolites in plants are tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have fungitoxic properties (Table 1).

Common name	Scientific name	Compound	Class	Activity
Apple	<i>Malus sylvestris</i>	Phloretin	Flavonoid derivative	General ^a
Betel pepper	<i>Piper betel</i>	Catechols, eugenol	Essential oils	General Bacteria, fungi
Ceylon cinnamon	<i>Cinnamomum verum</i>	Essential oils, others	Terpenoids, tannins	General
Garlic	<i>Allium sativum</i>	Allicin, ajoene	Sulfoxide	General
Grapefruit peel	<i>Citrus paradise</i>		Terpenoid	Fungi
Green tea	<i>Camellia sinensis</i>	Catechin	Flavonoid	General
Lantana	<i>Lantana camara</i>	6,7-dimethylesculetin	alkaloid	General
Mesquite	<i>Prosopis juliflora</i>	Phenethylamine	alkaloid	General
Olive oil	<i>Olea europaea</i>	Hexanal	Aldehyde	General
Orange peel	<i>Citrus sinensis</i>	d-limonene	Terpenoid	Fungi
Peppermint	<i>Mentha piperita</i>	Menthol	Terpenoid	General
Periwinkle	<i>Vinca minor</i>	Reserpine	Alkaloid	General
Potato	<i>Solanum tuberosum</i>	Solanine		Bacteria, fungi
Snakeplant	<i>Rivea corymbosa</i>	Ergine		General
Thyme	<i>Thymus vulgaris</i>	Caffeic acid	Terpenoid	Viruses, bacteria, fungi
		Thymol	Phenolic alcohol	
		Tannins	Polyphenols Flavones	
Physic nut	<i>Jatropha gossypifolia</i>	Curcin		General
Valerian	<i>Valeriana officinalis</i>	Essential oil	Terpenoid	General

Source: Cowan, 1999; "General" denotes activity against multiple types of microorganisms (e.g., bacteria, fungi, and protozoa)

Table 1. Plants and their phytochemicals containing antimicrobial activity

Antifungal action of plant extracts has great potential as they are easy to prepare and apply. Further, these are safe and effective in view of being systemic in their action and lack residual effect, easily biodegradable and exhibit stimulating effect on plant metabolism. Several authors have confirmed the antifungal properties of several plant parts and phytochemicals (Giridhar and Reddy, 1996; Benharref and Jana, 2006; Satish, 2007).

Plant fungicides have been reported to be safe to beneficial organisms such as pollinating insects, earthworms and to humans (Rotimi and Moens, 2002). Khalid *et al.* (2002) reported that their toxic effect is normally of an ephemeral nature disappearing within 14-21 days. Thus phytofungicides are environment-friendly. Due to very high and disproportionate monetary exchange rate, synthetic fungicides are now more expensive than they were before, thus making them unaffordable by most of the resource-poor farmers (Salako, 2002). Some synthetic fungicides such as methyl bromide are phytotoxic and often leave undesirable residues when applied on the growing crops (Anastasiah, 1997). Other deleterious effects include occupational hazards, mammalian toxicity and soil pollution. Thus, the search for an alternative or complement to synthetic fungicide is justified.

3. Toxigenic fungi and mycotoxin

Field and storage fungal contaminants of grains in Nigeria had previously been reported by Makun *et al.*, (2007) and in rice in India by Reddy *et al.*, (2004). They include *Alternaria alternata*, *Cladosporium cladosporioides*, *Curvularia* spp., *Phoma* spp., *Fusarium* spp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus tamarii*, *Aspergillus nidulans*, *Aspergillus candidus* and *Penicillium* spp. Fungal deterioration of seeds, grains and feed stuff is a chronic problem in the developing countries field and storage system as most of them are in tropical hot and humid climate. The presence and growth of fungi may cause spoilage of food and its quality and quantity (Candlish *et al.*, 2001; Rasooli and Abyaneh, 2004).

Aspergilli are the most common fungal species that can produce mycotoxins in seeds, food and feedstuffs. 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).

