EVALUATION OF PATULIN LEVELS AND IMPACTS ON THE PHYSICAL CHARACTERISTICS OF GRAINS

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Abstract

Seven grain samples – cowpea, sorghum, maize, groundnut, rice, millet, and acha – were obtained from all the LGAs in Owerri, and ground to flour after physical examination. The Patulin levels in the grains were evaluated. The Patulin concentration in the grains ranged from 0.11 to 13.19 µg/kg. The patulin levels in these grains were within the permissible limit recommended by WHO and European Union. The axial dimensions and appearance of the grain samples were not affected by the presence of Patulin. Results in Table 1 show the size of the length, width, and thickness of the grain samples, as well as the weight (g) of the grains per metric cup (250 ml). Figure 1 (a) and 1 (b) compared the sizes and weight of the grains, repectively, at a glance. There was no significant difference in the weight and size of the grains at p = 0.05.

Keywords: Patulin; Grains; Physical Examination; Mycotoxins

1. Introduction

Patulin is a mycotoxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular, rotting apples and figs (Moss, 2008: Trucksess and Scott, 2008). Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moss, 2008). The disruption of immune system in animals shows that humans are also at risk of patulin's immune system impairment. In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25 μg/kg in solid apple products used for direct consumption, and at 10 μg/kg for children's apple products, including apple juice (Moss, 2008: Trucksess and Scott, 2008).

Most commonly found in rotting apples, in general, the amount of patulin in apple products is viewed as a measure of the quality of the apples used in production. In addition, patulin has been found in other foods such as grains, fruits, and vegetables. While not considered a particularly potent toxin, a number of studies have shown patulin to be genotoxic, which has led some to theorize that it may be a carcinogen, although animal studies have remained inconclusive (Yi, 1997). Patulin has shown antimicrobial properties against some microorganisms. Several countries have instituted patulin restrictions in apple products. The World Health Organization recommends a maximum concentration of 50 μg/L in apple juice (World Health Organization, 2007). In the European Union, the limit is set to 50 micrograms per kilogram (μg/kg) in both apple juice and cider, and to half of that concentration, 25 μg/kg, in solid apple products and 10 μg/kg in products for infants and young children. These limits came into force on 1 November 2003. Frequently, patulin is found in apples and apple products such as juices, jams, and ciders. It has also been detected in other fruits including cherries, blueberries, plums, bananas, strawberries, and grapes (Llewllyn *et al.*, 1998). Fungal growth leading to patulin production is most common on damaged fruits. Patulin has also been detected in grains like barley, wheat, corn and their processed products as well as in shellfish (Llewllyn *et al.*, 1998). Dietary intake of patulin from apple juice has been estimated at between 0.03 and 0.26 μg/kg bw/day in various age groups and populations (Wouters et al.; WHO Food Additives Series 35). The content of patulin in apple juice is estimated to be less than 10–15μg/L. A number of studies have looked into comparisons of organic vs conventional harvest of apples and levels of patulin contamination (Pique *et al.*, 2013: Piemontese *et al.*, 2005: Pique *et al.*, 2013). For example, one study showed 0.9% of children drinking organic apple juice exceeded the tolerable daily intake for patulin. A recent article described detection of patulin in marine strains of Penicillium, indicating a potential risk in shellfish consumption.

A sub-acute rodent NOAEL of 43 μg/kg body weight as well as genotoxicity studies were primarily the cause for setting limits for patulin exposure, although a range of other types of toxicity also exists.Patulin is toxic primarily through affinity to sulfhydryl groups (SH), which results in inhibition of enzymes. Oral LD_{50} in rodent models have ranged between 20 and

100 mg/kg (Puel *et al.,* 2010). In poultry, the oral LD_{50} range was reported between 50–170 mg/kg. Other routes of exposure are more toxic, yet less likely to occur. Major acute toxicity findings include gastrointestinal problems, neurotoxicity (i.e., convulsions), pulmonary congestion, and edema. Studies in rats showed decreased weight, and gastric, intestinal, and renal function changes, while repetitive doses lead to neurotoxicity. Reproductive toxicity in males was also reported (Puel *et al.,* 2010). An NOAEL in rodents was observed at 43µg/kg. WHO concluded that patulin is genotoxic based on variable genotoxicity data, however, it is considered a group 3 carcinogen by the International Agency for Research on Cancer (IARC), since data was inconclusive.

