Effect of Powder and Essential Oil of Lemon grass on Aflatoxins Production in Dried Water Melon Seed

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Abstract: Antifungal activity of powder and essential oil from dried ground leaves of Lemon grass (*Cymbopogon citratus*) to control aflatoxin production by *Aspergillus flavus* NRRL 5096 on dried water melon seed by sun, oven, smoke and solar drying were studied. The powdered dry leaves and essential oil from lemon grass were mixed with seeds at levels ranging from 5-75 g and 0.1 to 2.0 % respectively. Obtained results revealed that the Minimal Inhibitory Concentration (MIC) on fungi was mixture of 75g powder and 0.1% oil. Also the moisture content ranged between 8.94-6.26% for dried water melon seed in addition, *A. niger* was the most frequent genus isolated from water melon seed before and after drying. Toxigenic of *A.flavus* were isolated from water melon seed dried by sun drying produced aflatoxin B_1 and B_2 at concentration 3.50 and 2.67 µg/L in liquid broth media respectively. Oven drying was the best method for drying water melon seed.
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Key words: powder and essential oil of *Cymbopogon citrates*, aflatoxins, dried water melon seed, Lemon grass.

1. Introduction

Aflatoxins are, the toxic metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. It is the most potent hepatocarcinogen and mutagen among mycotoxins. Several mycotoxins in agricultural products cause health hazards to people and animals and economical problem **(IARC, 1993).**

Dangerous mycotoxins are naturally present in foods, feeds and our environment **(Dragan *et al*., 2010).**

Water melon seed (*Citrullys vulgaris)* are smooth and black color. In Egypt, water melon seeds can be dried, roasted with salt and served as snacks. It is cultivated in the tropics for its seed which are a source of protein in important soup condiment in African diet **(Ekundayo, 1987).** Water melon seed are priced for highly nutritive contain protein, starch, vitamins, especially vitamin B and C and oils at level 27% (oleic, linoleic, palmitic and stearic) which used as vegetable oil. Also seeds are consumed in various forms such as (equsi) soup, melon ball snacks and ogori (fermented melon seed condiment used in seasoning) **(Odunfa, 1981).** Melon seed proteins contain low level of lysin, large amounts of glutamic acid, aspartic acid and arginine **(King and Onuora, 1984)**. Also, cooked Nigeria sausages with full fat egusi (melon seed) meal was produced with up 30% meat replacement, which had acceptable sausage with fat content and juiciness increased **(Akobundu, 1989).** A beverage from melon seeds in Turkey was produced to make a waste product available for human consumption which was a good source of iron, magnesium and a fair of source of protein, with a concentration of 0.31 mg/100g vitamin C (**Karakaya *et al*., 1995).**

Essential oils from different species of genus *Cymbopogon* are known for their antimicrobial activity **(Mishra and Dubey, 1994)**, antibacterial and antifungal activity as they contain some essential oils which inhibit mycotoxin formation **(Bullerman *et al*., 1984).** *Cymbopogon* had some important aromatic with remarkable commercial value. Also, essential oil of the *cymbopogon* are used in perfumery, cosmetic and soap industries and have antifungal and insecticide activity **Hajieghrari *et al*. (2006).**

Dry grains keep longer, safe from insects and moulds. Most African farmers spread their harvests to dry under the sun, which often require longer durations for the product to attain "safe" moisture level **(Begum 1991).** Since sun drying may be a difficult task due to the high rainfall at the time of harvest, a lot of work has been done on the design of solar and mechanical dryers for use by farmers in the tropics, These dryers are not in use by farmers because large capital investment in involved. So, other methods of drying that are much effective and rapid including microwave, oven but these could not be implemented in the sub-region because farmers do not have the requisite facilities, for that mechanical dryers could be set up in strategic locations, which farmers can utilize if sun drying is proven difficult. Smoking is also an efficient method of protecting maize against infestation by fungi and this practice was found to decrease aflatoxin levels in farmer's stores. The problem with smoking is that if not carefully applied, it may discolor the product and change the test **(Carruthers and Rodriguez, 1992).**

This study aimed to compare the traditional sun drying with other drying methods of water melon seed and to evaluate the activity of dry powdered leaves and essential oil from lemon grass

against toxigenic fungi and aflatoxins production in dried water melon seed.

