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Evaluation of Aflatoxins and Pesticide Residues in Fresh and Different Processed Mushrooms

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Abstract

Mushrooms are becoming popular to us due to their nutritional, medicinal and therapeutic values. Toxicity of aflatoxins, presence of DDT and heptachlor as pesticide residue are rare in edible mushrooms but not non-existent. So an attempt has been made to determine its presence and to the same quantify using HPLC and GC. Total seven categories of mushrooms and mushroom-based food products were analyzed. Among them (6 samples) 85.72% of the total was found to be free from aflatoxins. On the other hand, 5.53282 μgkg^{-1} of aflatoxin B_1 was found to be present in canned Button mushrooms (*Agaricus spp*).The results revealed that 14.28% (1 sample) of samples were toxicated by aflatoxin B_1, compare to the total samples analyzed including the imported was in the form of processed/preserved mushrooms. In the present experiments it was also observed that there were no aflatoxins in fresh-cut mushrooms and in the recently developed Mushroom-juice even in dried and powdered as processed Oyster mushrooms (*Pleurotus ostreatus*) which are being widely cultivated in Bangladesh. No pesticide residue such as DDT or heptachlor was detected in any of the samples examined.

Key words: Mushrooms, Oyster, Button, Aflatoxins, DDT, Heptachlor; Pesticide residue.

Introduction

Aflatoxins are a group of highly toxic secondary metabolites known to be produced by *Aspergillus flavus* and *Aspergillus pasiticus* (Codner 1963; Sargeant *et al*, 1971). These are ubiquitous in nature, associated with the spoilage and toxin production of stored, agricultural commodities (Diener,1977). Considerable importance is associated with the presence of aflatoxins in food and feed because of their carcinogenic, mutagenic and teratogenic effects (Golblatt, 1969; Healthcate, 1978). Approximately 300 to 400 substances are recognized as mycotoxins, comprising a broad variety of chemical structures (Sulyok *et al*, 2006). It has also been known that mycotoxins are toxic secondary metabolites produced by various mold species growing on many agricultural commodities and processed food, either in the field or during storage (Hussein and Brasel, 2001; Bennett and Klich, 2003). Mycotoxins have been ranked as the most important chronic dietary risk factor, higher than synthet ic contaminants, plant toxins, food additives or pesticide residue (Kuiper, 1998). Lately, additive and synergistic have been observed concerning the health hazard posed by mycotoxins (Mello *et al.*1999; Speijers, 2004; McKean *et al*, 2006). The nutritional and medicinal values of mushrooms have long been recognized (Lucas *et al*,1957; Suzuki and Oshima 1976). On accounts of their flashy nature and good flavor, it is being used various types of snacks and ready to eat products (Bruhn, 1995). By 2003, world mushroom production had reached three million tones and a trend to increase by 10% every year (Anon, 2003; Thaithatgoon, 1998). During the past few decades, it has been increased the demand of easier-to-prepare fresh vegetables to a healthy diet. However, mushrooms have assumed greater importance in the diets of both rural and urban dwellers. Most recently, these fresh mushrooms and mushroom-based food items are consumed by consumers of varied age groups (Valentine, 1995). Recently mushrooms are becoming popular in Bangladesh. An on average 3 tons of mushrooms are being imported per year (Anon, 2006). While 620-675 metric tons of mushrooms are produced in Bangladesh per annum, majority of 97% are Oyster mushrooms (*Pleurotus* spp). In Bangladesh, mushrooms are being cultivated mainly in polypropylene (pp) bags preparing spawn and few in making compost. The two main substrate or the basal components of

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the spawn are sawdust and paddy straw, other supplementary agro-wastes are rice husk and wheat bran (Kamal, 2008). It has been Reported that about 30 million tones of agricultural wastes like saw dust, paddy straw, wheat straw and sugarcane- bagasse are produced per annum in Bangladesh (Ahmed, 2001). As mushrooms can build up large concentrate of some of the toxins, there is possibility of present of heptachlor and DDT as pesticide residues in these agro-wastes. It has been pointed out by Mello (1997) that food processors must also recognize increased costs related to further processing requirements, in particular, analysis and monitoring for presence of the afflatoxins. However, little is known about aflatoxins and pesticide residues in mushrooms. So, an attempt has been taken with a view to evaluate aflatoxins, and pesticide residue in both of fresh and processed edible mushrooms.