Physical characteristics are part of the major parameters for ascertaining grain quality. Quantitative levels of Patulin in grains are not fully studied and the influences of the Patulin on the physical characteristics grains in the food systems are yet to be ascertained.The outcome of this study is useful to grain importers, exporters, handlers, and millers, and policy formulators on designing the bench levels for monitoring purposes. In this study, grain samples from markets in Owerri zone were investigated for patulin levels and the likelihood of their impact on the axial dimensions and appearance of the grains.

2. Materials and Methods

2.1. Source of Raw Materials:

The raw materials for the study; cowpea, groundnut, sorghum, millet, maize, acha, and rice, were obtained from markets in all the three local government areas in Owerri (Owerri Municipal, Owerri North, and Owerri West): Ekeonunwa, Relief, and Ama-hausa markets (Owerri Municipal): Orie Umuorii, Orie Uratta, and Ekeobi markets (Owerri North): Obinze, Nkwo Ukwo (Ihiagwa), and Umuerim markets (Owerri West).

2.2. Sample Collection and Preparation

Three representative samples were randomly collected (selected) for each of the grains from different markets in each of the local government areas in Owerri within October 2016 and July 2017. The samples were physically examined and ground into flour using Art's-Way's portable roller mill (PRM30: USA) and labeled accordingly as directed by AOAC (2000).

2.3. Physical Examination

One cup each of the samples was weighed with Yongkang Jieli sensitive weighing balance (100g to 1000g: China) with 0.01g accuracy and their values reported in gramme. A Louisware Electronic Digital Slide Caliper, IP54 (UK), with 0.02mm accuracy was used to measure the axial dimension (length, width, and thickness) of the grains. The shapes and appearance (colour) were visually examined as recommended by Codex Alimentarius Commission (FAO/WHO), 2007.

2.4. Determination of Patulin

The method of AOAC (2000) was used.

Extraction

Fifty grams (50 g) of grain flour was added to three 50 ml portions of ethyl acetate in 250 ml separator and extracts collected. Extracts were dried (upper phase) for about 30 minutes over anhydrous sodium sulfate, decanted into graduated 250 ml beaker. Sod sulfate was washed with two 25 ml portions of ethyl acetate and added to extract. Evaporation to less than 25 ml on the steam bath under a gentle stream of nitrogen (Did not evaporate to dryness) was done. It's allowed to cool to room temperature; adjusted to 25 ml mark with ethyl acetate and diluted to 100 ml with benzene.

Thin-layerChromatography (TLC)

Preparation of plates: 30 g silica gel was weighed into 300 ml glass stoppered flask; water was added. The solution was shook vigorously and poured into the applicator. Immediately five 20x20 cm plates with 0.25 mm thickness were coated. Plate was rested until gelled. Coated plates were dried for 1 hour at 110 ºC and stored in dessicating cabinet.

Preliminary Thin Layer Chromatography – Using a 10 µl syringe, two 5 µl spots and one 10 µl spot of test solution from above and 1, 3, 5, 7, and 10 µL of working standard on imaginary line 4 cm from the bottom edge of the plate, were spotted, followed by spotting 5 µL of the standard solution on top of the 5 µl test solution spot.

Plate was developed with toluene–ethyl acetate – 90% formic acid (5+4+1) contained in V-shaped metal trough inside the unlined equilibrated tank with silica gel facing maximum tank volume. When solvent front reached 4 cm from the top of the plate, the plate was removed and dried in air, preferably in hood Spray plate with 5% MBTH solution until layer appears wet, then heated about 15 minutes in 130 ºC oven. Plate was examined in transmitted and reflected long wave UV light.

NB: Patulin appeared as yellow-brown fluorescent spot at Rf about 0.5 – 1 µL standard solution.

NB: Patulin was also seen as a yellow spot under visible light if the concentration was more than 0.05 µg. TLC patterns were examined.

Calculation

$$\mu g \text{ Patulin} = \frac{S \times Y \times V}{50 \times X}$$

Where, S = uL standard equal to test solution, Y = Concentration of standard in µg/ml, V = Volume of test solution, X = µL test solution spotted giving fluorescent intensity equal to S (standard).

Sprayed TLC plate shall slowly turn to blue on standing in air for few hours unless covered by a second glass plate.

3. Results and Discussions

3.1. Results

Results in Tables 1 to 5 show the average levels of patulin, physical characteristics, nutritional composition, and functional properties of the grains, as well as the influences of patulin presence on the nutritional composition and functional properties of the grains.

