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EFFICACY OF HIGHER PLANT PRODUCTS IN MANAGEMENT OF STORAGE FUNGI AND AFLATOXIN B_1 PRODUCTION RESPONSIBLE FOR POST-HARVEST DETERIORATION OF HERBAL RAW MATERIALS: A REVIEW

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ABSTRACT

Herbal drugs have been widely used for the cure of several diseases since long due to their inherent biological active composition. Ancient literature revealed that biodiversity rich countries like India, China, Africa and Egypt have well documented record of traditional practice for the cure of several diseases and as an immunity booster. Even in modern era of modern medicine, as per an estimate by World Health Organization (WHO) nearly 80 % people are still depending on herbal raw material for their primary healthcare especially in developing countries. However, the recent report on fungal and mycotoxin contamination (especially aflatoxin B_1) impose the global threat for the safety of herbal drug. The fungal and aflatoxin B_1 contamination in raw material significantly deteriorate their bioactive components and also make them hazardous during their application. The article highlights the significance of herbal raw material in primary healthcare, problem of fungal and aflatoxin contamination and their solution using green chemical based on higher plant products.

Key words : Herbal raw material, Fungal contamination, Aflatoxin B_1, Higher plant products.

Introduction

Herbal drugs are crude preparations of medicinal plants. Herbal medicine has a long history and is quite popular among many people, particularly Asians and Northern Europeans. Herbal drugs are used by nearly 80% population in the world for primary health care. Besides their high demand in Asian countries, European people also prefer herbal drugs in curing various sufferings due to their no or minor side effects as compared to modern medicines (Roy, 2003). In Germany, over 80 per cent of all physicians regularly use herbal products. During the past decades, public interest in herbal medicines has increased dramatically not only in developing countries, but in industrialized countries also.

Phytotherapeutic agents are standardized herbal preparations consisting of complex mixtures of one or more plants. The adverse effects of standardized herbal drugs are less frequent compared with synthetic drugs. The low level of side effects has increased demand for these drugs. According to the World Health Organisation (WHO), as 80 per cent of the world's people rely for their primary health care on plant medicines. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available. Herbal drugs have been used since ancient times as medicines for treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances made in the field in modern medicine in recent decades, plants still make an important contribution to health care. Herbal medicines could be the natural answer to some ailments and they are gaining popularity in wealthy countries and their use remains widespread in developing countries. There are no doubts that herb– exporting countries will obtain more revenues following the widening market for herbal products in developed countries. Medicinal plants are most abundant in tropical and subtropical countries. Medicinal plants play a central role not only as tradition medicines but also as trade commodities, meeting the demand of distant markets.

Pharmaceutical companies have currently renewed their strategies in favour of natural product drug development and discovery (Seidl, 2002).Use of indigenous drugs of natural origin forms a major part of such therapies; more than 1500 herbals are sold as dietary supplements or ethnic traditional medicines (Patwardhan, 2005). India is sitting on a gold mine of well recorded and traditionally well practiced knowledge of herbal medicine. The country has rich diversity of medicinal plants.

Ironically, India has a very small share of this ever-growing global herbal market. Currently many large international companies are interested in commercializing herbal drugs. Contamination of raw materials of medicinal plants with fungi and mycotoxins represents a special hazard. The forests of India are estimated to harbour 90 per cent of India"s medicinal plants Over the past two decades, it has been well documented that raw materials of different medicinal plants frequently contain a certain number of microorganisms that mainly originate as the epiphytic flora of the plant itself. The productions of mycotoxins by associated moulds constitute a major health hazard to the consumers of herbal preparations.

The effect of fungal contamination is qualitative as well as quantitative. There may be a risk of mycotoxicoses to the patients after oral administration of drugs formulated from such raw materials. As a result of fungal contamination, the risk of mycotoxin production, specially aflatoxins, causing different adverse effects on the human systems, should be taken into consideration in the manufacturing process. It is ironical that by curing one disease with medicinal plants, the fungal contaminated raw materials cause others through mycotoxin secretion. The microbial contamination of raw materials of herbal drugs may be a major impediment preventing India and many tropical countries from becoming a herbal giant. The report on microbial contaminations of the raw materials of is a major reason of decline market of such countries. It has been felt that the stored drug samples harbour mycotoxin producing fungi in high frequency (Dubey *et al*, 2008). The raw materials of the drugs are quite often deteriorated by microorganisms before harvesting and during handling and storage. The raw materials are collected by unscientific methods and are commonly exposed to many microbial contaminants In India, the traditional methods of collection, storage and marketing coupled with humid climatic conditions make the raw materials of herbal drugs prone to fungal infestations. Hot and humid climate of the country is very conducive for growth, development and multiplication of microflora.

