KMITL Sci. Tech. J. Vol. 11 No. 1 Jan.-Jun. 2011

Control of Aflatoxins in Agricultural Products using Plant Extracts

Dusanee Thanaboripat*

Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand

Abstract

Aflatoxins, a worldwide health hazard to humans and animals, are among the most potent mutagenic and carcinogenic compounds known to be produced in nature. Various methods have been investigated for the control of aflatoxin producing fungi and aflatoxin production in agricultural products. This article reviews the application of herbal extract and essential oils for controlling the growth of aflatoxin producing fungi and aflatoxin production.

Keywords: Aflatoxin, *Aspergillus flavus, Aspergillus parasiticus*, herb, essential oil

1. Introduction

Aflatoxins, produced by *Aspergillus flavus*, *A. parasiticus, A. nomius, A. tamari, A. bombycis* and *A. pseudotamarii* are both acutely and chronically toxic to both humans and animals [1-4]. Some strains of *A. flavus* have been re-identified as *A. parasiticus* and *A. nomius* [5]. Various agricultural commodities have been found to be contaminated with either aflatoxin producing fungi or aflatoxins. Although the presence of *Aspergillus* mould does not necessarily indicate aflatoxin contamination, there is certainly an increased risk [6]. The foods at highest risk of aflatoxin contamination are corn, peanut and cotton seed [7]. Aflatoxin B1 has been detected in 80% of maize samples obtained from different locations in Southeast Nigeria [8] and similarly 92% of animal feed samples taken from commercial sources in Thailand were contaminated with aflatoxin B1 [9]. In at least three parts of the world, East Africa, the Philippines and Thailand, good epidemiological evidence has been collected showing a correlation between the incidence of liver cancer and exposure to aflatoxins [7]. Aflatoxins have also been identified as a potential biological weapon for food and water contamination [10].

Physical, chemical and biological methods have been investigated in order to prevent the growth of aflatoxin producing fungi and to eliminate or reduce the levels of aflatoxins or to degrade or detoxify aflatoxins in foods and feeds [11]. One of the most effective ways to control the problems caused by aflatoxins is to prevent the growth of fungi in the substrate, for example by the use of chemical inhibitors to suppress the spore germination of the fungi, as well as the development of the fungal mycelium, in the substrate susceptible to contamination by these toxins [12]. Because of aflatoxins' effects on health and economics, the search for antifungal agents is

*Corresponding author: Tel: 662-3298415 Fax: 662-3298412

Email: ktdusane@kmitl.ac.th

extensive and natural plant extracts may provide an alternative way to prevent fungal contamination of food or feed [13]. Control by naturally produced agents is becoming increasingly important because of consumers' mistrust of food and feed treatments that involve using synthetic xenobiotic substances. Natural plant compounds have been used traditionally to preserve foods in countries like Japan, India and Russia [14]. Extracts and powders of various spices, herbs and essential oils have been reported to have antimicrobial activity against aflatoxin producing fungi and some of them also inhibit aflatoxin formation [15-22]. Many essential oils have also been reported as effective inhibitors of fungal growth and aflatoxin production [23, 24]. Great success has been achieved to reduce mycotoxigenic fungi and mycotoxins in foods using plant products such as plant extracts and plant essential oils [25].