2. Materials and Methods

Aspergillus flavus NRRL 5906 was obtained from standard Association of Australia North Sydney.

Aflatoxins standards (B_1, B_2, G_1 and G_2) at concentrations 5ug/ml were purchased from Sigma and Chemical Company PO Box 4508 St. Louis, US.

Samples of water melon seed apparently healthy were collected from different house keeper in summer 2008. Seeds were manually extracted from the fruits, washed and mixed.

Natural leaves of lemon grass (*Cymbopogn citratus*) with no chemicals and aflatoxins were purchased from Siwa Oasis, Egypt. The Crispy dry leaves were powdered in a coffee grinder, and sieved with a 0.5mm size mesh.

Drying methods of water melon seeds

Seed were dried by four methods sun, oven, and smoke and solar dried. Sun drying was done by sum, at night the seeds were covered with polyethylene bags. The vacuum oven with hot air was done continuously for at 80°C. For smoke drying a wire mesh on which water melon seeds were spread above a burning wood fire, where the temperature was between 50°C to 60°C. Solar drying by the dryer absorber was made from black in plate with 0.4mm thickness, coated by black coating (70°C). Drying was terminated when the seeds had attained constant moisture, the time taking for the seeds to attain constant weight as 30, 9, 10, and 18 h for sun oven, smoke and solar drying respectively **(Bankole *et al.* 2005).**

Extraction of essential oil from lemon grass:

Two hundred grains of the powdered leaves of lemon grass was put in a round bottom flash, 1000ml of distilled water was added and then subjected to hydro distillation in modified clevenger apparatus for 8 hours**.** The oil recovered was dried over anhydrous sodium sulphate and kept in the refrigerator at 4°C before use **(Bankole, 1997)**.

Determination of moisture content (MC)

Moisture content (MC) was determined for water melon seed before and after drying according the method of **AOAC (2007).**

Aflatoxins analysis

The extraction and assay of aflatoxin (B_1, B_2, G_1 and G_2) were performed using the EEC method **(Bankole *et al*., 1996).** Five hundred grams of each seed samples was crushed with a Moulinex blender then homogenized in 250 ml of 7:3 methanol: water for 30 min and defatted twice with 25ml n-hexane in a separator funnel. Aflatoxins were qualitative and quantitative by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The average recovery of 86.5% for ten spiked melon seed samples.

Isolation of *Aspergillus spp*

100 seeds from each sample after drying by oven, sun, smoke and solar were surface sterilized for 1 min in 1% Na Cl , (5 per plate) were placed on Potato Dextraose Agar (PDA) plus chloromphoniced and incubated for 7 days at 28°C. The percentage of seeds with *Aspergillus spp.* was determined then identification was done according to **Barnett and Hunter (1987).**

Variability of aflatoxins production by *Aspergillus spp.*

Colonies of the isolated aflatoxins (B_1, B_2, G_1 and G_2) producing strains of *Aspergillus flavus* or *A. parasiticus* were characterized microscopically; colonies were suspended and grown in Yeast Extract Sucrose medium (YES) broth containing 15% sucrose and 2% yeast extract. After incubation for 2 weeks at 28°C under stationary culture conditions the culture filtrate and extracted twice with 3 volumes of chloroform and were analyzed for aflatoxins by HPLC **(Reddy and Farhana, 2011).**

Effect of drying methods on the growth of toxic *A. flavus* and aflatoxins production in vitro

Aspergillus flavus NRRL 5906 a high aflatoxins producing strain. The preparation of conidia suspension was done by flooding the surfaces of 7-10 day old cultures of fungi with 10 ml of 0.05% tween 80 in sterile distilled water **(Bankole, 1997).** 100g of each the dried water melon seeds (Sun, oven, smoke and solar) as well as the control (fresh seed) were distributed in each of stopper flask. The seed moisture content was equilibrated to approximately 14% by addition of sterile distilled water. The flasks were incubated in incubation at 28°C with manual shaking twice daily. After 14 days the aflatoxins level in each flask was determined according to **AOAC (2007).**

Effect of lemon grass powder and essential oil

The effect of lemon grass powder and essential oil on the growth and aflatoxins production of toxic *A. flavus* NRRL 5906 on water melon seeds by preparation on conidia suspension pure cultures was maintained on potato dextrose agar and the preparation of conidia suspension, the concentration of fungi was adjusted to approximately 10^6 Conidia/ml **(Bankole and Joda, 2004).**

Determination of Minimal Inhibitory Concentration (MIC)

Was conducted according to **(Bankole and Joda, 2004).**The MIC was determined as the lowest concentration at which no growth occurs. 500g of water melon seeds after drying (Sun oven, smoke and solar) were distributed in conical flasks the seed moisture content was equilibrated to approximately 14% by the addition of sterile distilled water. After autoclave and inoculated with 5 ml conidia suspension of the toxigenic *A. flavus* NRRL 5906.