The fungal contamination has been reported to affect the chemical composition of the raw materials and thereby decreases the medicinal potency of the herbal drugs (Roy, 2003). Despite several reports on fungal contamination and aflatoxin production on food items, limited information is available on microbial contamination of medicinal plant samples (Roy, 1988). There are some reports on export of aflatoxin contaminated herbal raw materials from India. Some of the samples have been reported to contain aflatoxin B_1 more than 20 µg/kg, the tolerance level fixed by WHO. The herbal drugs containing mycotoxins above the tolerance limit fixed by WHO, would be certainly rejected in the global market. Due to detection of aflatoxin B_1 in the black pepper procured from India, some foreign pharmaceutical firms decided to re-evaluate the suitability of Indian black pepper samples for formulation of phytomedicines (Seenappa and Kempton, 1980). High average temperature and relative humidity of the country are favourable for mould growth. Thus the quality control to prevent growth of fungi and mycotoxin are essential.

India can be a major player in the global herb based medicines if its raw materials are free from microbial contaminations. Since aflatoxins are extremely thermostable, the best remedy for is the checking its production is by antiaflatoxigenic chemicals. It is advisable to treat plant drugs with non toxic chemicals at various stages of storage and processing. However, it would be not proper to treat the herbal raw materials with synthetic chemicals in order to protect them from microbial contamination because the synthetic antimicrobials are mostly having mammalian toxicities and would cause side effects to different human systems in form of oral toxicity. The traditionally used medicinal plants and their components should be tested for their efficacy in protecting the herbal raw materials as botanical preservatives. The chemicals to be used in control of fungal infestation on stored drug commodities should not create any harmful effects to different human/animal systems. Many of synthetic antimicrobials used as agrochemicals in plant protection have been reported to incite adverse effects on different biological functions specially on liver and kidney function of human and animal beings and they are indirectly animal toxic in nature (Majumder, 1974; Marini-Bettolo, 1977; Gupta, 1983). The use of synthetic chemicals as antimicrobial has raised a number of ecological and medical problems due to residual toxicity, carcinogenicity and teratogenicity, hormonal imbalance (particularly sex harmones), spermatotoxin, impotency, infertility and abortions etc. (Bajaj and Ghose, 1975; Omura and Hirata, 1995

Prospects of higher plant-products as a green preservative agent for herbal raw material

In recent years, considerable attention has been directed to the research and application of alternative sources of antimicrobials (botanical antimicrobials / phytopesticides) in place of synthetic pesticides. There has been a renewed interest in botanical antimicrobials because of several distinct advantages. Botanicals, being natural derivatives are biodegradable, so they do not leave toxic residues or by products to contaminate the environment. Plant based antimicrobials are much safer than conventionally used synthetic ones. Antimicrobial plants have been in nature as its components for millions of years without any ill or adverse effects on ecosystem. Some plants have more than one chemical as active principle responsible for their biological properties. The biological activity in such plants may be due to synergistic effects of different active principles. They may impart different mode of action during their antimicrobial actions. These products may exhibit either for one particular biological effect or may have diverse biological effects (Varma and Dubey, 1999). Botanical antimicrobials have a very high level of safety for humans, animals, fish and other non target organisms principally because they have been reported to act by very different modes of action than most organic chemical pesticides that attack metabolic systems shared by both pest and non pest organisms (Sathynarayana and Sharma, 1993).

Higher plants contain a wide spectrum of secondary substances *viz*. phenols, flavonoids, quinines, tannins, essential oils, alkaloids, saponins and sterols. These plant chemicals may be exploited for their different biological properties (Kubo and Nakanishi, 1979; Mahadevan, 1982). For many years plant secondary metabolites have been neglected in science. Gradually, recognition of the important role in these compounds has increased, particularly in terms of their biological activity against different microorganisms (Harborne, 1998). More than 30,000 of these secondary metabolites have been reported from plants so far (Harborne, 1997) ,however, the overall total number of secondary compounds may probably exceed 100,000 (Wink, 1993). Such bioactive plant products are now a day used in pest management on large scale as botanical antimicrobials and are termed as third generation pesticides. Among the well represented plant antimicrobials Pyrethrins are a group of closely related esters obtained from the flower heads of *Chrysanthemum cinerariaefolium* (Asteraceae). Pyrethrum products represented 80% of the total market of botanical insecticides and are the insecticides favoured by organic growers because of their low mammalian toxicity and environmental non-persistence (Isman, 1994). The pesticidal plant receiving global attention for the last two decades, is the wonder tree of Indian origin, Neem (*Azadirachata indica*). Its seed are a rich storehouse of over 100 tetranortriterpenoids and diverse non isoperenoids (Devkumar and Sukhdev, 1993). The neem products are harmless to humans and other mammals. The attraction of modern society in ‘green consumerism’ (Tuley de Silva, 1996; Smis and Gorris, 1999) desiring fewer synthetic ingredients in recommendation of herbal products as ‘generally recognized as safe’ (GRAS) as stored commodities in the developed countries may lead scientific interest in essential oils as stored commodities.