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.*, 2010). 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 (IARC, 1993, Santos, Lopes, and Kosseki, 2001 Donmez-Altunta *et al.*, 2003; Negedu *et al.*, 2011) Aflatoxin B₁ (AFB₁) has been classified as a class 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1993).

4. Control of mycotoxigenic fungi and mycotoxins with plant products

In this section, the potentials of using plant-derived products to reduce toxigenic fungi and mycotoxin contamination of foods with particular emphasis on aflatoxins, ochratoxins and fumonisins are discussed.

4.1. Control of aflatoxigenic fungi and aflatoxins with plant products

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*. Aflatoxin contamination of crops is a worldwide food safety concern. An inhibitory effect of neem extracts on biosynthesis of aflatoxins (groups B and G) in fungal mycelia was reported by Bhatnagar *et al.* (1990). 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). 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*. Saxena and Mathela (1999) 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. In 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 the fungus 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 this fungus. 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 that clove and ginger are more effective than other plant extracts.

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*. 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 (Fan and Chen 1999). Pepper extracts have been shown to reduce aflatoxin production in *A. parasiticus* IFO 30179 and *A. flavus* var *columnaris* S46 (Ito *et al.*, 1994). Large-scale application of different higher plant products like 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 (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 *et al.*, 2006). 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%.

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.*, 2007; Jham *et al.*, 2005; Faria *et al.*, 2006) reported antifungal activity of cinnamon bark oil against *A. flavus*. Juglal *et al.* (2002) studied the effectiveness of nine essential oils in controlling the growth of mycotoxin-producing moulds 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.. 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 have 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 $\mu\text{g mL}^{-1}$ and AFB₁ production at 500 $\mu\text{g mL}^{-1}$. The oil completely inhibited the mycelial growth at 100 $\mu\text{g mL}^{-1}$ with significant reduction of AFB₁. From this plant extract they have identified 13 antifungal compounds. 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 cajuputi*) gave the highest inhibition followed by the essential oils of cinnamon (*Cinnamomum cassia*) and lavender (*Lavandula officinalis*), respectively.

In addition lemon and orange oils (at concentrations of 0.05-2.0%) effected more than a 90% reduction in aflatoxin formation by *A. flavus* has been demonstrated (Hasan, 2005). Kumar *et al.* (2009) studied the efficacy of essential oil from *Mentha arvensis* L. to control storage moulds of Chickpea. The oil effectively reduced mycelial 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.

4.2. Control of ochratoxigenic fungi and ochratoxins with phytochemicals

This mycotoxin can contaminate agricultural products, including cereals, coffee, dried fruits, wine and pork. Ochratoxin A (OTA) is a nephrotoxic and carcinogenic mycotoxin produced by certain species of *Aspergillus* and *Penicillium* (Reddy *et al.*, 2010). 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 was 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. The antitoxigenic potential of the spices was tested against OTA-producing strain of *A. ochraceus* Wilhelm. Clove completely inhibited the mycelial 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% (Bugno *et al.*, 2006). Reddy *et al.* (2010) reported the efficacy of certain plant extracts on mycelial growth of *A. ochraceus* and OTA biosynthesis.

Very few reports are available on effects of plant oils on growth of ochratoxigenic fungi and ochratoxin biosynthesis. 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).

4.3. Control of fumonisin producing fungi and fumonisins with phytochemicals

Fungi of the genus *Fusarium* are widely found in plant debris and crop plants worldwide (Reddy *et al.*, 2010). 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 (Makun *et al.*, 2010). 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.*, 2007). 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., *Azadiachta 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 efficacy of neem extract on the control of *F. verticillioides* in Maize. In another study, Amin *et al.* (2009) reported the efficacy of garlic tablet against *Fusarium* sp., associated with cucumber and found that garlic tablet effectively inhibited all the fungi tested.