2. Effect of medicinal plants on aflatoxin producing fungi and aflatoxin production

Various Southeast Asian medicinal plants such as Asiatic pennywort (*Centella asiatica*), betel nut (*Areca catechu*), betel vine (*Piper betle*), bitter cucumber (*Momordica charantia*), Chaa Phluu (*Piper sarmentosum*), Chinese radish (*Raphanus sativus*), clove (*Syzygium aromaticum*), eucalyptus (*Eucalyptus globules*), false coriander (*Eryngium foetidum*), hedge flower (*Lantana camara*), Indian mulberry (*Morinda citrifolia*), Madagascar periwinkle (*Catharanthus roseus*), mangosteen (*Garcinia mangostana*), mandarin (*Citrus reticulate*), onion (*Allium cepa*), pepper (*Piper nigrum*), pomegranate (*Punica granatum*), roselle (*Hibiscus sabdariffa*), Non Taai Yaak (*Stemona tuberosa*), tomato (*Lycopersicon esculentum*), Raang Chuet (*Thunbergia laurifolia*), Saab Sue (*Chromolaena odorata*), turmeric (*Curcuma longa*), water primrose (*Jussiaeda repens*) and wishing tree (*Cassia bakeriana*) were tested for their ability to control *A. flavus* [26]. The results showed that the crude ethanolic extracts of some medicinal plants inhibited fungal growth to various degrees. Betel vine, a traditional Thai medicine, gave the highest activity followed by false coriander, Indian mulberry, Chaa Phluu, Chinese radish and clove. Betel vine leaf extracts at concentrations of 6, 8 and 10% (w/w) completely inhibited the growth of *A. flavus* and aflatoxin production on maize for 28 days. The leaf of betel vine which is used by mouth as antiflatulent, antimicrobial and antipruritic, and topically for urticaria, contains eugenol and chavicol [27].

Crude ethanolic extracts of kaffir lime leaf, bitter cucumber fruit and tobacco leaf were compared for their ability to control the growth of *A. flavus* on Potato Dextrose Agar (PDA) [28] by the addition of appropriate amounts of extracts onto PDA to obtain the final concentration of 0, 2, 4, 6, 8, and 10% (w/v) and *A. flavus* was then point-inoculated into PDA. The results showed that all herbs had an inhibitory effect on fungal growth. Kaffir lime extract at 10% (w/v) and tobacco extracts at 8 and 10% (w/v) showed significantly higher inhibition than at lower concentrations and bitter cucumber extracts at 6, 8 and 10% (w/v) gave a similar inhibitory effect on the fungus. Kaffir lime at concentrations of 5 and 10% (w/v) inhibited fungal spore germination of *Colletotrichum gloeosporioides* and *Fusarium* sp., respectively [29]. The ethanolic extracts of kaffir lime leaves were also found to inhibit some strains of *Salmonella* [30]. The main compound in kaffir lime leaves is reported as citronellal (65.4%) whereas the major constituents in essential oil of kaffir lime peels are B-pinene (30.6%), limonene (29.2%), and sabinene (22.6%) [31]. The inhibitory effect on fungal growth of kaffir lime leaf might be due to citronellal.

Crude aqueous extracts of garlic, carrot and clove were tested for the inhibitory effect on aflatoxin production in rice by the addition of extracts into 50 g of rice to obtain the concentrations of 0, 2, 4, 6, 8 and 10% (w/v). The results showed that garlic, and clove at 10% (w/v) and carrot at 2% inhibited the fungal growth and reduced the level of aflatoxin in rice [20]. Reddy and colleagues [32] found that clove (*Syzygium aromaticum*) effectively inhibited the mycelia growth

of *A. flavus* and aflatoxin production. Among 22 plant extracts studied, clove and ginger were found to be more effective against food associated fungi [33]. Extracts from garlic bulbs, green garlic and green onions showed an inhibitory effect against *A. niger* and *A. flavus* [13]. However, green garlic and green onion lost their antifungal activity against *A. niger* after being heated at 80 and 60° C, respectively.

Dried ground leaves of *Cymbopogon citratus* (lemon grass) at 10% (w/w) reduced the deterioration and aflatoxin production in shelled melon seeds (*Colocynthis citrullus* L.) inoculated with toxigenic *A. flavus* [34]. Ethanolic extracts of olive callus tissues at 0.5 or 1.0% (v/v) inhibited aflatoxin production by 90% without inhibiting the growth of *A. flavus* [35]. Ryu and Holt [36] found that spice oils dissolved in soybean oil were less effective in reducing mold growth than when dissolved in water.