Materials and Methods

Collection of samples:

Fresh-cut Oyster mushrooms (*Pleurotus ostreatus*) are collected as of third flash from the Cropping room of IFST, BCSIR, Dhaka. The substrates were sawdust (FM_1) and paddy straw (FM_2) respectively. The sizes of mushrooms are in between 5 to 11cm in diameter on an average 8cm, and did not require use of pesticide in both of pre-harvest or post-harvest stages. Oven dried mushroom powder (MP1), mushroom soup powder (MP2) and recently developed Mushroom-Juice (MJ) are collected from the same laboratory as processed mushrooms. The canned (M;GM) White button mushrooms (*Agaricus* spp) were imported and collected randomized from mega shops of Dhaka city considered to be processed /preserved foods. Total seven categories of samples are collected and three replicates of each sample are analyzed.

Instruments/Apparatus

Detection and quantification of Aflatoxins, Ochratoxcins were performed with a High Performance Liquid Chromatograph (HPLC), Agilent1200, G1316A, CULCOM, German. Heptachlor and DDT were analyzed using Gas-Chromatography (GC-14B Shimadzu) with an electron capture detector (ECD), a manual sampler and solution software. Syringe (10µl;Hamilton co.). Concerned instruments/apparatus are rinsed with acetone prior to use.

Reagents

Acetone, diethyl ether, dimethyl formamide saturated with petroleum ether, n-hexane, petroleum ether (30-60^{O}C), petroleum ether (30-60^{O}C) saturated with dimethyl formamide, eluting mixture I (petroleum ether+diethyl ether 94:6v/v), standard solutions, eosin solution (2 mg in 100 ml), sodium sulfate solution (2g/100ml $NaSo_4$ 10 H_2O), sodium sulfate anhydrous (heated for at least 2 hour at 550^{O}C), florisil 60-100 mesh (heated for at least 2 hour at 550^{O}C, cool and stored in tightly stopper- container, prior to use was heated for at least 5 hour at 130^{O}C, cool and add 5% w/w water, the mixture was shaken for at least 20 min and was stored in a container for at least 10 hour), cotton wool.

Chemicals, reagents and solvents used for the analysis were procured form MERCK, Germany. DDT and heptachlor standards were obtained from Sigma Chemicals.

Sample preparation:

All the samples were blended while comminuting was avoided by brief chopping for several times. Titration was done with 25 g of sample and sodium sulfate to get dry powdery mixture, with the aid of an extraction thimble; the powdary mixture exhaustively with Petroleum Ether in Soxhlet apparatus. Concentrate and dilute to 25ml with petroleum ether saturated with dimethyl formamide (Hans and Zeumer, 1987).

Clean up

Preparation of injecting solution and clean up was performed according to the method described by Hans and Zeumer (1987).

Procedure for aflatoxins: To get injecting solution, the required amount of fresh-cut mushrooms or mushroom-based food products were weighted and then blended with the help of high-speed blender fitted with proof glass jar and explosion-proof mater to make slurry. Added acetone and distilled water to make it soluble using the formulae.

$$\text{Vol. of acetone to be added} = \frac{\text{Slurry wt x D. water: Meal x 4}}{\text{D. water: Meal + 1}}$$

Shacked 30 minutes using shaker (rm 521, DENLEY. UK) and filtered by whatman filter paper (Whatman-^{1}cat no 1001320; UK). Subsequently the solid phase extraction (SPE) was done by Vacuum manifold (Model No. 1223-4000) using Varian Bond Eluted column (PH 1210-2032) Followed wash columns for absorbing color and elution two additional columns for absorbing color and moisture (florisil magnesium tricillicate for cc and sodium sulphate for moisture) then eluted with elution buffer (Chloroform). Concentrated the extract with the help of concentrator (Techne, DB-3, UK) under nitrogen gas. Finally extracting were diluted with 300µl (.3ml) mobile phase (Methanol

22.5% +Acetonitrile 22.5% + 55% Deionized water) as injecting solution. In order to get results as computerized chromatogram, 30µl solution was injected into the HPLC with the help of 10µl syringe and run for 15 minutes.

Procedure for DDT and heptachlor

The DDT and heptachlor residues were analyzed by GC-148, Shimadzu with an electron capture detector (ECD), a manual sampler and GC solution software. A column of 3.1m X 3.2 mm; I. D glass spiral; stationary phase silicon OV 17, 5%, aging 300^{O}C, support chromosorb-W-AW-DMCS, mesh 80/100,1µm film thickness was used for the chromatographic separation of insecticides.

