Treated Melon Seeds and Aflatoxin Profile in Relation to Blood Parameters in Exposed Mice

*1Fapohunda SO, 2Esan A, 1Alabi OA, 1Adebote O, 1Kolawole O, 1Olayinka O, 3Atehnkeng J. and 4Kuhlmann J.

1Department of Biosciences and Biotechnology, Babcock University, Ilishan remo, Nigeria
2Biotechnology Department, Federal Institute of Industrial Research, Oshodi, Nigeria
3Pathology Unit, International Institute of Tropical Agriculture, Ibadan, Nigeria
4SGS Germany Gmbh, Hamburg, Germany

Email for Correspondence: oystak@yahoo.co.uk

ABSTRACT

The stored contaminated melon seeds (5kg) were treated with 250g of each of ginger, cinnamon and pepper for 35 days, to observe their possible antiaflatoxin potentials. The effect of contaminated untreated melon seeds and the ginger-, cinnamon- and pepper- treated melon seeds on blood parameters (PCV, WBC, lymphocytes, monocytes, eosinophils, basophils, neutrophils and platelets) in mice were also carried out. The mice were fed with ground melon seeds from each group alongside the normal rat chow for 14 days after which the blood parameters were assessed. The LC-MS² analyses showed a significant induction of aflatoxin B₁, B₂ and deoxynivanelol in the melon seeds used. Ginger, cinnamon and pepper were able to reduce the concentrations of these mycotoxins significantly, with ginger being the most effective followed by cinnamon and then pepper. The mycotoxin contaminated untreated melon seeds induced severe effects on blood parameters in exposed mice while treatments significantly reversed some of the mycotoxic effects on blood parameters. The 3 treatments showed promise as preservatives against mycotoxin contamination in stored melon seeds. This detection of some Fusarium toxins in Nigerian melon seeds is worthy of special note. The result of this study is important because mycotoxin control will result in economic gains as well as health improvement in Africa. Reducing mycotoxin levels in foods will confer international trade advantages as well as offer long-term health benefit to the local population.

Keywords: cinnamon, pepper, ginger, melon, aflatoxin, Fusarium toxins blood parameters

INTRODUCTION

Melon seeds (Colocynthis citrullus L.) are very important in making Nigerian local soup and constitute a very valuable source of oil and protein (Bankole and Joda, 2004). Melon seed, an important oilseed in West Africa has been shown to be prone to fungal and aflatoxin contamination at largely unsafe levels (Bankole et al., 2006). Also Opadokun (1992) reported high incidence (73%) of aflatoxin in Nigerian melon seed at mean content of 19µg/kg.

A variety of factors contribute to the susceptibility of melons becoming contaminated during harvest, packing, and storage. These include direct contact with the soil, which can be a potential source of contamination with human pathogens that may be present in the soil (Richards and Beuchat, 2005). Rind characteristics also play a role in susceptibility of contamination given that melons may have netted surfaces (cantaloupes), a characteristic that would make it more difficult to remove the pathogen just by washing alone, if contaminated. Mechanical damage can also be a problem since melons are quite heavy, and wounds incurred through punctures, cracks, bruising, making an excellent
entry point for pathogens (Fleming et al., 2005). Fungi of the genus *Aspergillus* and *Penicillium* are widely distributed storage fungi of melon seeds, causing seed discolorations, decreased nutritive value, increase in free fatty acid and peroxide values, decreased seed germination and producing a number of toxic metabolites including aflatoxin (Aboaba and Amasike, 1991; Bankole et al., 1999). Aflatoxins have been associated with elevated rate of liver cancer, growth stunting and immunotoxicity in West Africa (Gong et al., 2002; Turner et al., 2003). With the growing health hazards to livestock and man, concerted effort is now being directed at finding very cheap and reliable methods of minimizing mycotoxin production in stored food items. Prevention of pathogenic and spoilage microorganisms in food is usually achieved by using chemical preservatives but they are responsible for many carcinogenic and teratogenic attributes as well as residual toxicity and with growing concern of microbial resistance towards conventional preservatives, consumers tend to be suspicious of chemical additives and thus the exploration of naturally occurring antimicrobial for food preservations receives increasing attention (Schuenzel and Harrison, 2000). Many plant derived products such as spices, fruit preparations, vegetable preparations or extracts have been used for centuries for the preservation and extension of the shelf life of foods (Chatopadhyay and Bhattacharyya, 2007). Spices are used as substances that increase the taste and variation of food (Bulduk, 2004), they include leaves (mint and coriander), flower (clover), bulbs (garlic, turmeric), fruits (pepper), stem (cinnamon) and rhizomes (ginger and turmeric) (Shelef, 1983).