Crude aqueous extracts of neem (*Azadirachta indica*) were found to have an inhibitory effect on the growth of *A. flavus* and *A. parasiticus* [21]. By adding the appropriate amount of neem extracts into PDA to obtain the final concentrations of 0, 2, 4, 6, 8, and 10% (w/v) and fungal cultures were inoculated into PDA, the results showed that neem leaf extracts at 2 and 6 % (w/v) were the lowest concentrations for reducing the growth of *A. parasiticus* and *A. flavus*, respectively whereas neem branch extracts at concentrations of 4 and 2% (w/v) were the lowest concentrations for reducing the growth of *A. parasiticus* and *A. flavus*. Bhatnagar and McCormick [37] and Bhatnagar *et al.* [38] reported that neem leaf extract prepared by blending fresh leaves (50 g wet weight) in 1 L of 10mM potassium phosphate and added directly to submerged culture of *A. parasiticus* at concentration greater than 10% (v/v) did not affect fungal growth but blocked aflatoxin biosynthesis (>98%). Zeringue and Bhatnagar [39] observed that dosing fungal culture with neem-derived volatiles by passing microbe-free compressed air through an enclosed system containing fresh neem leaves and the emitted volatiles were passed over the surface of submerged liquid cultures of *A. parasiticus* for 3-day incubation period resulted in 90% overall reduction in aflatoxin production and a 51% reduction in fungal mass when compared with cultures without neem-derived volatiles. The tetranotriterpenoids and volatile compounds in neem are reported to be responsible for its antiaflatoxigenic properties [40].

Capsanthin and capsaicin, the colouring and pungent principles of red chilli, *Capsicum annum*, respectively, were tested against the growth and aflatoxin producing potentials of *A. flavus* in liquid medium by adding the appropriate amounts of crystalline capsanthin and pure capsaicin into 50 ml of liquid medium to obtain the final concentrations of 0.02, 0.06 and 0.1% (w/v) [16]. The results showed that capsanthin completely inhibited both the growth and toxin production at all concentrations up to the fourth day of incubation whereas capsaicin showed some inhibitory effect up to the fourth day of incubation.

The extracts of several other wild and medicinal plants have been tested against aflatoxin producing fungi [41]. Aqueous extracts of *Lupinus albus* (Leguminosae), *Ammi visnaga* (Umbelliferae) and *Xanthium pungens* (Compositae) were found to inhibit mycelial growth and aflatoxin formation of *A. flavus* [42]. The inhibitory effect was proportional with the applied concentration and the plant extracts also affected the ratio of aflatoxins B_1 to B_2. The extracts of these plants inhibited aflatoxin production by inhibiting the growth of *A. flavus*. Masood and Ranjan [43] also found that extracts of *Argemone mexicana* and *Cyperus rotundus* inhibited aflatoxin production by inhibiting the growth of *A. flavus*. Our results also showed that aflatoxin formation was inhibited due to the inhibition of the growth of *A. flavus* [22, 26].

El-Shayeb and Mabrouk [44] reported that by adding the appropriate amount of powdered liquorice roots (*Glycyrrhiza glabra*), lupine seeds (*Lupinus termis*), fenugreek (*Trigonella foenum-graecum*), artemisia flower heads (*Artemisia herba-alba*), roselle flower heads (*Hibiscus subdariffa*) and fennel-flower seeds (*Nigella sativa*) into 50 ml of liquid medium to obtain the final concentrations of 0.1, 0.5, 2.0, 5.0 and 10% (w/v), these plants inhibited aflatoxin formation by 85-90% of that of control at a concentration of 10% (w/v). Their effects on mycelial

growth were less pronounced. The activities of the six plants investigated were more anti-aflatoxigenic than fungistatic. The inhibitory activity of liquorice roots on aflatoxin formation might be attributed to the presence of triterpenes and triterpene derivatives [45]. Alderman and Marth [46] and Mabrouk and El-Shayeb [47] also reported that terpenes and their oxidized derivatives present in citrus oil and lentils were responsible for inhibiting aflatoxin formation.

