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Seasonal variation in aflatoxins levels in edible seeds, estimation of its dietary intake and vitamin E levels from Southern Areas of Punjab, Pakistan

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Abstract: A total of 779 samples of edible nuts (melon seeds, watermelon seeds, pumpkin seeds, and cantaloupe seeds) from southern cities of Punjab, Pakistan were collected from the summer and the winter seasons. The natural occurrence of aflatoxins (AFs) and vitamin E (tocopherols) levels were investigated using HPLC. The results have shown that 180 (43.4%) samples from the winter season and 122 (33.4%) samples from the summer season were found positive with AFs. The elevated average levels of total AFs ($20.9 \pm 3.10 \mu\text{g/kg}$, dry weight) were observed in watermelon seeds without shell and the lowest average amount ($15.9 \pm 3.60 \mu\text{g/kg}$) were documented in melon seeds without shell samples from the winter season. The elevated average amount of total AFs $17.3 \pm 1.50 \mu\text{g/kg}$ were found in pumpkin seeds available as without shell. The results have documented a significant difference in total AFs levels in edible seeds available as shells versus without shells ($\alpha = 0.05 \& 0.01$). The highest dietary intake of $6.30 \mu\text{g/kg/day}$ was found in female individuals from pumpkin seeds (without shell) in the winter season and the value of $3.00 \mu\text{g/kg/day}$ were found in pumpkin seed without shell in summer season in female individuals. The highest amount of total tocopherol levels was $22.2 \pm 7.70 \text{ ng}/100 \text{ g}$ in pumpkin seeds samples from winter season and $14.5 \pm 5.50 \text{ mg}/100 \text{ g}$ in melon seeds samples from summer season. The variation of total tocopherol levels in edible seeds among the winter and summer seasons showed significant difference ($p \leq 0.0054$), except watermelon seeds samples with non-significant difference ($p \geq 0.183$).

Keywords: AFs, Edible seeds, Tocopherols levels, Dietary intake

1. Introduction

In recent years, owing to its beneficial nutritional value linked with the management of acute diseases such as cardiovascular conditions, cancer, obesity, and diabetes, the use of seeds as dietary additive has increased substantially [1,2]. The functional and nutraceutical properties are focused on a high content of important proteins, fatty acids, synthetic fibers, antioxidants, carotenoids, minerals and vitamins [2]. The seeds of the Cucurbitaceae family (containing watermelon or pumpkin) are mostly thrown out, however they are utilized for food enrichment or nutraceutical development. [3]. Herbaceous plant Chia seeds are

considered as one of the most nutritious foods due to their bioactive peptides and proteins [4]. The majority of edible seeds are usually consumed as vegetable oils [5]. The seeds of pumpkin, winter melon, and watermelon are huge, plentiful, and edible and pumpkin seeds are roasted with relish in some geographic areas [6]. Charmagaz (watermelon seeds, melon seeds, cantaloupe seeds and pumpkin seeds) is extremely popular in Pakistan and believed to help in brain development and rejuvenation. It is mostly used to make different types of drinks such as Sardie (cold drink) and used in making of sweet dishes, curries, a type of cold drink in summer and halwaa.

The edible seeds may be affected due to bad weather conditions, poor pre-harvest and post-harvest conditions. The processes of drying, storage, and transportation are critical and may contribute for fungal attack [7]. Insect and pests are the main cause of damage, but moulds are substantially identified as a major contributor of damage to agricultural goods during storage [8]. These fungi produce mycotoxins which may cause lethal and carcinogenic impacts on human and animal health [9].