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 from the Brazilian epazote (*Chenopodium ambrosioides* L.) against postharvest deteriorating fungi *F. oxysporum* and *F. semitectum*. Growth of the fungi was completely inhibited at 0.3% concentration.

More recently Dambolena *et al.* (2010) investigated the constituents and the efficacy of essential oils of *O. basilicum* L. and *O. gratissimum* L. from different locations in Kenya against *F. verticillioides* infection and fumonisin production. 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 $\mu\text{L mL}^{-1}$ clove oil.

The anti fungal effects of 75 different essential oils on *F. oxysporum* f. sp., *cicer* (FOC) were evaluated. The most active essential oils found were those of lemon grass, 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 B₁ (FB₁) 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 play an important role in FB₁ 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 (Marin *et al.*, 1998).

5. Challenges in the production and usage of fungicidal botanicals

The effective control by fungitoxic plant products in developing countries remains poor and seriously hampered by several factors including lack of proper legislative authority; shortage of trained personnel in natural pesticide regulatory procedures; lack of infrastructure, transportation, equipment and materials; lack of product and phyto pesticide residue analysis facilities and capabilities (Akunyili and Ivbijaro, 2006). However, the inadequate availability of raw materials, formulation of quality, potent products and their commercialization are among the constraints facing phytofungicides.

At the present time in most developing countries, research on product quality is uncoordinated, with quality research projects conducted in isolation and the results often not widely disseminated. Knowledge transfer from academia to industry and government, and information dissemination between industry members is quite limited. Fostering the development of a cohesive quality research network could make significant inroads in addressing this problem, and could generally assist in the successful execution of strategies to close quality research gaps (Anjorin, 2008).

There are relatively few standard commercialized botanical fungicides produced in developing countries despite several reports of *in vitro* fungicidal activities of several plant products. Only the resource-poor farmers are left with the usage of home-produced plant fungicides. Anjorin and Salako (2009) reported the following constraints faced by Nigerian farmers in the preparation and usage of home-produced plant fungicides:

- Collection and utilization of natural products seemed to be expensive in terms of time and labour.
- Crude and inadequate processing tools and implements such as grinding stone instead of a grinder or a blender thus making their preparation full of drudgery. However, 65%

of the farmers agreed that if sophisticated facilities are available, they might not be able to afford it.

- Scarcity of certain plant materials especially those that people compete for because of its efficacy such as *Erythrophleum suaveolens* or those that are of commercial value such as cashew nut.
- Low efficacy of most botanicals due to their brief persistence or short shelf-life as they are easily prone to microbial, thermal or photo degradation. This often leads to a repetitive or frequent application of these plant products for optimal efficacy. Thus many of the farmers indicated that if they have the cash, they would rather switch to synthetic products because of ease of handling and efficacy.
- Bulkiness of some botanical materials during collection, preparation and application, such as 10 kg of neem leaf powder required for amending 100 m² of tomato field per time. Thus up to 65% of farmers used plant materials exclusively as protectants of stored seeds because of the limited quantities needed while lower percentage of the farmers applied it only on small gardens not more than one-tenth of a hectare
- Washing away of extracts on foliage or leaching during rainfall which often warrant repetitive application thus increasing labour involvement. Thus addition of surfactants might be required.

These weaknesses of home-produced plant fungicides in developing countries necessitate an improvement so that natural fungicides could be standardized and commercialized. Some local pesticidal plants used by Nigerian farmers and their constraints are as shown in Table 2. Production of commercial phytofungicides is more sophisticated than the home-produced crude form. That might have been the reason why the patented natural fungicide is relatively scarce and expensive in the open-markets in developing countries. The standard procedure involved in phytofungicide production is the use of variety of solvents such as hexane, ethanol, pentane, methanol or ether singly or in a mixture for fractionating the components or extracting the active compounds in the fungicidal plants. Once the active ingredient has been extracted and purified, it has to be added to inert compounds to produce a fungicide product with a known stable plant pesticide concentration. During the process of isolating the active ingredients, it should be bioassay guided (McGuffin, 2001).