3. Effect of essential oils on aflatoxin producing fungi and aflatoxin production

Essential oils from 16 aromatic plants, i.e., safflower (*Carthamus tinctorius*)**,** marigold (*Tagetes erecta*), coriander (*Coriandrum sativum*), pomelo (*Citrus maxima*), mangosteen (*Garcinia mangostana*), *Kaempferia parviflora*, ginger (*Zingiber officinale*), pepper (*Piper nigrum*), Boraphet (*Tinospora crispa*), aloe (*Aloe vera*), lavender (*Lavendula officinalis*), rosemary (*Rosemarinus officinalis*), cinnamon (*Cinnamomum cassia*), eucalyptus (*Eucalyptus globules*), thyme (*Thymus vulgaris*), and white wood (*Melaleuca cajuputi*) were tested for their inhibitory effect on *A. flavus* IMI 242684 on PDA by agar diffusion test [48]. Two hundred and fifty µl of each essential oil diluted by ethanol to give the concentrations of 50, 25, 12.5, 6.25%, were placed into cylinder cup (6mm dia) on agar plate seeded with *A. flavus*. The results showed that the essential oil of white wood gave the highest inhibition followed by the essential oils of cinnamon and lavender, respectively. The essential oil of white wood at 25% (v/v) completely inhibited the growth of *A. flavus* IMI 242684 on PDA for 28 days. The major constituents of white wood oil are monoterpene compounds such as terpinolene (24.74%) and ϒ-terpinene (22.84%) [49]. Mahmoud [23] reported that 100 ppm of five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), each completely suppressed growth of *A. flavus* and consequently prevented aflatoxin synthesis in liquid medium. Some of these essential oils could prevent fungal growth and toxin formation for 8 days. The hydrosols of anise, cumin, fennel, mint, pickling herb, oregano, savory and thyme showed a strong inhibitory effect on mycelial growth of *A. parasiticus* NRRL 2999 [50].

Sinha *et al.* [51] tested the inhibitory effect of clove and cinnamon oils on the growth of *A. flavus* by adding these oils into 50 ml of liquid medium to give the final concentrations of 0, 0.005, 0.01, 0.015, 0.02 and 0.025% (w/v). Clove oil at 0.005 and 0.01% (w/v) and cinnamon oil at 0.005% (w/v) stimulated the growth of *A. flavus* in liquid media whereas higher concentrations reduced the mycelial growth. Bullerman *et al.* [52] stated that clove and cinnamon oils and their principal components such as eugenol and cinnamic aldehyde respectively, inhibited growth and aflatoxin production by *A. parasiticus*. Cinnamic aldehyde and eugenol at 0.02% (200 ppm) could inhibit fungal growth for 4-6 weeks.

Thanaboripat *et al*. [22] reported that by adding appropriate amount of citronella oil into PDA to obtain the final concentration of 0.2% (v/v) could inhibit the growth of *A. flavus* IMI 242684, *A. flavus* M113, *A. flavus* S 156 and *A. parasiticus* IMI 102566 for 21, 7, 7 and 21 days, respectively. Essential oils of cinnamon (*Cinnamomum zeylanicum*), peppermint (*Mentha piperita*), basil (*Ocimum basillicum*), origanum (*Origanum vulgare*), the flavoring herb *epazote* (*Teloxys ambrosioides*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*) caused a total inhibition of *A. flavus* on maize kernels. The optimum dosage for protection of maize varied from 3 to 8% (v/w) [53]. Mahmoud [23] reported that 0.01% (100 ppm) of five oils, namely geraniol, nerol and citronellol (aliphatic oils), cinnamaldehyde (aromatic aldehyde) and thymol (phenolic ketone), completely suppressed growth of *A. flavus* and consequently prevented aflatoxin synthesis in liquid medium.