Mycotoxins are recognized as naturally occurring secondary metabolites produced by filamentous fungi with various chemical structures [10,11]. The Food and Agricultural Organization (FAO) reported that globally 25 percent crops are infected with mycotoxins [12,13]. Major classes of mycotoxins with highest influence on human beings and agriculture losses are aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), and fumonisins (FB) [14,15]. However, nuts and seeds are mostly contaminated with aflatoxins [16]. The most studied class of AFs is a group of compounds formed primarily by *Aspergillus flavus* and *Aspergillus parasiticus* [17,18]. Previous studies have shown that 20 different types of AFs are known, but the most commonly known classes are AFB₁, AFB₂, AFG₁, and AFG₂ [19,20]. Health effects like teratogenic, mutagenic, and hepatocarcinogenic are caused due to these toxins mostly by attack on liver [21]. The International Agency for Research on Cancer (IARC) has classified AFs as carcinogenic to humans (Group 1). The levels of toxicity in various forms of AFs are as AFB₁ > AFG₁ > AFB₂ > AFG₂ [22]. Most important and deeply studied class of AFs is AFB₁ because it is considered as highly toxic and its widespread occurrence documented in previous studies in various staple foods and feeds. Currently, almost about 119 countries have established regulations for AFs in international trade and defined their regulatory limits [23]. The maximum acceptable limits for total aflatoxins (AFB₁ + AFB₂ + AFG₁ + AFG₂) are 15 µg / kg for hazelnuts, groundnuts, Brazilian nuts, apricot kernels, almonds, and pistachios for food practices [24]. However, no regulations are implemented for these toxins by Pakistan.

Vitamin E, commonly occurring in various food like edible oils, cereals, margarines, nuts, fatty fishes and egg yolk contains numerous essential lipophilic organic compounds which are necessary to maintain smooth functions of human body. It is also used as food additives, because of their antioxidants properties and their ability to protect against fat rancidity [25]. There were studies which have documented that antioxidants were found effective to inhibit or control aflatoxigenic fungi and the production of AFB₁ and fumonisins in stored maize [26-27].

The environmental conditions of Pakistan are favorable for fungal propagation and consequently for the production of AFs [28]. The seasonal variation may affect aflatoxins contamination during storage or selling in supermarket or open shops, the humid environment may increase moisture level and provide favorable conditions for fungal growth. In our previous studies, a considerable amount of AFs in dry fruits was documented (highest levels of total AFs 7.30 ± 1.80 µg/kg in peanuts without shell, the lowest mean of 2.90 ± 1.50 µg/kg in watermelon seeds with shell samples) [29,30] and high amount of AFs were observed in peanut and peanut products [31]. Therefore, the present research is aimed to examine; the natural presence of AFs levels in edible seeds, to compare the amounts of AFs in edible seeds with the European Union (EU) recommended limits, to determine the dietary intake of AFs in local population, and to investigate the vitamin E content in selected edible seeds. The findings of the current work will be beneficial for farmers, consumers and traders to create awareness, about the health risks related to these toxins e.g. to purchase edible seeds in proper packing and the seeds available in shells have less levels as compared to without shell samples.

2. Materials and methods

2.1 Sampling

Total 779 samples of edible seed (melon seeds, watermelon seeds, pumpkin seeds and cantaloupe seeds) samples (414 from winter season and 365 from the summer season) were collected from various cities of Southern Punjab (Multan, Bhakkar, Layyah, and Muzaffargarh) Pakistan. The seeds samples were directly purchased from market, open shops and superstore. The edible seed samples were obtained randomly. The terms of seasons i.e., the summer season is comprised of (May 2019 to August 2019) and the winter season (November 2019 to January 2020). The deshelling of edible samples was done from a market, where samples were purchased. The sample size of each seed was kept of 1 kg and the samples were ground in fine a particle size with a grinding mill (Retsch, Dusseldorf, Germany). The mill was cleaned properly after grinding each sample. After grinding, the samples were stored in plastic polyethylene bags and placed in the laboratory (Food Safety and Food Toxicology, Department of Applied Chemistry, GCUF) at -20 °C in a freezer.

2.2 Chemicals and reagents

Aflatoxins standards (in acetonitrile 1 µg/ml) and the chemicals like methanol (HPLC grade), chloroform (HPLC grade), cupric carbonate (HPLC grade), anhydrous sodium sulfate (HPLC grade) were acquired from (Sigma-Aldrich, Steinheim, Germany). The other chemicals and reagents used in current research, were of at least of high purity grade ($\geq 90\%$) and the double distilled water was used for analysis.