Table 1: Mean Values of the Weight and Size (Axial dimension) of Grain Samples

Grains		*Weight (g/250ml)*	*Length (mm)*	*Width (mm)*	*Thickness (mm)*
Rice	**A**	**183.66 ± 0.32[a]**	**7.53 ± 0.76[a]**	**2.99 ± 0.48[a]**	**2.32 ± 0.56[a]**
	B	**184.58 ± 0.81[a]**	**7.16 ± 0.33[a]**	**2.73 ± 0.28[a]**	**1.74 ± 0.17[a]**
	C	**183.25 ± 0.49[a]**	**7.87 ± 0.82[a]**	**3.68 ± 0.48[a]**	**2.14 ± 0.23[a]**
	Total	**183.83 ± 0.77**	**7.52 ± 0.66**	**3.13 ± 0.56**	**2.07 ± 0.40**
Cowpea	**A**	**178.32 ± 0.30[a]**	**16.71 ± 1.71[a]**	**11.53 ± 2.40[a]**	**7.76 ± 1.47[a]**
	B	**179.13 ± 0.89[a]**	**16.99 ± 0.71[a]**	**13.26 ± 0.90[a]**	**9.88 ± 1.22[a]**
	C	**178.34 ± 0.91[a]**	**16.61 ± 1.14[a]**	**11.95 ± 2.23[a]**	**9.21 ± 2.13[a]**
	Total	**178.60 ± 0.77**	**16.77 ± 1.10**	**12.25 ± 1.87**	**8.95 ± 1.71**
Acha	**A**	**134.60 ± 0.78[a]**	**1.89 ± 0.04[a]**	**0.81 ± 0.02[a]**	**0.39 ± 0.08[a]**
	B	**134.58 ± 0.64[a]**	**1.93 ± 0.04[a]**	**0.86 ± 0.04[a]**	**0.38 ± 0.02[a]**
	C	**134.13 ± 0.26[a]**	**2.00 ± 0.06[a]**	**0.99 ± 0.17[a]**	**0.37 ± 0.05[a]**
	Total	**134.44 ± 0.57**	**1.94 ± 0.06**	**0.89 ± 0.12**	**0.38 ± 0.05**
Maize	**A**	**178.17 ± 0.27[a]**	**15.32 ± 1.29[a]**	**8.47 ± 2.35[a]**	**5.26 ± 0.50[a]**
	B	**178.20 ± 0.86[a]**	**15.27 ± 0.64[a]**	**9.66 ± 0.92[a]**	**5.20 ± 0.36[a]**
	C	**179.20 ± 1.02[a]**	**16.11 ± 0.83[a]**	**10.23 ± 0.90[a]**	**5.60 ± 0.68[a]**
	Total	**178.52 ± 0.85**	**15.57 ± 0.92**	**9.45 ± 1.55**	**5.35 ± 0.49**
Millet	**A**	**198.82 ± 0.19[a]**	**3.29 ± 0.41[a]**	**1.83 ± 0.23[a]**	**1.11 ± 0.18[a]**
	B	**198.25 ± 0.27[a]**	**3.19 ± 0.39[a]**	**1.72 ± 0.11[a]**	**1.07 ± 0.20[a]**
	C	**198.62 ± 0.56[a]**	**3.45 ± 0.42[a]**	**1.85 ± 0.24[a]**	**1.01 ± 0.21[a]**
	Total	**198.56 ± 0.41**	**3.31 ± 0.37**	**1.80 ± 0.18**	**1.06 ± 0.17**
Sorghum	**A**	**204.12 ± 0.53[a]**	**6.02 ± 1.20[a]**	**4.86 ± 0.90[a]**	**2.18 ± 0.33[a]**
	B	**204.10 ± 1.48[a]**	**6.32 ± 0.66[a]**	**4.73 ± 0.34[a]**	**2.02 ± 0.25[a]**
	C	**203.90 ± 0.92[a]**	**5.54 ± 0.56[a]**	**3.76 ± 0.50[a]**	**1.80 ± 0.18[a]**
	Total	**204.04 ± 0.92**	**5.96 ± 0.81**	**4.45 ± 0.75**	**2.00 ± 0.28**
Groundnut	**A**	**158.57 ± 0.45[a]**	**20.20 ± 1.33[a]**	**11.77 ± 1.58[a]**	**8.26 ± 0.51[a]**
	B	**158.75 ± 0.55[a]**	**19.25 ± 1.85[a]**	**12.82 ± 1.85[a]**	**9.40 ± 1.26[a]**
	C	**159.90 ± 0.95[a]**	**20.97 ± 0.15[a]**	**11.30 ± 0.39[a]**	**8.18 ± 0.30[a]**
	Total	**159.07 ± 0.86**	**20.14 ± 1.36**	**11.96 ± 1.41**	**8.61 ± 0.91**
Total		**176.72 ± 22.16**	**10.17 ± 6.78**	**6.28 ± 4.64**	**4.06 ± 3.43**