There has been speculation on the contribution of the terpene fraction of the oils to their antimicrobial activities [54]. The antimicrobial activity varies widely, depending on the type of spice or herb, test medium and microorganism [55]. Contents of essential oils in different species is influenced by genetic variations between cultivars, culture conditions, environment and by crop and post-crop processing [56, 57].

According to Farag *et al.* [58], commonly used concentrations of cumin and clove oils decreased aflatoxin production from *A. parasiticus* by 99%. Most of the compounds, known for their inhibitory effect against aflatoxin production at the early stage, become almost ineffective on prolonged incubation [43, 59]. Some fungitoxicants stimulated aflatoxin synthesis after long periods of incubation [43]. Antimicrobial activity of essential oils depends not only on their components but also on the chemical structure of these components [60].

4. Conclusions

Even though the prevention of fungal growth is still the best practice to prevent contamination by aflatoxins in foods and feeds, other measures are also necessary. The advantage of using-plant-produced compounds as a source of safer and more effective control substances than synthetically produced antimicrobial agents can be demonstrated both practically and in terms of consumer acceptance. Other procedures such as the elimination or decomposition of aflatoxins are also required because the prevention alone may not always be successful. Several studies have focused on the potential use of essential oil applications in biological control of aflatoxin producing fungi and insect pests. Certain essential oils can be applied as mold inhibitors in order to prevent the growth of aflatoxigenic fungi in stored food. However, the appropriate application of essential oils should further be investigated. While dealing with grain protection, fumigation is the preferred method for applying substances into the bulk in order to control the biotic factors which damage the grain.

5. Acknowledgements

The author wishes to thank Dr. B. J. B. Wood, University of Strathclyde, U.K. for his help in proofreading this paper.

References

[1] Kurtzman, C. P., Horn, B. W. and Hesseltine, C. W. **1987**. *Aspergillus nomius*, a new aflatoxin producing species related to *Aspergillus flavus* and *Aspergillus tamarii*. *Antonie van Leeuwenhoek*, 53, 147-158.

[2] Dvorackova, I. **1990**. *Aflatoxins and Human Health*. Boca Raton: CRC Press.

[3] Goto, T., Wicklow, D. T. and Ito, Y. **1996**. Aflatoxin and cyclopiazonic acid production by a Sclerotium producing *Aspergillus tamarii* strain. *Applied and Environmental Microbiology*, 62(11), 4036-4038.

[4] Peterson, S. W., Ito, Y., Horn, B. W. and Goto, T. **2001**. *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*. *Mycologia*, 93(4), 689-703.

[5] Pitt, J. I. **1993**. Corrections to species names in physiological studies on *Aspergillus flavus* and *Aspergillus parasiticus*. *Journal of Food Protection*, 56, 265-269.