2.3 Aflatoxins extraction

The extraction of AFs from edible seeds was carried out following the method of Schuller and Van Egmond [32] with some modifications. The edible seeds were kept at 40 °C for dryness in a vacuum oven and then milled. Then, 40 g of dried ground edible seeds were taken and added in 200 mL of methanol/water (80:20 v/v) solution. Afterwards, 5 g of NaCl was added to this mixture and blended for 2 min. The Whatman no.1 filter paper was used to filter the mixture. After carrying the filtration, 50 mL of the filtrate was taken in a separatory funnel and 50 mL of chloroform was added shaken gently for 1 min on shaker and left to develop phases. Then 5 g of cupric carbonate was taken in a beaker and the aqueous chloroform layer was separated and filtered using anhydrous sodium sulfate. After filtration, the chloroform extract was taken in a vial and dried using nitrogen stream with controlled temperature (50 °C). The samples were derivatized by adding 100 µL of trifluoroacetic acid (TFA) to 400 µL of mixture of samples and then vortexed for 30 s on vortex (Scilogex SCI-FS, USA), and allowed the mixture to stand for 15 min. Lastly, the solution of 900 µL (water/acetonitrile) with a ratio of (9: 1 v/v) was added and vortexed for 30 s and subjected for HPLC analysis.

2.4 Extraction of oil

The extraction of oil from each edible seed was determined following the method of Gliszczynska-Swiglo et al. [33]. In a cotton thumb bell, 20 grams of ground sample was taken, and soxhlet apparatus (at 110 °C, for a duration of 24 h) was used with 250 mL of n-hexane to extract the oil of edible seed sample. Approximately (0.04 to 0.12 g) of the sample was dispersed in 1 mL of 2-propanol and vortexed for 2 min and stored in a vial for HPLC analysis.

2.5 HPLC conditions

The analysis of AFs and tocopherols were performed on HPLC instrument (Shimadzu, Model- LC-10A, Kyoto, Japan) with a C₁₈ column (250 mm x 4.6 mm, 5 µm) (Discovery, HS, Bellefonte, PA, USA) provided with a fluorescence detector (RF-530). The mobile phase for tocopherols was consisted of 50% acetonitrile (solvent A) and 50% methanol (solvent B) with a 1 mL/min flow rate and a 20 µL of injection volume. The fluorescence detector was set at 325 nm of an emission wavelength and 295 nm of excitation wavelength. The flow rate of 1.5 mL/min of isocratic mobile phase with composition of (water/methanol/acetonitrile) (60:20:20 v/v/v) was used for the analysis of AFs. The emissions and excitation wavelengths were 440 and 360, respectively. The final injection volume was 20 µL.

2.6 Dietary Intake Evaluation

According to the formula used by FAO/WHO [34], the estimated daily intake (EDI) can be calculated as,

$$\text{Estimated Daily Intake (EDI) } \mu\text{g/kg/day} = \frac{\text{Intake rate of edible seeds (g)} \times \text{AFs mean level } \mu\text{g/kg}}{\text{Average weight (kg)}}$$

A frequency questionnaire was used to estimate the consumption data of edible seeds used in rice, sweet dishes and in cold drinks to approximately 650 participants and asking them about the edible seeds utilized in the last 4 weeks. The questionnaire was completed by considering all aspects of the intake of seeds, their dietary supplements and consistency. The exact quantity of edible seeds used in food products was evaluated from the survey. The male and female participants had an average weight of 71.5 and 50 kg, respectively.

2.7 Statistical analysis

The data was analyzed statistically as triplicate replicates, and the mean levels were given as standard deviations. The seven-point standard curve was constructed, a straight-line equation was obtained, and the coefficient of determination (R²) was determined using linear regression. The significant differences in AFs levels in edible seeds from the summer and the winter seasons were determined using one-way ANOVA ($\alpha = 0.05$) using SPSS (IBM, USA).

3. Results and discussion

3.1 HPLC Method Validation

The HPLC parameters were evaluated in terms of limit of detection (LOD) and the limit of quantification (LOQ). The LOD and LOQ of AFB₁ and AFB₂ were 0.05 and 0.15 µg/kg, and 0.08 and 0.24 µg/kg, for AFG₂ and AFB₂, respectively. The LOD and LOQ were measured as signal-to - noise ratio of S/N (3), as shown in Table 1. The precision and accuracy were calculated using recovery analysis. The recovery examination was carried out by adding 4, 8 and 20 µg /kg levels of AFB₁ and AFG₁ and levels of 2, 4 and 12 µg /kg of AFB₂ and AFG₂ were added to negative samples of mixed edible seeds. The current procedure has revealed a good recovery, ranging from 75 to 110% with RSD (relative standard deviation) from 10 to 19%. A previous study [29] reported recoveries of AFs in dry fruits and edible seeds samples varied from 83-90% with RSD 8 to 19%, comparable to the results of present study. The values of LOD and LOQ for AFB₁ and AFG₁ were 0.04 and 0.12 µg/L and 0.06 and 0.18 µg/L for AFG₂ and AFB₂, respectively. The natural occurrence of individual AFB₁, AFG₁, AFG₂ and AFB₂ in pumpkin seed sample is shown in Figure 1.