The structures of natural plant products are normally too complex, illusive and very expensive to pursue. The process of simplification and purification are often slow and cumbersome; may lead to loss of activity (Dayan *et al.*, 1992). A standard of active ingredient from fungicidal plant is required for the registration of commercial fungicide and their subsequent use on a commercial scale. Most of these natural products should be subjected to rigorous mammalian toxicity testing as it is done to synthetic pesticide before it is confirmed safe to man. Nicotine has been reported to be highly toxic to man and pyrethrum and derris were toxic to fish and should not be used near water (Asogwa, 2009). The aqueous extract from *Azadirachta indica* leaf, neem oil from the kernel, neem cake have all been reported to cause infertility (e.g. by retarding spermatogenesis) in studies with male rats, mice, rabbits and guinea pigs. Oral administration of neem oil to female rats caused infertility or had abortive effect (Moravati, *et al.* 2008).

Name	Family	Local names	Parts used	Use	Constraints
1. Iron weed (<i>Blumea perotitiana</i>)	<i>Asteraceae</i>	Gw*: <i>Sinmisinmi</i> Ba: <i>Gbagbaje</i> Ha: <i>Tabataba</i>	Whole leaf Leaf powder	For seed dressing	Difficult to renew; scattered in the wild
2. Bush tea (<i>Hyptis suaveolens</i>)	<i>Lamiaceae</i>	Gw: <i>Basamsin</i> Ba: <i>Adabwa</i> Ha: <i>Dadoya</i>	Whole leaf Leaf powder/ slurry	Fumigant in the farm produce store	Offensive odour produced
3. Ground star weed (<i>Mitracarpus villosus</i>)	<i>Rubiaceae</i>	Gw: <i>Jiji pampi</i> Ba: <i>Olugodotondo</i> Ha: <i>Gogamasu</i>	Leaf powder/ extract	Seed/tuber dressing at low concentration	Tiny leaves difficult to harvest
4. Lophira (<i>Lophira lanceolata</i>)	<i>Ochnaceae</i>	Gw: <i>Gbonrii</i> Ba: <i>Zhimya</i> Nu: <i>Gbetseti</i>	Leaf powder/ extract	Yam sett dressing	Difficult to processing because of the tough leaves
5. Neem (<i>Azadirachta indica</i>)	<i>Meliaceae</i>	Gw: <i>Sawaki</i> Ba: <i>Kunini</i>	Whole leaf/leaf power/extract	Used to protect foliage, seed/soil treatment	Seed collection laborious and bitter taste residue
6. Tobacco (<i>Nicotiana tabacum</i>)	<i>Solanaceae</i>	Gw: <i>Taba</i> Ba: <i>Taba</i>	Whole leaf/leaf power/extract	Seed /store treatment	Leaf has other competitive demand/market value.
7. Stinking cassia (<i>Senna alata</i>)	<i>Leguminosae: Caesalpinioidea</i>	Gw: <i>Wampin</i> Ba: <i>Kpe tesuusu</i>	Whole leaf/leaf power/extract	Store protectant	Low efficacy
8. Olax (<i>Olax subscorpiodea</i>)	<i>Leguminosae: Caesalpinioidea</i>	Gw: <i>Wazigege</i> Ba: <i>Ombolaawe</i> Ha: <i>Gwanonkurmi</i>	Leaf powder/ extract	Store pesticide	Not wide spread. Found beside stream/river or in the forest
9. Sodom apple (<i>Calotropis procera</i>)	<i>Asclepiadaceae</i>	Gw: <i>Kokekoke</i> Ba: <i>Obiyawae</i>	Leaf extract	Seed treatment	Not easily available
10. Mushroom (<i>Amanita phalloides</i>)	<i>Amanitaceae</i>	Gw: <i>Munu</i> Ba: <i>Tsatsigba</i> Ha: <i>Ganganzomo</i>	Powder/ cap extract	Seed treatment	Cap not renewable; seasonal and sparsely distributed in the wild
11. Lippa (<i>Lippa multiflora</i>)	<i>Verbanaceae</i>	Gw: <i>Minsin</i> Ba: <i>Bukamburu</i> Ha: <i>Agwantaaki</i>	Leaf powder/ extract	Store protectant	Causes itching when it touches the skin.