Antimicrobial activities of cinnamon (Muthuswami et al., 2008), ginger (Ababutain, 2011) and pepper (Ram and Pranay, 2010) have been reported against many bacterial and fungal strains. However, the ability of these spices as stored-food preservatives has not been fully explored. Therefore, the objective of this study was to evaluate and compare the protective properties of these three spices against mycotoxin production in stored melon seeds. The effect of the treated melon seeds on certain blood parameters of exposed mice was also carried out.

### MATERIALS AND METHODS

#### Spices sample

Ginger (*Zingiber officinale*), cinnamon (*Cinnamomum zeylanicum*) and crushed red pepper (*Capsicum annuum*) were purchased from 3 local supermarkets in Lagos, Nigeria.

#### Experimental design

Melon seeds (30 kg) were purchased from the main markets in Ibadan, Lagos and Ilisan remo, South west Nigeria and induced for mycotoxin as follows: The melon seeds were placed in a perforated tray, sprinkled with water and exposed for 24 hrs at room temperature (23 ± 2°C) in the laboratory. Thereafter, it was covered with polythene nylon and left to stay for 6 days to induce possible fungal growth. Aflatoxin production was induced by inoculation with *Aspergillus parasiticus* NRRL 2999 and ELISA analysis was carried out to determine aflatoxin content. The mycotoxin-induced melon seeds were then divided into 4 groups: Group A contained 5kg of the mycotoxin contaminated melon seeds alone (untreated control), group B contained 5kg of the contaminated melon seeds mixed with 250g of dried ground pepper, group C contained 5kg of the contaminated melon seeds mixed with 250g of ground ginger while group D contained 5kg of the contaminated melon seeds mixed with 250g of ground cinnamon. The samples (groups A-D) were covered with polythene nylon and left to stay for another 35 days.

#### Determination of mycotoxin and moisture content, temperature and proximate analysis

The mycotoxin and moisture content, temperature and proximate analysis of the melon seeds were carried out twice: immediately after purchase before the melon seeds were induced for mycotoxin, and after 35 days of treatment with the various spices. The samples were sent to SGS, in Germany where multimycotoxin analyses were carried out as follows: Ten grams (±0.05g) ground and homogenized melon seeds were extracted with a mixture of acetonitrile and water by horizontal shaking for 45 min. An aliquot of the sample was centrifuged and the supernatant was subsequently diluted with an aliquot of 13C Stable isotope marked Aflatoxin B1, B2, G1, G2 and Water. In case of turbidity, the sample was cleaned by means of a 0.20 µm regenerated cellulose filter. The diluted sample was analyzed by Liquid chromatography–mass spectrometry (LC-MS²) in ESI+ and quantification was achieved under consideration of the 13C internal standards.

The temperature was measured using mercury bulb thermometer placed in the experimental sample while the moisture
and ash contents were determined according to the method of AOAC as described by Pearson (1976). The crude fibre and protein contents were determined by the Weende method (Pearson, 1976) and Kjedahl method (AOAC, 1997) respectively.

Laboratory animals

Male Swiss albino mice (26-31g) obtained from the Nigeria Institute of Medical research (NIMR), Lagos, Nigeria were used for this study. All mice were housed in common metallic cage under 25 ± 2 °C. They were quarantined in a pathogen-free, well ventilated room to enable them acclimatize to the environment for about 2 weeks. Supply of food and water was *ad libitum*. The animals were maintained under 12 hrs light-dark cycle throughout the duration of the study.

Administration of melon seeds to the mice

The mice were exposed to the melon seeds after it has been ground into fine powder using laboratory blender. Mice (5 per cage) were grouped into nine: group 1 was allowed to eat normal rat chow throughout the experiment (control), group 2 was fed with rat chow mixed with ground ginger (10:1 w/w; rat chow/sample), group 3 was fed with rat chow mixed with melon-treated with ginger (10:1 w/w), group 4 was fed with rat chow mixed with aflatoxin contaminated melon (10:1, w/w), group 5 was fed with rat chow mixed with ground pepper (10:1, w/w), group 6 was fed with rat chow mixed with melon treated with pepper (10:1, w/w), group 7 was fed with rat chow mixed with healthy (non-mycotoxin contaminated) melon (10:1, w/w), group 8 was fed with rat chow mixed with ground cinnamon (10:1, w/w) while group 9 was fed with rat chow mixed with melon treated with cinnamon (10:1, w/w). The mice were fed for 14 days.