Table 1. Analytical parameters for the determination of AFs and tocopherols.

Aflatoxins	Linearity µg/ml	LOD µg/kg	LOQ µg/kg	R ²	Precision (%RSD)	
					Reproducibility	Repeatability
AFB ₁	1-120	0.05	0.15	0.9981	17	15
AFB ₂	0.5-25	0.08	0.24	0.9947	14	16
AFG ₁	1-120	0.05	0.15	0.9972	12	17
AFG ₂	0.5-25	0.08	0.24	0.9972	10	16
α	0.5-60	0.04	0.12	0.9986	11	19
γ	0.1-30	0.07	0.21	0.9883	16	18
δ	0.1-30	0.07	0.21	0.9891	14	18

RSD: Relative Standard deviation; LOQ: Limit of Quantification; LOD: Limit of Detection. (α , γ , δ are the isomers of tocopherols)

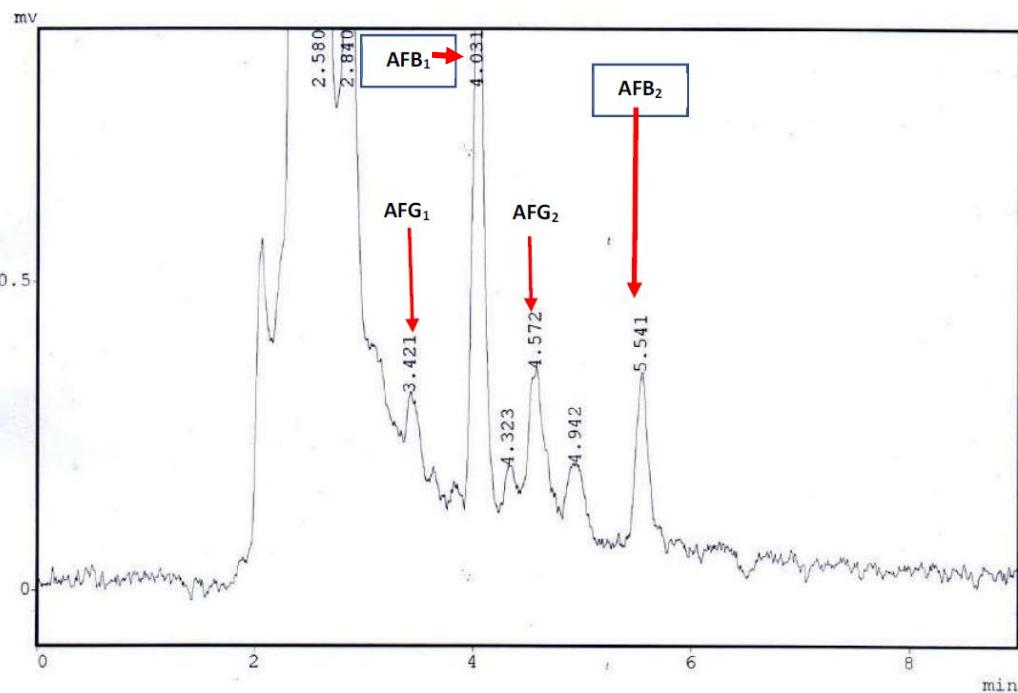


Figure 1: The natural occurrence of individual representation of AFB₁, AFG₁, AFB₂ and AFG₂ in pumpkin without shell seed sample.