* Gwa= Gwari languages; Ba= Bassa; Ha= Hausa

Table 2. Plant leaves locally used for crop protection and their constraints among Abuja, Nigerian farmers

It should be noted that despite the vast literature on the efficacy of plant material in controlling mycotoxigenic moulds in developing countries, there has not been any concerted effort on its commercial production for a large-scale use on farmers' field. Jaryum *et al.*, 2002 believed that botanicals are most suitable for seed protection than on the crop field or stored farm produce. They cited the complaints of farmers on the residual bitter taste on the grains treated with neem seed powder.

Udoh *et al.* (2000) were of the view that caution must be exercised in using plant materials to control mycotoxins, because some of these materials are natural media for *A. flavus* growth. Hell *et al.* (2000) found that the use of *Khaya senegalensis* bark to protect maize against insects increased the risk of aflatoxin development, and that even the farmers in Benin, West Africa were aware of the low efficiency of the indigenous products, but were being compelled to use them because of their inaccessibility to chemical products. Some toxigenic *A. flavus* have been found to grow and produce mycotoxins in herbal plants. Also *C. odorata*, which has been reported to be potent against insects, was found to be an excellent substrate for the growth of storage fungi (Efuntoye, 1996, 1999). This might be due to the fact that phytochemicals are prone to photo-, microbial- and thermal-degradation if not properly stored. For an effective control of toxigenic fungi and mycotoxins with the use of fungicidal botanicals, integrated approach by adoption of good agronomic/cultural practices is imperative. This is by reduction of insect pest, early harvesting, rapid drying of agricultural products to a safe low moisture content of about 15% and the use of improved storage structures. Other complementary methods of control could be by manually or electronically sorting out of physically damaged, discoloured and infected grains from the apparently healthy ones.

5.1. Demanding plant fungicide regulations in developing countries

The introduction and use of natural pesticides in developing countries require proper regulation. In Nigeria for instance, Section 1 of the Pesticide Registration Regulations Decree, 1996 prohibited the manufacture, formulation, import, export, advertisement, sale, and distribution of any pesticide, unless it has been registered in accordance with the provisions of these regulations. Included among the essential parts of the regulations are the following:

5.1.2. Issuance of certificate of registration

For a pesticide registration, the completed application form shall include the original certificate of analysis of the pesticide product. The form shall include product chemistry which shall state the product composition, normal concentration, physical and chemical characteristics as well as standard laboratory analytical methods for each active ingredient, and impurity or inert ingredient that is toxicologically significant (NAFDAC, 1996).

5.1.3. Reports required by the regulations agency

Other studies demanded include environmental fate (fate in air, soil, and water); mobility and distribution; persistence and bioaccumulation (half life and degradation); hazards to

human or domestic animals; toxicity whether by oral, dermal, and or inhalation (acute toxicity or chronic toxicity) reproductive studies; effects on non-target flora and fauna, including birds and fishes; mutagenicity; product performance including efficacy trials in the country of usage. Other requirements include dosage and direction for use of the fungicidal natural pesticide; fields of application; and methods of application. The registration of any pesticide product shall be valid for a period of five years, thus it is subject to periodical renewal

5.1.4. Default and penalty

In the event of a default in compliance with the requirements of these regulations, the individual concerned may be prohibited from carrying on this business either absolutely or for a given period declared by National Agency for Food and Drug Administration and control (NAFDAC) in addition to a fine of one hundred thousand naira (\$625.00).

6. Strategies for effective fungitoxic phytochemicals production and usage

In this section, some strategies for effective fungitoxic phytochemicals production and usages are discussed.