[6] Robertson, A. **2005**. Risk of aflatoxin contamination increases with hot and dry growing conditions. [Online] Available at: http://www.ipm.iastate.edu/ipm/icm/node/ 182/print
[7] Smith, J. E. and Moss, M. O. **1985**. *Mycotoxins. Formation, Analysis and Significance.* Chichester: John Wiley & Sons.
[8] Aja-Nwachukwu, J. and Emejuaiwe, S. O. **2006**. Aflatoxin-producing fungi associated with Nigerian maize. *Environmental Toxicology and Water Quality,* 9(1), 17-23.
[9] Charoenpornsook, K. and Kavisarasai, P. **2006**. Mycotoxins in animal feedstuffs in Thailand. *KMITL Science and Technology Journal*, 6(1), 25-28.
[10] Smith, J. E. **2004**. *Biotechnology*. 4th ed. Cambridge: University Press.
[11] Thanaboripat, D. **2002**. Importance of aflatoxin. *KMITL Science Journal*, 2(1), 38-45.
[12] Moreno-Martinez, E., Vazquez-Badillo, M. and Facio-Parra, F. **2000**. Use of propionic acid salts to inhibit aflatoxin production in stored grains of maize. *Agrociencia*, 34(4), 477-484.
[13] Yin, M.-C. and Cheng, W.-S. **1998**. Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. *Journal of Food Protection*, 61(1), 123-125.
[14] Wilson, C. L. and Wisniewski, M. E. **1992**. Further alternatives to synthetic fungicides for control of postharvest diseases. In E. T. Tjamos, ed. 1992. *Biological Control of Plant Diseases*. New York: Plenum Press, pp.133-138.
[15] Krishnamurthy, Y. L. and Shashikala, J. **2006**. Inhibition of aflatoxin B_1 production of *Aspergillus flavus* isolated from soybean seeds by certain natural plants products. *Letters in Applied Microbiology*, 43, 469-474.
[16] Masood, A., Dogra, J. V. V. and Jha, A. K. **1994**. The influence of colouring and pungent agents of red chilli (*Capsicum annum*) on growth and aflatoxin production by *Aspergillus flavus*. *Letter in Applied Microbiology*, 18, 184-186.
[17] Prasad, G., Sahay, S. S. and Masood, A. **1994**. Inhibition in aflatoxin biosynthesis by the extract of *Amorphophallus campanulatus* (OL) and calcium oxalate. *Letter of Applied Microbiology*, 18, 203-205.
[18] Thanaboripat, D. **2003**. Mycotoxins: occurrences and control in foods. In International Union of Food Science and Technology, ed., 2003. *The International Review of Food Science and Technology*. U.K.: IUFoST, pp. 130-133.
[19] Thanaboripat, D., Naranong, N. and Peerapakorn, N. **1989**. Effect of some herbs on growth of *Aspergillus flavus* and Aflatoxin Production. *Srinakarinwirot Journal of Science*, 5, 33-39.
[20] Thanaboripat, D., Nontabenjawan, K., Leesin, K., Teerapiannont, D., Sukcharoen, O. and Ruangrattanametee, V. **1997**. Inhibitory effect of garlic, clove and carrot on growth of *Aspergillus flavus* and aflatoxin production. *Journal of Forestry Research*, 8, 39-42.
[21] Thanaboripat, D., Cheunoy, W., Petcharat, U., Ruangrattametee, V. and Kraisintu, K. **2000**. Control of aflatoxigenic fungi by Thai neem. *Government Pharmaceutical Organization Journal,* 21, 41-49.
[22] Thanaboripat, D., Mongkontanawut, N., Suvathi, Y. and Ruangrattametee, V. **2004**. Inhibition of aflatoxin production and growth of *Aspergillus flavus* by citronella oil. *KMITL Science Journal*, 4(1), 1-8.
[23] Mahmoud, A.-L. E. **1994**. Antifungal action and antiaflatoxigenic properties of some essential oil constituents. *Letter in Applied Microbiology*, 19, 110-113.
[24] Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Yoshinari, T., Rezaee, M.-B., Jaimand, K., Nagasawa, H. and Sakuda, S. **2008**. Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. *International Journal of Food Microbiology*, 123, 228-233.
[25] Reddy, K. R. N., Nurdijati, S. B. and Salleh, B. **2010**. An overview of plant- derived products on control of mycotoxigenic fungi and mycotoxins. *Asian Journal of Plant Science*, 9, 126-133.

[26] Thanaboripat, D., Prugcharoen, P. and Ruangrattanametee, V. **2005**. Inhibitory effect of some medicinal plant extracts on the growth and aflatoxin production of *Aspergillus flavus*. In Q.Yang and Z.,Yu, eds. 2005. *Study on Plant Pest and Disease Biological Control and Bio-technology*, Harbin: Heilongjiang Science and Technology Press, pp.52-62.

[27] Saralamp, P., Chuakul, W., Temsiririrkkul, R. and Clayton, T. **1996**. *Medicinal Plants in Thailand*. Vol. 1. Bangkok: Department of Pharmaceutical Biology, Faculty of Pharmacy, Mahidol University.