Experiments were carried out to determine the potential of using the powder, essential oil and mixed of powder plus essential oil .The powder leaves was used at level 5, 10, 25, 50 and 75 g (W.W)**.** Different dosages of the oil to give concentrations of oil, 0.1, 0.25, 0.5, 1 and 2% (V/W). Powder leaves and oil were put together. The control was also similarly set up but without powdered leaves or essential oil. The aflatoxins were determined after 14 days incubation in incubator at 28°C according to **Bankole *et al*. (1996).**

Statistical analysis

Data analysis of variance using the SUPER ANOVA (Abacus Concepts Inc CA, USA) computer and significant differences between means were determined by the least significant difference technique at 95% confidence level according to **Peterson (1985).**

3. Results and Discussion

Concerning aflatoxins detection; obtained results revealed that aflatoxins (B_1, B_2, G_1 and G_2) were not detected in any of the investigated samples. These results were agreement with **Ogunsamwo *et al*. (1989)** and **Ubani *et al*. (1993)**, they screened for aflatoxins in melon seed and they not detected aflatoxins in any of melon seed. Also, 27% of melon seed samples from farmer's stored contained aflatoxin B_1 with mean levels of 14 µg/kg in forest and 11 µg/kg in savanna of Nigeria were detected **(Bankole and Adebanjo, 2004).**

Concerning moisture content; as shown in table (1). It was significantly lowest for oven dried seeds, followed by that with smoke dried and solar-dried, while moisture content was highest in sun dried. Nearly similar results were reported by **Bankole *et al* (2005),** who Found seed moisture content of one hundred and thirty seven samples of melon seed from Nigeria Varied ranged between 5.3 to 10.4%.

The obtained results also revealed that percentage of seeds infected with *Aspergillus spp*. Samples after drying showed that *A. niger* was the most frequent genus flowed by *A. flavus* (table 2). The results presented in Table (2) shows that, *A. niger* was isolated from water melon seed before drying had largest count (70.0%). Also, water melon seed after drying by solar energy had the four species belonged to *Aspergillus*. The most frequently associated with melon in Nigeria **(Bankole, 1993 and Bnkole *et al*., 2004).** In these respect, Fungi associated with three cultivars of melon seed (*Colocynthis citrullus, Citrullys vulgaris* and *Citrullus lanatus*) in Nigeria were investigated by **Chiejina (2006)** who found the genus, *Aspergillus* was the most predominant, *A. niger* was the topping the list of fungi.

Concerning identification of *Aspergillus* isolates and their ability to produce aflatoxins, it was found that, aflatoxin B_1 and B_2 at concentration 4.50 and 3.67 µg/L respectively, were isolated from dried melon seed by sun drying. On the other hand all *A. parasiticus* isolated from water melon seed dried by solar energy produced aflatoxins B_1, B_2, $G_1$and G_2 at concentration 8.11, 4.00, 6.29 and 3.37 µg/L, respectively. In these respect, Dorner **(2004)** found toxigenic of *A.flavus* could produce only two aflatoxins, B_1 and B_2, but most of *A.parasiticus* could produce all of the four types of aflatoxins.

These results may be due to the time taking for drying which was 30 and 18 h for sun and solar drying, respectively make spores of fungi activation. **Hell *et al*. (2009)** studied the mycoflora and occurrence of aflatoxin in dried vegetables include melon seed in Benin, Mali and Togo, West Africa and found mycotoxigenic fungi belong to *Aspergillus* species had ability to produce aflatoxin was found on dried vegetable products sample from African markets.