3.2 Occurrence of AFs in edible seeds

The research was conducted for the examination of AFs in 414 samples of edible seeds (melon seeds, watermelon seeds, cantaloupe seeds and pumpkin seeds) from the winter season and 365 samples of edible seeds from the summer season and results are represented in Table 2. The maximum average of AFB₁ and total AFs (16.5 ± 2.45 and 20.9 ± 3.10 µg/kg) was found in without shelled watermelon seeds samples from winter season. Furthermore, the maximum average of AFB₁ (14.4 ± 1.90 µg/kg) and total AFs (17.3 ± 1.50 µg/kg) was found in without shell samples of pumpkin seed from the summer seasons. In shelled pumpkin seeds the amount of AFs was found 17.3 ± 1.50 µg/kg, was found from summer season. In shelled watermelon seeds the minimum level of AFB₁ and total AFs were 6.70 ± 1.90 and 8.4 ± 1.90 µg/kg respectively, from summer season. The findings showed that out of a total of 365 summer season experiments, 122 (33.4 percent) were aflatoxin infected and 180 (43.5 percent) were aflatoxin poisoned out of 414 winter season experiments. The results have shown that 27.2% samples of edible seeds having the levels of AFB₁ higher than EU permissible limit (≥ 5 µg/kg), and 12.2% samples having total AFs levels

greater than $\geq 20 \mu\text{g/kg}$ from the summer season as represented in Figure 2. Furthermore, 32.8% samples found levels of AFB₁ greater than ($\geq 5 \mu\text{g/kg}$), and 11.5% samples have total AFs amount higher than 20 $\mu\text{g/kg}$, in the winter season as shown in Figure 3.

Table 2: Occurrence of AFB₁ and total AFs (μg/kg) in edible seeds from summer and winter season from Punjab, Pakistan.

Seasons	Type	Melon Seeds		Watermelon Seeds		Pumpkin Seeds		Cantaloupe Seeds		Total
		Shelled	Without shell	Shelled	Without shell	Shelled	Without shell	Shelled	Without shell	
Winter Season	Total sample (n)	60	65	42	32	75	60	40	40	414
	Positive Sample n (%)	23 (38.33)	20 (30.76)	18 (42.85)	20 (62.5)	23 (30.66)	28 (46.66)	23 (57.5)	25 (62.5)	180 (43.4)
	AFB ₁ (μg/kg) ± SD	10.5±2.10	12.6±2.50	8.90 ±2.80	16.5±2.45	12.9±2.60	12.6±3.40	7.9±2.60	11.8±3.20	
	Total AFs (μg/kg) ± SD	13.5±3.40*	15.9±3.60*	11.1±2.10**	20.9±3.10**	13.5±2.90**	18.5±2.90**	13.5±3.10*	16.5±3.50*	
	Range (μg/kg)	0.05 – 25.40	0.05 – 35.5	0.05 – 20.5	0.05 – 35.5	0.05 – 28.5	0.05- 38.9	0.05- 50.5		
Summer Season	Total sample (n)	50	60	40	40	60	50	30	35	365
	Positive Sample (%)	18 (36)	15 (25)	12 (30)	15 (37.5)	19 (31.7)	15 (30)	10 (33.3)	18 (51.4)	122 (33.4)
	AFB ₁ (μg/kg) ± SD	8.20±2.50	10.9±2.50	6.70 ±1.90	10.1±1.90	9.8±2.50	14.4±1.90	6.3±1.90	10.2±2.80	
	Total AFs (μg/kg) ± SD	11.80±2.10*	13.2±2.80*	8.40 ±1.95*	11.6±1.80*	15.0±2.50*	17.3±1.50*	10.5±1.95*	13.9±2.10*	
	Range (μg/kg)	0.05 – 25.5	0.05 -39.8	0.05 -23.6	0.05 – 45.6	0.05 -29.8	0.05 – 39.7	0.05-20.5	0.05 – 33.2	

*= The significant difference of variation in total AFs levels in edible seeds available shelled versus without shell ($\alpha = 0.05$). **= The significant difference of variation in total AFs levels in edible seeds available shelled versus without shell ($\alpha = 0.01$).