[28] Thanaboripat, D., Chareonsettasilp, S., Pandee, K. and Udomwongsup, K. **2006**. Inhibitory effect of kaffir lime, bitter cucumber and tobacco extracts on the growth of *Aspergillus flavus*. *KMITL Science and Technology Journal*, 6(1), 18-24.

[29] Noengpa, K., Prayoonrat, P. and Chingduang, S. **2006**. Efficiency of certain medicinal plants to inhibit *Colletrotrichum gloeosporiodes* and *Fusarium* sp. [Online] Available at: http://plantpro.doea.go.th/

[30] Nanasombat, S. and lohasupthawee, P. **2005**. Antibacterial activity of crude ethanolic extracts and essential oils of spices against Salmonellae and other Enterobacteria. *KMITL Science and Technology Journal*, 5(3), 527-538.

[31] Lawrence, B. M., Hogg, J. W., Terhune, S. J. and Podimuang, V. **1971**. Constituents of the leaf and peel oils of *Citrus hystrix* D.C. *Phytochemistry,* 10, 1404-1405.

[32] Reddy, K. R. N., Reddy, C. S. and Muralidharan, K. **2009**. Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. *Food Control*, 20, 173-178.

[33] Pundir, R. K. and Jain, P. **2010**. Antifungal activity of twenty two ethanolic plant extractsagainst food-associated fungi. *Journal of Pharmacy Research*, 3, 506-510.

[34] Bankole, S. A. and Joda, A. O. **2004**. Effect of lemon grass (*Cymbopogon citratus* Stapf) powder and essential oil on mould deterioration and aflatoxin contamination of melon seeds (*Colocynthis citrullus* L.). *African Journal of Biotechnology*, 3(1), 52-59.

[35] Paster, N., Juven, B. J. and Harshemesh, H. **1988**. Antimicrobial activity and inhibition of aflatoxin B_1 formation by olive plant tissue constituents. *Journal of Applied Bacteriology*, 64, 293-297.

[36] Ryu, D. and Holt, D. L. **1993**. Growth inhibition of *Penicillium expansum* by several commonly used food ingredients. *Journal of Food Protection*, 56, 862-867.

[37] Bhatnagar, D. and McCormick, S. P.**1988**. The inhibitory effect of neem (*Azadirachta indica*) leaf extracts on aflatoxin synthesis in *Aspergillus parasiticus. Journal of the American Oil Chemists' Society*, 65,1166-1168.

[38] Bhatnagar, D., Zeringue, H. and McCormick, S. P. **1990**. Neem leaf extracts inhibit aflatoxin biosynthesis in *Aspergillus flavus* and *Aspergillus parasiticus*. In US. Department of Agriculture, 1990. *Proceedings of the USDA Workshop*. Beltsville, Maryland.

[39] Zeringue, H. J. and Bhatnagar, D. **1994**. Effects of neem leaf volatiles on submerged cultures of aflatoxigenic *Aspergillus parasiticus*. *Applied and Environmental Microbiology*, 60(10), 3543-3547.

[40] Choudary, P. L. **2002**. Effects of antimicrobials from neem leaf extract on aflatoxigenic molds. *Journal of Mycology and Plant Pathology*, 32, 266.

[41] Bilgrami, K. S., Misra, R. S. Sinha, K. K. and Singh, A. **1980**. Effect of some wild and medicinal plant extracts on aflatoxin production and growth of *Aspergillus flavus* in liquid culture. *Journal of Indian Botany Society*, 59, 123-126.

[42] Mahmoud, A.-L. E. **1999**. Inhibition of growth and aflatoxin biosynthesis of *Aspergillus flavus* by extracts of some Egyptian plants. *Letter in Applied Microbiology*,29, 334-336.