As shown in Table (3), the lowest concentration of total aflatoxins Produced by *A. flavus* NRRL 5906 which grow on dried seed by oven which recorded 3.204 µg/kg aflatoxins (B_1 and B_2). While it was highest in water melon seed before dried which recorded 17.540 µg/kg, in this respect, **Ekundayo and Idzi (1990)** found the mean quality 0.40 µg/g of aflatoxin in healthy shelled seed infected with spores of *A. flavres* after 14 days of inoculation, also he reported the shelled melon seeds served as a utilizable nutrient source for growth of the fungi hence the rapid colonization by the diverse range of fungi.

On the other hand *A. flavus* NRRL 5906 produced aflatoxins at level 9.270µg/kg when grow on water melon seed dried by smoking. These findings not agree with those reported by **Bankole and Adebanjo (2003),** who reported that 12% of farmers in various ecological zones in Nigeria used smoke to preserve their grains and decrease aflatoxin levels in farmer's stores. Also the efficacy of smoking was also confirmed by **Hell *et al*. (2000)** in survey conducted in Benin.

Concerning the effect of different concentrations of powder and essential oil of *cymbopogon citrates,* results in figures 1-3, the powdered dry leaves and essential oil from lemon grass were mixed with the inoculated seed at level 75 g and 0.1% respectively and had the highest

effect to inhibition the effect of toxigenic *A. flavus* in dried water melon seed. **Adegoke and Odelusola, (1996),** found that, the essential oil and powder extracts of *cymbopogon citratus* inhibited the growth of fungi including toxigenic species such as *A. flavus***.** Moreover, dried seed with powder lemon grass at concentration 50g decreased the growth of *A. flavus* in dried seed by oven, smoke and solar drying, followed by dried seed by oven and solar drying with extracted oil (0.25%) treatment. Similar results were obtained by **Singh *et al*. (2010), Helal *et al*. (2007), Bonkole & Joda (2004)** and **Bankole & Adebanjo (2003).**

Citral, geraniol and citronellol showed the highest antifungal activities among terpenoids, the main component found in lemon grass oil was citral 68.4%. **(Viollon and Chaumont, 1994).**

Table (1) moisture content % of water melon seed dried by different methods

Moisture content (%)			
Drying methods			
Oven	Sun	Smoke	Solar
6.26±0.41[d]	8.50±0.26[b]	7.00±0.08[c]	7.35±0.17[b]
Moisture content (%) of water melon seed before drying was 8.94±0.52[a]			

Mean of three determination ± Standard deviations.
The same letter is not significantly different according to Duncan's Multiple Range test at, the 95% confidence level.

Table (2) Effect of drying methods on the percentage of *Aspergillus spp.* isolated from water melon seed.

Aspergillus spp.	Water melon seed before dying	Water melon seed after drying by			
		Oven	Sun	Smoke	Solar
A. flavus	10.20	25.00	33.33	14.29	33.33
A. niger	70.00	68.75	66.67	71.43	57.15
A. parasiticus	7.50	6.25	ND	ND	4.76
A. Ochraceus	12.30	ND*	ND	14.28	4.76

*ND: Non Detected

Table (3): Quantitative estimation of aflatoxins content of water melon seed

Dried water melon seed by	Aflatoxins content µg/kg				Total aflatoxins
	B_1	B_2	G_1	G_2	
Oven	2.001	1.203	ND*	ND	3.204
Sun	5.388	3.507	3.146	3.075	15.116
Smoke	3.450	2.019	3.280	0.971	9.720
Solar	4.001	2.112	3.753	1.304	11.170
Water melon seed before drying	6.951	3.282	5.053	2.54	17.540

ND*: Non Detected.

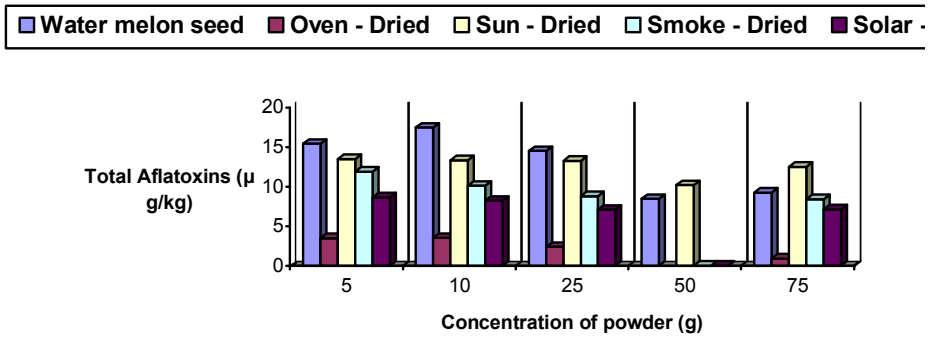

Figure 1 Effect of different concentration of powder of *cymbopogon citrates* and aflatoxins production on water melon seed