Haematological investigation

Blood collection was performed by placing each animal in airtight dissector jar with cotton soaked in diethyl ether. Blood was collected from each animal by cardiac puncture using sterile needle and 5ml syringe. The sample was put in an ethylene-diamine-tetraacetic acid (EDTA) bottles to prevent adhesion proteins (coagulation factors) in cell-cell and cell-matrix interactions for hematological determinations (Gabriel et al., 2008). Using URIT 3000 automated haematology analyzer, hematological parameters including total counts of WBC, haemoglobin (Hgb), packed cell volume (PCV), monocytes, lymphocytes, eosinophils, basophils, neutrophils and platelets were measured. All the animals received humane care (handling and restraining) according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals’ prepared by the National Academy of Science and published by the National Institute of Health. Ethical regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments (PHS, 1996).

Statistical analysis

The SPSS® 15.0 statistical package was used for data analysis. Data obtained were expressed as mean of triplicates and mean ± standard deviation of triplicates. Differences between the negative control-group and individual groups were analyzed at the 0.05 probability level.

Results

The results of the moisture content and temperature of the melon seed both at the beginning and at the end of the 35 days treatment period is shown in Table 1. There was a significant increase in moisture content from the initial reading at the beginning of the 35 days treatment compared to the last day of treatment in all the groups. This increase was not significantly different between the ginger and cinnamon treated melon and the untreated contaminated melon (control). However, pepper-treated melon had the least moisture content after 35 days (18.16) which was significantly lower than the moisture content of the control, the ginger and cinnamon treated groups.

There was no significant change in temperature of the untreated melon (control) and the ginger and cinnamon treated groups, both at the beginning of the treatment and after 35 days of treatment. However, pepper-treated melon had a
significant increase in temperature (from 28.75 to 31.50 °C) compared to the other groups.

The result of the proximate analysis of the melon seeds both before the experiment and at the end of the 35 days is shown in Figure 1. There was a significant increase in the fat content of the aflatoxin contaminated melon seeds compared to the initial reading before induction of mycotoxin. However, a considerable further increase in fat content was recorded in the ginger-treated melons followed by cinnamon-treated melons and the least in pepper-treated melons compared to the mycotoxin contaminated untreated melon seeds. The protein content significantly increased in the mycotoxin contaminated untreated melon compared to the initial reading before the induction of the mycotoxins, but there was a significant reversal of the protein content in the treated groups, with pepper reducing the protein content most, followed by ginger and then cinnamon. The results further showed a drastic reduction in the fiber content of the mycotoxin contaminated untreated melon compared to the initial reading before the induction of the mycotoxin, and subsequent further reduction in the treated groups with ginger having the least fiber content followed by cinnamon and pepper. There was no significant difference in the ash content of the melon seeds before the experiment and in all the groups. Table 2 showed the result of the mycotoxin levels in the untreated and treated melon seeds. Ten different mycotoxins were identified, namely: aflatoxin B₁, B₂, G₁ and G₂, fumonisin B₁, B₂ and B₃, ochratoxin A, deoxynivanelol and zearalenone. There was significant induction of mycotoxin in the melon seeds especially aflatoxin B₁, B₂ and deoxynivanelol within the 35 days of the experiment. The result showed a significant decrease in the aflatoxin B₁ level in the ginger, cinnamon and pepper treated groups compared to the untreated melon seeds. The cinnamon-treated group reduced the aflatoxin B₁ concentrations more than three times lower than the untreated melon seeds followed by the ginger- and pepper-treated groups, which were more than twice lower than the control (untreated melon seeds). There was a significant reduction in the concentration of deoxynivanelol in the ginger and pepper treated groups, but an increase in the cinnamon treated group compared to the untreated group. A slight reduction in aflatoxin B₂ was also recorded only in ginger and cinnamon- treated melon seeds compared to the untreated melon. The concentrations of the rest of the tested mycotoxins were not significantly different among the treated and untreated groups.

The effect of the aflatoxin contaminated and spices treated melon seeds on haematological parameters of blood are illustrated in Table 3. The PCV and haemoglobin levels of the animals in all the groups were within the normal haematological values although the mice fed with mycotoxin contaminated melon had the lowest mean PCV and
Table 3. Blood parameters (mean±SD) of mice exposed to uncontaminated and mycotoxin contaminated ground melon seeds and the treatment with ginger, pepper and cinnamon.