*****A, B and C represent samples from Owerri Municipal, Owerri North, and Owerri West, respectively. *P= 0.05

Table 2: Appearance (Colour and Shape) of Grain Samples

Grain	*Sample*					
	A		**B**		**C**	
	Colour	**Shape**	**Colour**	**Shape**	**Colour**	**Shape**
Rice	**White**	**Cylindrical**	**White**	**Cylindrical**	**White**	**Cylindrical**
	White	**Cylindrical**	**White**	**Cylindrical**	**White**	**Cylindrical**
	White	**Cylindrical**	**White**	**Cylindrical**	**White**	**Cylindrical**
Cowpea	**Whitish cream**	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**
	Whitish cream	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**
	Whitish cream	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**	**Whitish cream**	**Kidney-shaped**
Acha (Fonio)	**White**	**Finger-shaped**	**White**	**Finger-shaped**	**White**	**Finger-shaped**
	White	**Finger-shaped**	**White**	**Finger-shaped**	**White**	**Finger-shaped**
	White	**Finger-shaped**	**White**	**Finger-shaped**	**White**	**Finger-shaped**
Maize	**White**	**Large and flat**	**White**	**Large and flat**	**White**	**Large and flat**
	White	**Large and flat**	**White**	**Large and flat**	**White**	**Large and flat**
	White	**Large and flat**	**White**	**Large and flat**	**White**	**Large and flat**
Millet	**Yellowish white**	**Ovoid**	**Yellowish white**	**Ovoid**	**Yellowish white**	**Ovoid**
	Yellowish White	**Ovoid**	**Yellowish white**	**Ovoid**	**Yellowish White**	**Ovoid**
	Yellowish white	**Ovoid**	**Yellowish white**	**Ovoid**	**Yellowish white**	**Ovoid**
Sorghum	**Red**	**Spherical**	**Red**	**Spherical**	**Reddish brown**	**Spherical**
	Red	**Spherical**	**Light red**	**Spherical**	**Light red**	**Spherical**
	Light red	**Spherical**	**Red**	**Spherical**	**Red**	**Spherical**
Groundnut	**Red**	**Round-shaped**	**Red**	**Long-shaped**	**Red**	**Long-shaped**
	Red	**Long-shaped**	**Red**	**Long-shaped**	**Red**	**Long-shaped**
	Red	**Long-shaped**	**Red**	**Round-shaped**	**Red**	**Long-shaped**

***A, B and C represent samples from Owerri Municipal, Owerri North, and Owerri West, respectively.

Table 3: Mean values of Patulin

Grains		*Patulin (µg/kg)*	*Grains*		*Patulin (µg/kg)*	*Grains*		*Patulin (µg/kg)*
Rice	A	0.83 ± 0.08 [b]	***Maize***	A	2.50 ± 0.37 [b]	***Groundnut***	A	12.03 ± 1.43 [a]
	B	1.00 ± 0.03 [a]		B	1.95 ± 0.11 [c]		B	9.55 ± 0.55 [b]
	C	0.53 ± 0.11 [c]		C	3.89 ± 0.16 [a]		C	12.25 ± 0.52 [a]
	LSD	0.06		LSD	0.20		LSD	0.76
	Total	0.79 ± 0.22		Total	2.78 ± 0.89		Total	11.28 ± 1.53
Cowpea	A	2.37 ± 0.58 [a]	*Millet*	A	2.50 ± 0.12 [a]			
	B	2.44 ± 1.00 [a]		B	1.16 ± 0.20 [c]	*Total*		2.93 ± 3.60
	C	2.49 ± 0.12 [a]		C	1.87 ± 0.05 [b]	*WHO/EU/ FAO/CODEX Limits*		50.00 in adult food
	LSD	0.55		LSD	0.11			
	Total	2.44 ± 0.58		Total	1.84 ± 0.59			
Acha	A	0.51 ± 0.04 [c]	***Sorghum***	A	1.17 ± 0.20 [a]			10.00 in infant food
	B	0.70 ± 0.07 [b]		B	0.72 ± 0.03 [b]			
	C	0.91 ± 0.11 [a]		C	0.10 ± 0.02 [c]			DI of 0.40 BW
	LSD	0.06		LSD	0.09			
	Total	0.71 ± 0.19		Total	0.66 ± 0.47			