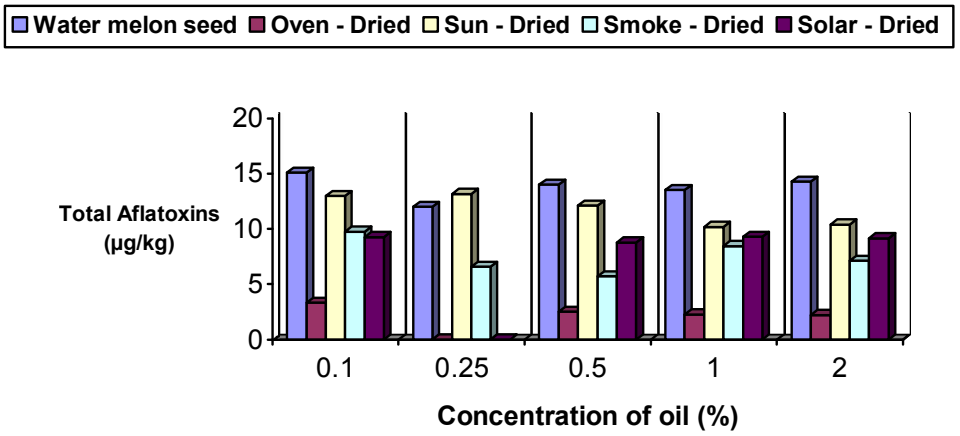

Figure 2 Effect of different concentration of essential oil of *cymbopogon citrates* and aflatoxins production on water melon seed.

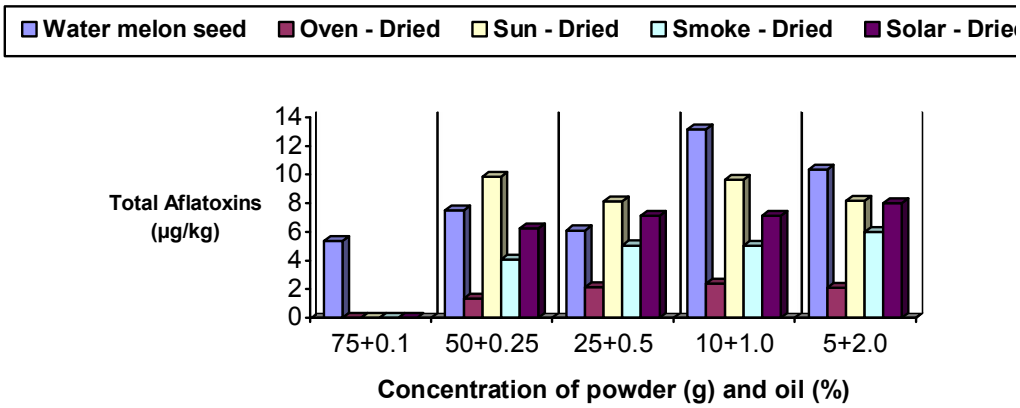

Figure 3 Effect of different concentration of powder plus essential oil of *cymbopogon citrates* and aflatoxins production on water melon seed.

The plasma membrane of *A. flavus* in the presence of 1µl/ml *C. citratus* essential oil was seemed to be irregular. The marked action of terpenic alcohols E-*citraltus* myrecene and Z-citral (the main constituents of *C. citrtus* oil), may be attributed to the polarity of the OH-group, making these compounds relatively soluble in water, which confers these molecules their lipophilic properties and the ability to penetrate the plasma membrane (Knobloch *et al*. 1989).

On conclusion, Oven drying is the best method for drying the crops and *Cymbopogon citratus* (powder, essential oil and powder plus oil), owing to its antifungal, anti-aflatoxigenic properties, may be recommended for its practical application as a botanical fungi toxicant for enhancing the shelf-life of food commodities.

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