<table>
<thead>
<tr>
<th>Sample</th>
<th>PCV (%)</th>
<th>Hgb (g/dl)</th>
<th>WBC (x10^9/L)</th>
<th>Neutro (%)</th>
<th>Lympho (%)</th>
<th>Monocytes (%)</th>
<th>Eosino (%)</th>
<th>Platelets (x10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal hematology values*</td>
<td>35.1-45.4</td>
<td>11.0-15.1</td>
<td>1.8-10.7</td>
<td>6.6</td>
<td>55.8-91.6</td>
<td>0.0-7.5</td>
<td>0.0-3.9</td>
<td>592-2972</td>
</tr>
<tr>
<td>Mice chow (control)</td>
<td>39.00±2.65</td>
<td>13.00±0.89</td>
<td>10.23±0.38</td>
<td>11.33±1.16</td>
<td>86.00±5.29</td>
<td>2.00±3.46</td>
<td>NIL</td>
<td>630</td>
</tr>
<tr>
<td>Mice chow + ginger</td>
<td>38.33±7.02</td>
<td>12.77±2.36</td>
<td>11.57±3.31</td>
<td>24.67±3.86</td>
<td>75.33±3.86</td>
<td>0.00</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Chow + melon + ginger</td>
<td>41.00±2.64</td>
<td>13.67±0.85</td>
<td>12.83±1.31</td>
<td>18.00±6.00</td>
<td>78.67±6.11</td>
<td>3.33±1.16</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Chow + Contaminated melon</td>
<td>36.67±1.15</td>
<td>12.23±0.40</td>
<td>9.60±0.52</td>
<td>32.00±0.78</td>
<td>66.67±1.93</td>
<td>1.33±1.16</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Chow + melon + pepper</td>
<td>36.67±6.11</td>
<td>12.23±2.04</td>
<td>10.50±2.29</td>
<td>18.67±6.42</td>
<td>79.33±7.02</td>
<td>2.00±2.00</td>
<td>NIL</td>
<td>680</td>
</tr>
<tr>
<td>Mice chow + pepper</td>
<td>38.67±2.08</td>
<td>12.90±0.72</td>
<td>12.73±4.87</td>
<td>13.33±5.03</td>
<td>85.33±3.05</td>
<td>0.00</td>
<td>4</td>
<td>360</td>
</tr>
<tr>
<td>Chow + uncontaminated melon</td>
<td>41.67±1.53</td>
<td>13.87±0.51</td>
<td>13.23±0.29</td>
<td>18.00±8.00</td>
<td>84.00±8.72</td>
<td>0.67±1.15</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Chow + melon + cinnamon</td>
<td>40.00±0.00</td>
<td>13.30±0.00</td>
<td>10.73±2.27</td>
<td>14.00±2.00</td>
<td>85.33±3.05</td>
<td>0.67±1.54</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Chow + clove</td>
<td>38.00±3.00</td>
<td>12.70±1.00</td>
<td>10.90±1.99</td>
<td>24.67±3.06</td>
<td>73.33±3.06</td>
<td>2.00±0.00</td>
<td>NIL</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>


Figure 1. Proximate composition of melon seeds before and after treatment with ginger, cinnamon and pepper for 35 days.
haemoglobin of 36.67 and 12.23 respectively. The total WBC increased above the normal haematological values in all the groups except in the group fed with rat chow alone (control), with the group fed with mycotoxin contaminated melon reducing the WBC from 10.23 (rat chow alone) to 9.6. There was significant increase in PCV, haemoglobin level and WBC in the groups fed with melon treated with ginger, followed by cinnamon and then pepper compared to the group fed with mycotoxin contaminated melon alone. The neutrophils level increased above the normal haematological values in all the groups. The group fed with mycotoxin contaminated melon increased the neutrophils levels to 32.00 which is about three times the level in mice fed with rat chow alone (11.33) and more than twice the level in mice fed with healthy (uncontaminated) melon (18.00). There was significant reduction in the neutrophils level in the groups treated with cinnamon compared to ginger and pepper. The values for the lymphocytes and monocytes were similar to the result of PCV and haemoglobin with no significant difference in the levels of eosinophils and platelets within the groups.

DISCUSSION

The quality and safety of food is of great importance so that markets are not compromised by the sale of low quality or unsafe food. For the safety of human food, food-borne bacteria constitute the greatest hazard, followed by mycotoxins. Conversely in terms of livestock feeds, mycotoxins pose the greatest threat. Mycotoxins have attracted worldwide attention due to the significant losses associated with their impact on human and animal health, and consequent national economic implications (Bhat and Vashanti, 1999).