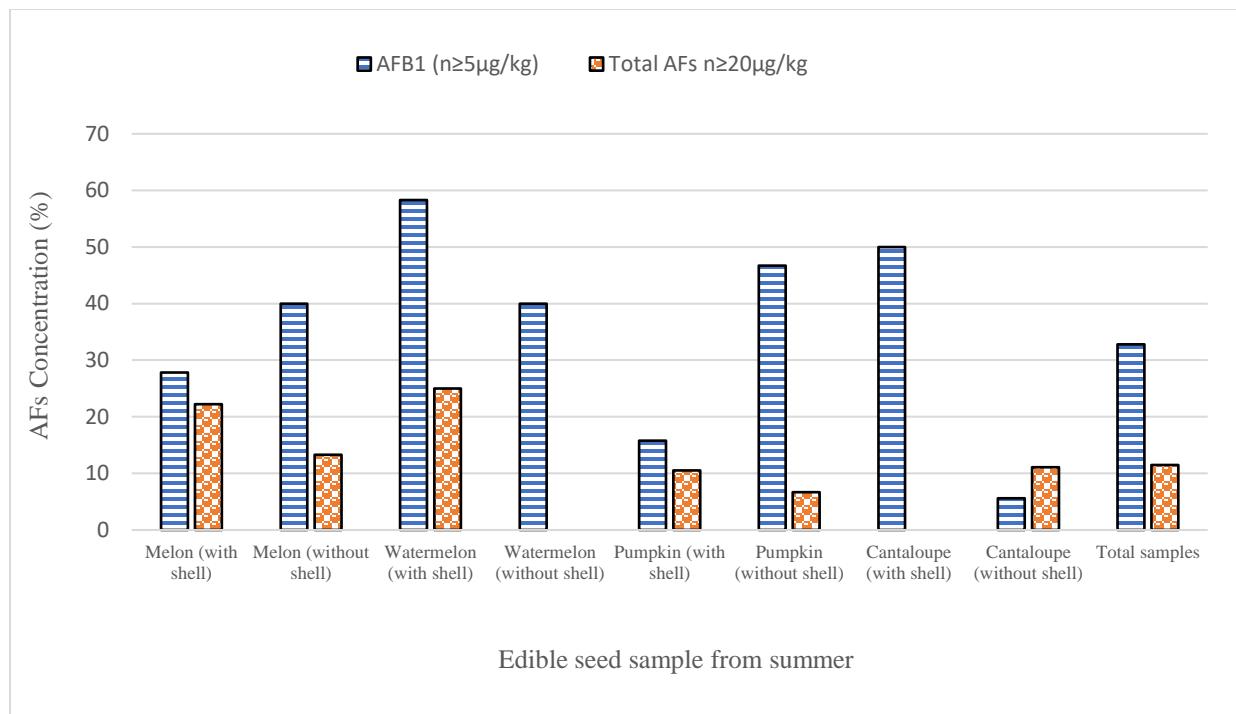


Figure 2. The number of AFB₁ and total AFs ($\mu\text{g}/\text{kg}$) samples higher than the recommended EU limits from summer season.