6.1. Fungicidal plant formulation and quality control

Several laboratories have found literally thousands of phytochemicals, which have inhibitory effects on most toxigenic fungus *in vitro* but have not been formulated for the protection of crops and animal produce against fungi and mycotoxin. The aspect of quality control of phytochemicals, which is practically low or non-existent in most developing countries, should be taken seriously. It would be advantageous to standardize methods of extraction, and *in vitro* testing so that the search could be more systematic and interpretation of results would be facilitated. The development and validation of relevant bioassays pose significant challenges in developing countries. There is necessity for validated biological assays with a demonstrated high correlation between *in vitro* activity and field efficacy in order to provide the most reliable laboratory measurement of product potency/ strength (Anjorin and Salako, 2009)

In the formulation of plant fungicide, biological activity and its efficacy should be stabilized and further enhanced by the addition of stabilizers, antioxidants and synergists. Certain additives could be added to increase the shelf-life and ease of handling. Sun screens such as para-amino benzoic acid (PABA) could be added to reduce the photoxidation of most active ingredient by ultra-violet light (Zubkoff, 1999). Also, as phytochemicals such as azadirachtin, the principal bioactive ingredient in neem, is heat sensitive and cold processing technology for neem seed would be needed. Dark-coloured, sterile containers with a lid are required to minimize photodegradation and microdegradation respectively.

6.2. Proper product labelling

Labeling of commercial phytofungicidal products and following label directions should be enforced in developing countries of the world just as it is being done in some developed countries. During the registration processes of botanicals, a label created should contain directions for proper use of the material labeling and package insert shall be informative, accurate and in *lingua franca* or in local language. Minimum requirements on a package label shall include name of product, brand name and common or chemical name of active ingredients, batch number, manufacture date, expiry date; precautions for storage and handling in transit; leaflet insert, giving full description for application and safe use, and pictograms. Many stores also sell whole dried plants, which have been found occasionally to be misidentified, with potentially disastrous consequences. The new rules, issued in late 1997, require products to be labeled as a botanical fungicide and carry a "fungitoxicity facts" panel with information similar to the "synthetic pesticides" panels appearing on the formulated botanical product. The rules also require that products containing botanical ingredients specify the part of the plant used (Food and Drug Administration (1997). This is not often done in the markets in the developing countries.

6.3. Phytochemical application techniques

Home-produced plant fungicide should be timely applied, not in a bright sunlight. Otherwise, it should also be stored appropriately. Also, improved methods of application such as mechanical mixers for uniform and bulk coating of oil on grain should be adopted. The 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.

The active life and efficacy of natural products in the soil is determined by factors such as soil temperature and structure, water stress, microbial action, and fertilizer applied (Breland, 1996). Combination of two or more plant parts or species could make the plant fungicides to have broad spectrum. Anjorin, *et al.* (2008) reported that combination of two plant extracts proved more effective and could reduce the risk of resistance developing by the target fungi.

6.4. Identification of phytofungitoxic plant species

Plant species and botanical characterization is very important for the sake of quality control. Proper identification of fungicidal botanicals can be achieved by using morphological differences or anatomic microscopy to show the difference between any two plants at the time of cultivation while collecting the plant material.

There should be botanical monographs that provide specific tests for identity - usually at least three tests per botanical: macroscopic identity, microscopic identity and chromatography. The personnel making the identifications of botanicals must have recognized expertise and the procedures must be stringent, with sufficient safeguards to

discourage and detect falsification. Moreover, the required reference standards encompass pure chemicals, authenticated herbarium voucher specimens, raw and powdered herb samples, and prepared microscopy slides. An electronic inventory of herbal pesticide reference materials should be created. Reference texts are also indispensable resources (Jackson and Snowdon, 1990).

The main disadvantage of organoleptics and microscopy is that a significant investment in human resources is required to train personnel. Also problems associated with the selection and use of fungitoxic material standards is instability, special handling or storage requirements and shelf life (Flaster and Lassiter, 2001). Recombinant DNA analysis and gene chip technology are superior methods of identification. DNA methods for species characterization and adulterant detection have been published (Wolf *et al.*, 1999).