*****A, B and C represent samples from Owerri Municipal, Owerri North, and Owerri West, respectively.
*P= 0.05 *BW = Body Weight *DI = Daily Intake

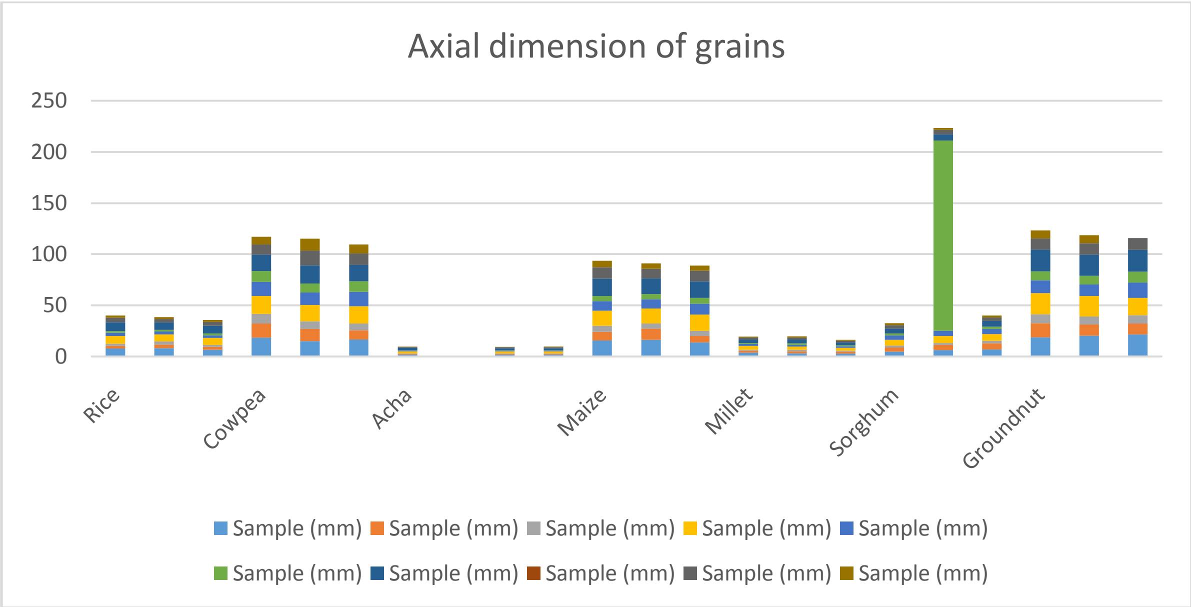

Figure 1 (a): Size (axial dimension) of grains

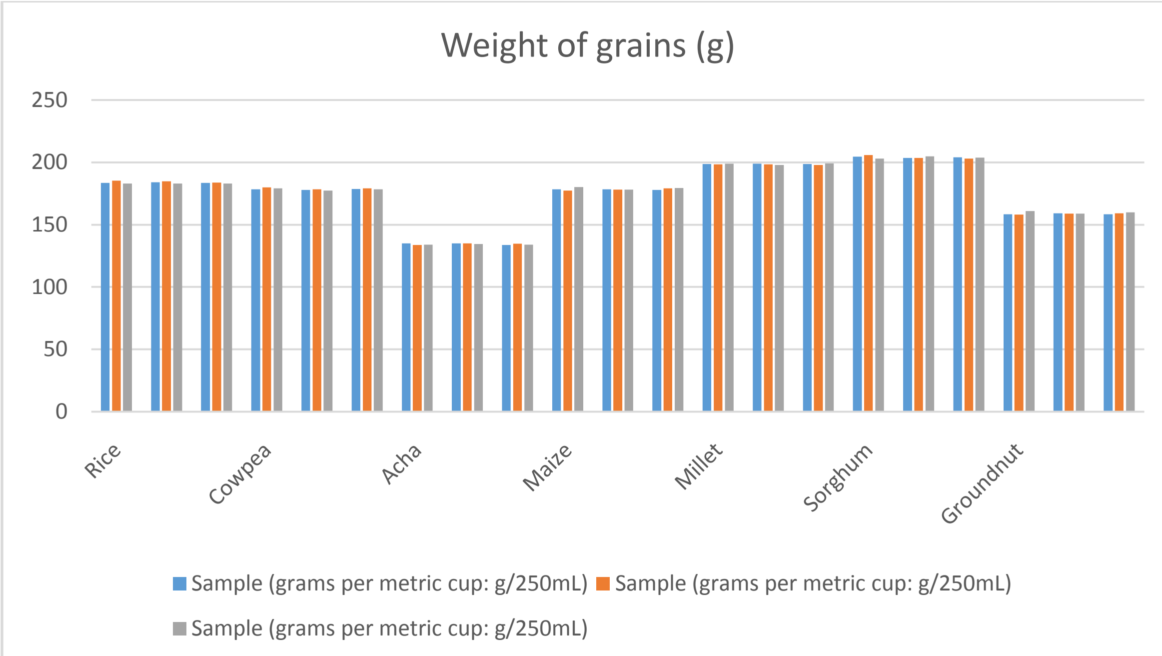

Figure 1 (b): Weight of grains

3.2. Discussions

3.2.1. Physical examination

The results of the physical examination of the grains are shown in Tables 1 and 2. Results in Table 1 show the size of the length, width, and thickness of the grain samples, as well as the weight (g) of the grains per metric cup (250 ml). Figures 1 (a) and 1 (b) compared the sizes and weight of the grains, repectively, at a glance. From Table 1, it can be seen that there was no significant difference in the weight and size of the grains at $p = 0.05$. Figure 1 (b) shows that the weight of the grain samples remained consistent. The colour and shape of the grains are shown in Table 1 (b). There was no difference in colour and shape of the grains.

3.2.2. Patulin

Patulin results are shown in Table 3. Patulin is a mycotoxin produced by a variety of molds, in particular, *Aspergillus, Penicillium*, and *Byssochlamys* (Puel *et al*., 2010). The World Health Organization and European Union recommend a maximum concentration of 50 micrograms per kilogram (µg/kg) in adult foods and 10 µg/kg in products for infants and young children (Moss, 2008; World Health Organization, 2007). As regards patulin, the Scientific Committee on Foods (SCF) endorsed in its meeting on 8 March 2000 the provisional maximum tolerable daily intake (PMTDI) of 0.4 µg/kg body weight for patulin (European Food Safety Authority, 2000). Patulin is destroyed by the fermentation process. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals (Moss, 2008). The Patulin concentration in the grains range from 0.11 to 13.19 µg/kg. These levels are within the WHO/EU limits. The highest and lowest average Patulin concentration were seen in groundnut and sorghum flour, respectively.