Among the different plant products, application of essential oils is a very attractive method for controlling post harvest diseases. In recent years, the essential oils have received much attention as resources of potentially useful bioactive compounds. (Gerasimos *et al*, 1997). The essential oils produced by different plant genera are in many cases biologically active, endowed with antimicrobial, allelopathic, antioxidant and bio regulatory properties (Elakovich, 1988; Deans *et al*, 1995, Vaughn and Spencer, 1991; Caccioni and Guizzardi, 1994; Holley and Patel, 2005). The volatility, ephemeral nature and biodegradability of flavour compounds of angiosperm will be especially advantageous if they are developed as antimicrobials (French, 1985). There may be least chance of residual toxicity by treatment of drug commodities with volatile substances of higher plant origin. Girisham and Reddy (1987) have suggested the use of volatile compounds which move faster as gases at physiological temperature and biologically more active at extremely low concentrations. This is important, in particular to organisms like fungi, which have, virtually every single vegetative cell exposed to the surrounding medium and where consequently the surface to volume ratio is very high. Furthermore, these compounds sublimate to form a protective atmosphere for combating the growth of microorganisms. Norrman (1968), Fries (1973), Rytch and Zyska (1977), Mallic and Nandi (1982) have recommended the use of volatile compounds in the control of mould infestation during storage. The essential oils are volatile substances generally composed of mono and sesquiterpinoids aldehydes, esters, acids, ketones, alcohols, coumarins and their composition varies within the same species as a result of genetic and environmental factors. Numerous studies have documented the

Table 1 : Efficacy of plants products in inhibition of fungal growth and aflatoxin secretion.

Investigators	Plant parts / Products	Investigation
Alderman (1976)	*Citrus* oils	Lemon at 2000 ppm & orange at 3000 ppm, maximum suppression of mycelium growth and toxin formation.
Khan (1978)	Tolnaflate 2-napthyl-N-methyl an antifungal drug	Changed morphology of *A. parasiticus* to yeast like form and inhibited aflatoxin formation.
Mabrouk and El-Shayeb (1980)	Black pepper, peppermint, cumin, ginger & clove	Clove 0.1%, black pepper & ginger (10%) completely inhibited AFB_1 production.
Chou and Yu (1984)	*Piper betle* extract	0.5% grind leaf powder completely inhibited the growth & aflatoxin production
Paster *et al*. (1988)	Constituents of olive plant ti	Ethanolic extract inhibited 90% aflatoxin production without inhibiting fungal growth.
Azaizeh *et al*. (1990)	Tannin extracts from *Arachis hypogea* L.	Inhibited aflatoxin production.
Bilgrami *et al*. (1992)	Eugenol, onion, garlic extracts	Garlic extract significantly inhibited mycelium growth (61%) while aflatoxin inhibition was highest 60% in onion extract and eugenol.
Patkar *et al*. (1994)	EO *Cinnamomum* sp.	Inhibited AFB_1 and ochratoxin production.
Mahoney and Rodriguez (1996)	Pistachio extract of hulls (mesocarp & epicarp) seed coat (testas)	Both seed coat and water soluble extract of hulls, suppressed aflatoxin production in *A.flavus.*
Awuah and Kpodo (1996)	Plant extracts of *Xylopia aethiopica*, *Monodera myristica*, *Cinnamomum* sp. and *Piper nigrum.*	Suppressed aflatoxin synthesis.
Bankole (1997)	Oil of *Azadiarachta indica*, *Morinda lucida*	Both inhibited *in vivo* and *in vitro* AFB_1 production in maize. Reduction of mycelial weight and aflatoxin production decreased as concentration increased consequently.
Fan and Chen (1999)	Ethanolic extract of onion	10 mg/ml inhibited aflatoxin production and mycelium growth of *A. flavus* and *A. parasiticus.*
Kim *et al*. (2000)	Korean soya bean paste	Effective inhibitor of aflatoxin production and fungal growth of *A. parasiticus.*
Zeringue *et al*. (2001)	Neem oil	Reduced fungal radial growth and AFB_1 production.
Goncalez *et al*. (2001)	Biflavonoids from *Ouratea* sp.	Four biflavonoids showed inhibitory activity on AFB_1 & B_2 production but did not inhibit fungal growth at tested concentration.
Allameh (2002)	Neem leaf extract	50% (v/v) neem extract inhibited 90% aflatoxin production. There is positive correlation between G-ST (Glutathione s- transferase) activity and aflatoxin production in fungi.
Paranagama *et al*. (2003)	EO *Cymbopogon citratus*	Fungistatic, fungicidal and 100% antiaflatoxigenic.
Selvi *et al*. (2003)	*Garcinia indica* extract	Exhibited antiflatoxin activity used as biopreservative in food application and neutraceuticals.
Gonçalez *et al*. (2003)	*Polymnia sonchifolia*	Ethanol, ethylacetate, hexane & methanolic extract inhibited AFB_1, AFB_2 and fungal growth.
Mellon and Moreau (2004)	Polyamine mixture of diferuloylputres-cine and p-coumaroylferuloylputres-cine (85:15 w:w) in pericarp of *Zea mays.*	Thin polyamine mixture inhibited aflatoxin production of *A.flavus.*