[43] Masood, A. and Ranjan, K. S. **1991**. The effect of aqueous plant extracts on growth and aflatoxin production by *Aspergillus flavus*. *Letter in Applied Microbiology*, 13: 32- 34.
[44] El-Shayeb, N. M. A. and Mabrouk, S. S. **1984**. Utilisation of some edible and medicinalplants to inhibit aflatoxin formation. *Nutrition Reports International*, 29(2), 273-281.
[45] Canonica, L., Giovani, R. and Attilio, B. **1966**. Triterpenes of Glycyrrhiza glaba. I. Two new lactones with an oleanone structure. *Gazzatta Chimina Italiana,* 96, 772.
[46] Alderman, G. G. and Marth, E. H. **1976**. Inhibition of growth and aflatoxin production of *Aspergillus parasiticus* by citrus oils. *Zeitschrift für Lebensmittel-Untersuchung und – Forschung,* 160, 353.
[47] Mabrouk, S. S. and El-Shayeb, N. M. A. **1982**. Isolation of inhibitors of *Aspergillus flavus* from lentils (*Lens culinris* Medicus). In *Proceedings of the Fifth International Symposium on Mycotoxins and Phycotoxins,* Vienna, Austria 1-3 September 1982.
[48] Thanaboripat, D., Suvathi, Y., Srilohasin, P., Sripakdee, S., Patthanawanitchai, O. andChareonsettasilp, S. **2007**. Inhibitory effect of essential oils on the growth of *Aspergillus flavus*. *KMITL Science and Technology Journal*, 6(1), 18-24.
[49] Brophy, J. J., Thubthimthed, S., Kitirattrakarn, T. and Anantachoke, C. **2002**. Volatile leaf oil of *Melaleuca cajuputi*. In *Proceedings of the Forestry Conference*, pp. 304-313.
[50] Ozcan, M. **2005**. Effect of spice hydrosols on the growth of *Aspergillus parasiticus* NRRL 2999 strain. *Journal of Medical Food,* 8(2), 275-278.
[51] Sinha, K. K., Sinha, A. K. and Prasad, G. **1993**. The effect of clove and cinnamon oils ongrowth of and aflatoxin production by *Aspergillus flavus*. *Letters in Applied Microbiology*, 16, 114-117.
[52] Bullerman, L. B., Lieu, F. Y. and Seier, S. S. **1977**. Inhibition of growth and aflatoxin production by cinnamon and clove oils, cinnamic aldehyde and eugenol. *Journal of Food Science*, 42(4), 1107-1110.
[53] Montes-Belmont, R. and Carvajal, M. **1998**. Control of *Aspergillus flavus* in maize with plant essential oils and their components. *Journal of Food Protection*, 61, 616-619.
[54] Conner, D. E. **1993**. Naturally occurring compounds. In P. M. Davidson and A. L. Branen, eds, 1993. *Antimicrobials in Foods*. New York:Marcel Dekker Inc., pp. 441-468.
[55] Snyder, O. P. **1997**. *Antimicrobial Effects of Spices and Herbs*. [Online] Available at: http://www.hi-tm.com/Documents/Spices.html
[56] Charles, D. J. and Simon, J. E. **1990**. Comparison of extraction methods for the rapid determination of essential oil content and composition of Basil. *Journal of American Horticultural Science,* 115, 458-462
[57] Paakkonen, K., Malmsten, T. and Hyvonen, L. **1990**. Drying, packaging and storage effects on quality of Basil, Marjoram and Wild Marjoram. *Journal of Food Science*, 55, 1373-1382.
[58] Farag, R.S., Daw, Z.Y., Abo-Raya, S.H. **1989**. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *Journal of Food Science*, 54, 74-76.
[59] Ansari, A. A. and Shrivastava, A. K. **1991**. The effect of eucalyptus oil on growth and aflatoxin production by *Aspergillus flavus*. *Letters in Applied Microbiology*, 13(2), 75-77.
[60] Farag, R. S., Daw, Z. Y., Hewedi, F. M. and El-Baroty, G. S. A. **1989**. Antimicrobial activity of some Egyptian spice essential oils. *Journal of Food Protection,* 52, 665-667.