The temperature was fixed for the injector at 250^{O}C, column at 280^{O}C, detector at 280^{O}C. The carrier gas was nitrogen with a 60 ml/min-flow rate. 1.0 µl sample was injected for each run and the running time was 25min. Standards peak were identified by injecting high concentration of the standard (0.5 ppm and 0.25ppm) and the retention time for DDT and heptachlor were determined. Then calibration was done at 3 points (25 ppb, 50 ppb and 100ppb) by composite stock standard solution. GC system was calibrated using external standard technique. Individual standard stock solution (100mg/l) was prepared by weighing appropriate amounts of Teflon-lined screw cap and dissolving the weighed standard in HPLC grade hexane. Stock standard solution was used to prepare primary dilution standards. Appropriate volume of each individual stock solution was taken in a volumetric flask and mixed the solutions to obtain composite stock standard solution.

Detection and quantification

Gas chromatograph equipped with ECD was checked for linearity. Instrumental limit of detection for GC-ECD was 1.0µg/l for organochlorine pesticides. An aliquot of mushroom samples which were collected as blank and treated exactly as a sample including exposure to all glassware, equipments, solvents and regents was used with the sample matrix. No analytic peak was detected in laboratory reagent blank. Aliquot of fortified samples matrix were prepared to which known quantities of the pesticides were added in the laboratory in ppb range. This laboratory fortified matrix was analyzed exactly like the sample. Extraction and clean up were done as mentioned and the recoveries formed untreated control samples of mushrooms, fortified with the analyzed compounds at level of 25ppb were 96-100% for heptachlor and 98-100% for DDT.

Prior to injection of the first sample solution, a standard solution was injected at least three times to check the operating conditions and the constancy of the detector signals. Further linearity of the ECD signal was checked by injecting serial dilutions of DDT and heptachlor. A standard solution injected after at least every other sample solution due to column contamination so that any alterations of the gas chromatographic system could be recognized.

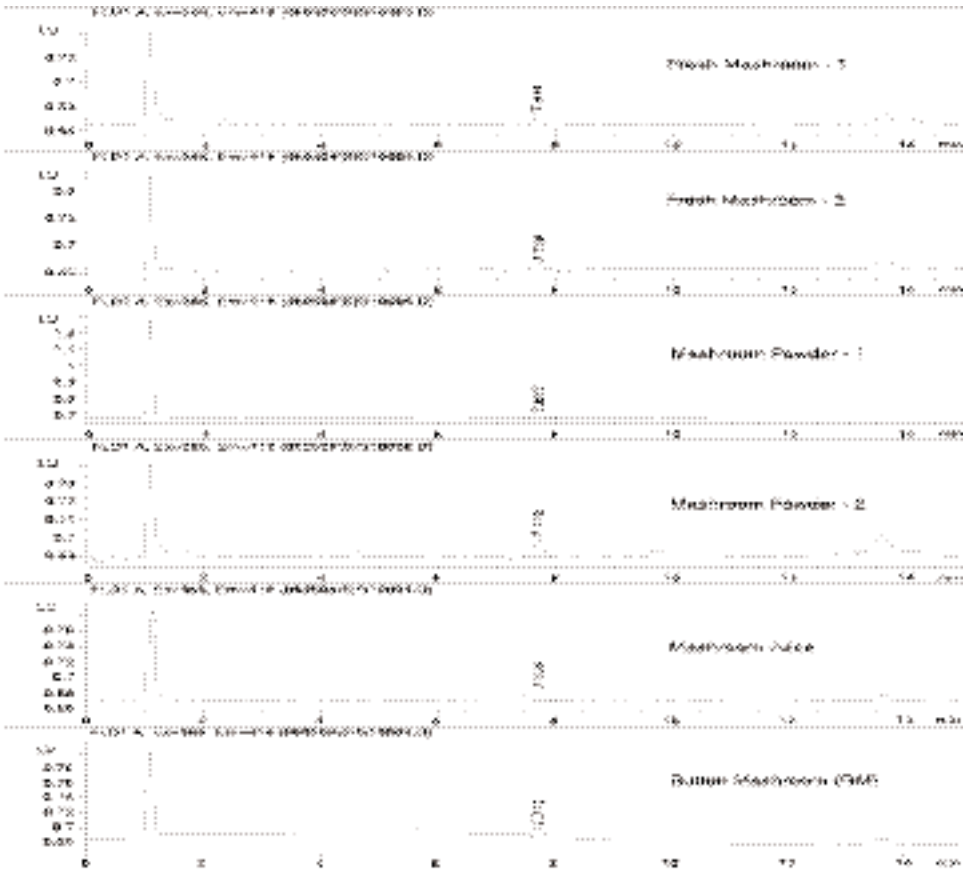
Results and Discussion

Results obtained from the experiments are summarized in Table I. Fig.-1 showed that there was no peak for afflatoxins in any sample (FM-1, FM-2, M, GM, MJ, and MP-1&MP-2) considering retention time as specified for mentioned HPLC. It indicated that there was not found any aflatoxins in fresh or processed Oyster mushrooms or might be present below the detection limit (.01ppb), generally recognized as insignificant. Bano *et al.* (1986) have been studied 12 mycotoxins including aflatoxins B_1; B_2 in the spent straw after development of *Pleurotus sajor-casu* fruting bodies. When fed to albino rats and mice of both sex no biological or histopathological abnormalities were found. No toxic

Table I. Tabular form of the results of Aflatoxin and Pesticide residue in frest and different Processed and preserved mushrooms.