Major acute toxicity findings on Patulin include gastrointestinal problems, neurotoxicity (i.e., convulsions), pulmonary congestion, and edema. Studies in rats showed decreased weight, and gastric, intestinal, and renal function changes, while repetitive doses lead to neurotoxicity. Reproductive toxicity in males was also reported (Puel *et al.,* 2010). An NOAEL in rodents was observed at 43µg/kg. WHO concluded that patulin is genotoxic based on variable genotoxicity data, however, it is considered a group 3 carcinogen by the International Agency for Research on Cancer (IARC), since data was inconclusive. Patulin decreased sperm count and altered sperm morphology in the rat (Selmanoglu, 2006). Also, it resulted in abortion of F1 litters in rats and mice after i.p. injection (Puel *et al.,* 2010). Embryotoxicity and teratogenicity were also reported in chick eggs (Puel *et al.,* 2010). Although there are only very few reported cases and epidemiological data, the FDA has set an action limit of 50 ppb in cider due to its potential carcinogenicity and other reported adverse effects. In humans, it was tested as an antibiotic intra-nasally for use against the common cold with few significant adverse effects, yet also had negligible or no beneficial effect. Patulin exposure can be successfully contained by following Good Agricultural Practices (GAP) such as removing mold, washing, use of fungicides, and not using rotten or damaged apples for baking, canning, or juice production.

4. Conclusion

Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom, commonly known as molds. These mycotoxins are found in grains before, during and after harvest. The patulin levels in these grains were within the permissible limit of 50 µg/Kg recommended by WHO and European Union. There was no significant difference in the physical characteristics of the grain samples. Patulin was present in all the grains at various quantities. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. The Patulin concentration in the grains ranged from 0.11 to 13.19 µg/kg. These levels are within the WHO/EU limits. The highest and lowest average Patulin concentration were seen in groundnut and sorghum flour, respectively. The physical properties of the grains were consistent.

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