6.5. Collection of information

There should be databases which can supply information about plants with fungitoxic potential in each ecological zone. An example is the Grainge and Ahmed Handbook of Plants with Pest-Control Properties (1988). A database has been developed in National Pharmaceutical Institute, Idu, Abuja, Nigeria and in Zimbabwe (Elwell, pers. comm.) which could provide a valuable source for their respective region. Linking the information obtained from the database to the knowledge of the indigenous flora could draw rings on the most promising plants which deserve further studies. Important aspects to consider are not only fungitoxic properties of the plants but also their proper identification, distribution, abundance and easiness to be propagated. Cooperation with botanists at the National Herbaria and Universities would facilitate the work.

7. Summary

The potentials and constraints in controlling mycotoxigenic fungi and mycotoxins in developing countries with phytochemicals were looked into in this chapter. It was indicated from this review that natural fungicides application would result in more efficient control of toxigenic fungi and may lead to reduction of mycotoxins in the stored products. Vast fields in developing countries are blessed with abundant plants with fungicidal potential with local preparation and application attracting lower capital investment than synthetic fungicides. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimycotic and antimycotoxin properties. However, there are several challenges involved. These include the low fungitoxic efficacy of several phytochemicals, some are hazardous to beneficial non-target organisms and humans; while few could even support the growth of pathogens including fungi. In view of the constraints involved in the preparation and application of these botanicals and in order to enhance their efficacy, there is a need for standardizing the production, formulation, commercialization and application of these fungitoxic phytochemicals commonly used in developing countries.

8. Conclusion

The development of fungitoxic botanicals in developing countries is rather slow or it often terminates in the laboratory, in the experimental fields or locally used by rural farmers. There is yet very few or no locally produced quality marketable phytofungicide in developing countries. This situation is unsatisfactory thus commercial production of phytofungicide is strongly advocated. More research on toxigenic fungi control with natural products should be undertaken; provision of appropriate processing facilities and some of the marketing strategies for the products should be carefully planned. Also protocols on production of fungitoxic compounds for large scale production should be developed. Communication between researchers and extension organization should be intensified. Through this, phytofungicide research can be directed at farmers need and the knowledge concerning their use will be provided. Both vertical and horizontal information exchange on plant pesticide related issues should be intensified at local, national and international level. This is to confirm, collaborate and upgrade technical innovations toward commercializing phytofungicide as it has been in Ghana and India. Plant fungicide production could be sequentially integrated into a sustainable crop protection system in the developing countries. Integrated Disease Management strategy of prevention and control of toxigenic fungi and mycotoxins should be considered.

An ideal fungitoxic plant is expected not to compete with crop land, not act as weeds, not support crop pests and the products from it should be easily prepared. It is recommended that reliable toxigenic fungi and mycotoxin control methods that are attractive and safe should be developed. With a time perspective of four stages the following recommendations are given:

- to compile information on plants with potential fungi and mycotoxin control properties, to identify crops and target fungi and mycotoxin and to formulate projects in developing countries;
- to concentrate on some promising fungitoxic plants, including different aspects, to establish work groups focusing on one or a few plants with a key-person as a coordinator and advisor, to carry out comparative studies with emphasis on the mechanisms;
- to publish and to organize a workshop where new findings are presented and the methods are critically analysed in relation to the feedback from farmers
- and to produce extension material on fungicidal phytochemicals preferably in the form of leaflets, one for each plant, to organize workshops at the village level.

Research priorities on fungicidal plants such as investigation of the taxonomic status and agronomy of existing plants with fungicidal potentials, and initiation of a selection/breeding programme with multilocation testing of promising provenances is necessary. The use of modern genetic and molecular techniques such as cell culture, genetic engineering and biotechnology in boosting active ingredients should be considered. This could be by employing recombinant DNA technology or metabolic engineering strategies to breed plant species with higher quantity of fungitoxic bioactive compounds in them.

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