Sl. No.	Code	Sample	Packet	Origin	Substrate	Aflatoxin				Pesticide residue
						B_1	B_2	G_1	G_2	
1	FM-1	Fresh oyster	100g polypack	Local	Sawdust	A	A	A	A	ND
2	FM-2	Fresh oyster	100g polypack	Local	Straw	A	A	A	A	A
3	GM	Button	450g cane	Imported	Unknown	A	A	A	A	A
4	M	Button	450g cane	Imported	Unknown	P	A	A	A	ND
5	MJ	Juice (oyster)	250ml bottle	Local	Straw	A	A	A	A	ND
6	MP-1	Powder (oyster)	100g polypack	Local	Straw	A	A	A	A	ND
7	MP-2	Soup powder	100gm polypack	Local	Sawdust	A	A	A	A	ND

Key: A= Absent; P = Present; ND= Not done (As because, in case of sl. No-1, substrate was not susceptible and sl. No 4-7 samples were derived from Fresh Oyster mushrooms).



effects or illness sign occurred in chick or pigs. A number of literatures revealed that mushrooms have the ability to accumulate toxic substances from substrate through mycelium. In such finding we can assumed that the observation was similar with that of Bano *et al.* findings in case of Oyster mushrooms (*Pleurotus* spp) and these types of mushroom-based food products. As can be seen in Fig. 2, aflatoxin-B_1 has found to be present in White button mushroom. The amount was 5.53282µgkg-$_1$. Mori *et al*, (1978) have already been reported about aflatoxin-b toxicity as mycotoxin which is closely related to hepatocarcinogenicity from *Aspergillus* flavus in several mushrooms and Agaricus bisporus is included in this group. It was evident that toxins in edi-ble mushrooms are rare but not non-existent specially in White button mushrooms. Considering the carcinogenic effects of afflatoxins on animal and human, there are statuary limits or in some cases advisory guidelines for the maximum levels of the afflatoxins permitted in foods and feeds through out the world (Friesen and Garren 1982). The Food and Drug Administration (FDA) has established more specific guideline on acceptable levels of afflatoxins in human foods and animal feeds.

Within the EC, individual member states have already adopted regulations and recommendations respecting maximum permitted levels of afflatoxins $B_1$and M_1.The intake limits for humans of about 0.15ng/kg/day for afflatoxins B_1 and 0.20ng/kg/day for afflatoxins M_1(CSHPE,1999).There are no such type of limiting value specified by Bangladesh. Usually, Bangladesh follows the recommendation of FDA. Our observed value was much higher than that of specified limits. It would be much helpful for us to consumption of imported White button mushroom but it should keep in mind that the results do not represents the whole samples. It may occurre in the particular collected sample or laid with in the specified lot.

Now, It is clear that when afflatoxin contamination does occur at levels above the legal limit it can lead to significant loss and may also be detrimental for health and health policy. From the processing and preservation point of view, we do agree with the opinion passed by Charmley that the extent of afflatoxins presence and concomitant economic loss is now becoming more generally recognized (Charmley *et al*, 1995). In the course of these experiments, it was observed

RetTime [min]	Type	Area LU *s	Amt/Area	Amount [ppb]	Grp	Name
6.052		-	-	-		Aftx G2
6.958		-	-	-		Aftx G1
8.474		-	-	-		Aftx B2
9.929	BPA	1.61403e-1	34.27958	5.53282		Aftx B1
Totals :				5.53282		

that between 7.701 to 7.953 retention times there was a peak in both of Oyster and Button mushrooms. It seemed that the indicated peak may be a characteristic of fungi.

As per Fig. 3 and Fig. 4, it was clear that there was no DDT and Heptachlor as pesticide residue are not to be found in any sample examined. Absent of DDT and Heptachlor also indicated that the components of the substrates were free from pesticide residues.

Conclusion

Afflatoxins and pesticide residues have been viewed as an unavoidable contaminants in course of practices. Hence, more attention as to purity of import commodities like mushrooms processed or preserved are to be paid and at the same time, care should be taken for using of the wastes needed as substrate for cultivating edible mushrooms. It is also to be noted that if there present such type of toxins in fresh or processed foods, then must be considered it's recommended daily intake (RDI).

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