Mycotoxicosis is the consequence or effect (disease or pathological abnormalities) of ingesting toxin-contaminated foods by man and animals. It may also result indirectly from consumption of animal products such as milk from livestock exposed to contaminated feed. However, based on extensive analytical studies (IARC, 1993) and detailed study of the distribution of fungi in nature, the five agriculturally important toxins from fungi are aflatoxins, fumonisins, ochratoxin A, zearalenone and deoxynivalenol. Fungal toxins can cause acute or chronic intoxications, depending on the animal, sex, breed and dosage (Coker, 1979). The only toxin that has gained prominence in scientific literature in food products from the West African sub-region is aflatoxin, while there are few studies conducted on fumonisin and ochratoxin A.

Analytical studies (Yusuf et al., 2006; Kyari, 2008; Akubugwo et al., 2008; Obasi et al., 2012) have been carried out on melon seeds primarily because of the extensive and increasing demands for them both for human consumption and for numerous industrial applications. However, high rate of spoilage due to fungal contamination and the effect of chemical preservatives on non-target organisms has raised issues of economic importance, hence the need for identification of natural preservatives of melon seeds. The results of this present study showed that cinnamon, ginger and pepper have varying degree of preservative potential on stored melon seeds.

The results highlighted a significant decrease in aflatoxin B1 and deoxynivalenol concentrations in the cinnamon, ginger and pepper treated seeds. Aflatoxins are a group of mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus. The naturally occurring aflatoxins are B1, B2, G1 and G2 of which B1 is known to be the most toxic (Schmidt and Esser, 1985). Therefore, the reduction of aflatoxin B1 by the tested spices is of great economic importance. This reduction in aflatoxin concentrations is probably because certain herbs have antifungal properties (Ababutain, 2011; Barbosa-Canovas et al., 1998; Awuah, 1996), which could inhibit the growth of the fungi responsible for the identified mycotoxin or alter the process of mycotoxin production in the organisms. The fungistatic or fungicidal effect of spices is due to the inhibitory action of natural products and the mechanisms involved are cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating in mycelium germination inhibition (Cowan, 1999) and with plant lytic enzymes acting on the fungal cell wall causing breakage of β-1,3 glycan, β-1,6, glycan and chitin polymer (Brull and Coote, 1999).

The ability of the 3 tested treatments to inhibit the level of aflatoxin in this study is in accordance with previous reports on some spices. Some traditionally useful plants have been shown to exhibit fungitoxic properties. Awuah (1996) reported that Ocimum gratissimum, Cymbopogon citratus, Xylopia aethiopica, Monodera myristica, Syzygium aromaticum, Cinnamum verum and Piper nigrum are effective in inhibiting formation of a critical precursor in aflatoxin synthetic pathway. Leaf powder of Ocimum sp has been successfully used in inhibiting mould development on stored soybean for 9 months (Awuah, 1996). The essential oil and powder extracts of Cymbopogon citratus inhibited the growth of fungi including toxigenic species such as A. flavus and A. fumigatus (Adegoke and Oduluso, 1996). Awuah and Ellis (2002) reported the effective use of powders of leaves of O. gratissimum in combination with some packaging materials to protect groundnut kernels artificially inoculated with A. parasiticus.

The results further showed a decrease in the haemoglobin level in the control, due to defective haemopoiesis, inhibited erythropoiesis or an increase in destruction of red blood cells (Selmanoglu et al., 2001; Choudhari and Deshmukh, 2007). This decrease was however reversed by the 3 treatments, an indication of the ability of these spices to either neutralize the mycotoxin or boost haemopoiesis. The white blood cell count shows a significant increase in all
the groups. WBC is responsible for both specific and non-specific immunity. WBC increase when there is an infection, they cooperate with each other first to recognize the pathogen as an invader and then to destroy it. An enhanced WBC level is normally observed during infections and allergic responses of the host (Willey et al., 2008). The toxins in this experiment acted as antigens or allergens capable of eliciting the production of an increased WBC level.

The results obtained further confirmed the link between moisture and temperature on the one hand and aflatoxin production on the other (Viquez et al., 1994). *Aspergillus flavus/parasiticus* thrive at moisture levels of 13-18% (Christensen, 1982).

In conclusion, cinnamon, ginger and pepper showed promise in preserving melon seeds against aflatoxin production. However, the report of the occurrence of DON and patulin in some of the analysed samples has raised further alert on the human and animal health. The possible additive effect of these *Fusarium* toxins on blood parameters profile can also not be ruled out.

This study is important because user-friendly control measures will result in economic gains and health improvement in Africa. Reducing mycotoxin levels in foods will confer international trade advantages as well as offer long-term health benefit to the local population and livestock.

REFERENCES