Table 1 continued...

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Investigators	Plant parts / Products	Investigation
Gowda *et al*. (2004)	Turmeric, onion and clove oil	Clove oil 0.5-1% completely inhibited aflatoxin production while onion and turmeric moderately reduced aflatoxin production.
Rasooli and Abyaneh (2004)	EO *Thymus eriocalyx, T. porlock*	Inhibition of fungal growth and aflatoxin production.
Bankole *et al*. (2005)	EO and leaf powder of *Cymbopogon citratus*	Essential oil completely inhibited aflatoxin (AFB_1) production and prevented the deterioration of melon seed up to 6 months while leaf powder reduced infestation level.
Sanchez *et al*. (2005)	Flower extract of *Agave asperima* and *A.striata*	Inhibition of growth of *A.flavus* and aflatoxin production.
Kumar *et al*. (2005)	EO *Mentha arvensis*	*Mentha* oil completely inhibited mycelial growth at 100 ppm and AFB_1 production at 50 ppm.
Kumar *et al*. (2005)	EO *Chenopodium ambrosioides*	Oil completely inhibited mycelial growth and AFB_1 production.
Kumar *et al*. (2007)	EO *Chenopodium ambrosioides*	Evaluation of oil as a potential sourceof antiaflatoxigenic and antioxidant activity.
Srivastava *et al*. (2008)	EO *Cinnamomum camphora* and *Alpinia galanga*	Inhibition of aflatoxin production from a toxigenic strain of *A. flavus*
Singh *et al*. (2008)	EO *Cinnamomum camphora*	Oil of *C. camphora*is recommended as herbal fungitoxicant against the fungal infection and aflatoxin B_1 ontamination of some herbal drugs.
Kumar *et al*. (2008)	EO *Thymus vulgaris*	*T. vulgaris* essential oil as a safe botanical preservative against post harvest fungal infestationand aflatoxin inhibition

antifungal (Suhr and Nielson, 2003; Mishra and Dubey, 1994) and antibacterial (Canillac and Mourey, 2001) effect of plant essential oils. Examination of indigenous local herbs and plant materials have also been reported particularly from India (Ahmad and Beg, 2001), Australia (Cox *et al*, 1998), Argentina (Penna *et al*, 2001) and Finland (Rauha *et al*, 2000).

Production of essential oils by plants is believed to be predominantly a defense mechanism against pathogens and pests (Oxenham, 2003) and indeed, essential oils have been shown to possess antimicrobial and antifungal properties (Tripathi and Dubey, 2004; Kumar *et al*, 2007; Srivastava *et al*, 2008). Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by the consumers and their exploitation for potential multi-purpose functional use.

Although, various essential oils have been screened for their antimicrobial activity against different microorganisms, but detailed studies *viz*. antifungal, aflatoxigenic activity, phytochemistry and safety limit profile have not been done properly with most of the oils. Therefore, there is urgent need to bioprospect the pesticidal property of different essential oils and detailed *in vitro* and *in vivo* investigations are required for the recommendation of their practical application as botanical antimicrobials for the control of post harvest biodeterioration of drug commodities and thereby enhancing shelf life of the commodities. Although, a large number of plants and their products have been tested as antiaflatoxigenic properties, most of the earlier investigations have been confined only to *in vitro* studies. Only a few *in vivo* investigations have been carried out with plant products to inhibit aflatoxin production. Table 1 summarised the detailed investigations on higher plants and their products in inhibition of aflatoxin B_1 production.

Conclusion

Fungal and aflatoxin B_1 contamination is one of the major causes responsible for the deterioration of quality of herbal raw materials used for the preparation of Ayurvedic drug. The use of synthetic chemicals for the control of fungal contamination is generally not accepted due to their residual toxic effects to the healths. In this context, plant products possess significant efficacy against aflatoxin secretion by the toxigenic strains of *A. flavus* and fungal growth could be used as a green chemical as a preferred alternative to the synthetic ones. However, a detailed investigation on dose stabilization, *in-situ* efficacy during practical application and safety profile are needed.

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