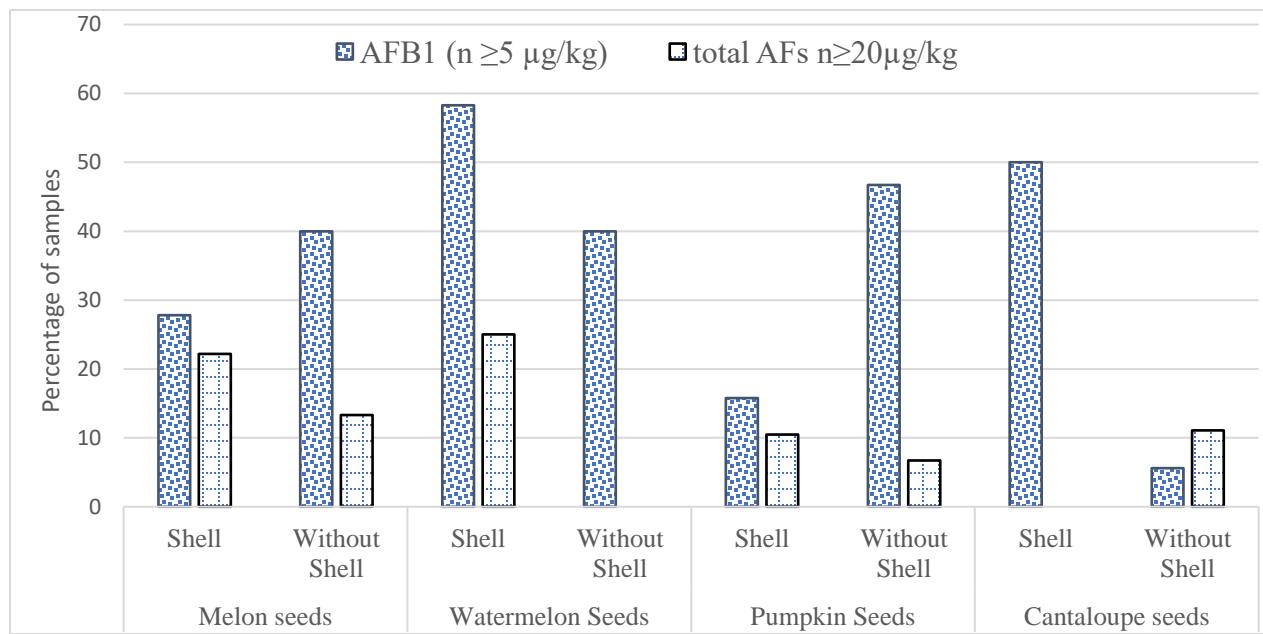


Figure 3. The number of AFB₁ and total AFs ($\mu\text{g}/\text{kg}$) samples higher than the recommended EU limits from winter season.

In a previous study, Iqbal et al. [29] have examined 320 samples of edible nuts (peanut, poppy seed, pistachio, almonds, cashew) and dry fruits (figs, plum, raisins, apricot, dates, watermelon seed, pomegranate seeds and melon seeds) and observed that 128 (40%) samples were contaminated with AFB₁,

and total AFs, the incidence was comparable to the results of current findings. The samples have levels of total AFs greater than 4 $\mu\text{g}/\text{kg}$ and 10 $\mu\text{g}/\text{kg}$ in 34 and 25% of samples and the elevated average amount of total AFs ($7.30 \pm 1.80 \mu\text{g}/\text{kg}$), was comparatively low to the results of present study. In another study, Masood et al. [30], from Pakistan have investigated 307 samples of dried fruits and edible nuts and observed that 132 (43%) samples were found to be contaminated with AFB₁, and total AFs. The elevated average amount of total AFs i.e. $7.89 \pm 0.99 \mu\text{g}/\text{kg}$ in peanuts without shell samples and the lowest average amount ($2.45 \pm 0.11 \mu\text{g}/\text{kg}$) was observed in watermelon without shell samples. Huang et al. [35], from China have observed a very high percentage of samples found to be contaminated with AFs i.e. 93.9% of peanut butter and documented an average amount ranging from 0.3 $\mu\text{g}/\text{kg}$ to 95.9 $\mu\text{g}/\text{kg}$, much higher range than the results of present findings. Iqbal et al. [31] have analyzed 198 samples of peanut and peanut products, from Pakistan and found that 61% of roasted peanut (shell samples), 68% of roasted peanut (without shell), 59%, of raw peanut (with shell), 55% of raw peanut (without shell), 50% of peanut butter, 42% of peanut cookies and 20% of peanut nemko were observed to be contaminated with AFs. The average concentration in raw peanut shell samples was 6.4 $\mu\text{g}/\text{kg}$, in roasted peanut with and without shell (10.4 and 12.3 $\mu\text{g}/\text{kg}$), in raw peanut without shell 9.6 $\mu\text{g}/\text{kg}$, peanut butter 2.4 $\mu\text{g}/\text{kg}$, peanut nimko (3.4 $\mu\text{g}/\text{kg}$), and in peanut cookies 4.6 $\mu\text{g}/\text{kg}$, much lower than the findings of present study. A low percentage of AFs contamination and average amount from Turkey have documented by Kabak et al. [36]. The results demonstrated that 300 samples of both dried figs, and hazelnuts have found 6 (12%) samples of hazelnut kernel (ranging from 0.09 to 11.3 $\mu\text{g}/\text{kg}$) and 5 (8.3%) samples of roasted hazelnut kernel with average 0.17 to 11.2 $\mu\text{g}/\text{kg}$ were found contaminated by AFs. Bankole et al. [37] from Nigeria have analyzed 137 melon seed samples and documented mean level of AFB₁ (14.8 $\mu\text{g}/\text{kg}$) from forest melon seed and 11.3 $\mu\text{g}/\text{kg}$ in savanna melon seeds. In another study, Juan et al. [38] from Morocco have analyzed 100 sample of dried fruits and nuts and documented very high mean level of AFB₁ (2500 $\mu\text{g}/\text{kg}$) from walnut samples and found average level of (1430 $\mu\text{g}/\text{kg}$) from pistachio samples, very high amount compared to the results of present findings. In summer season during the cultivation the environmental conditions, especially the climatical variations i.e. rainfall, broadly affects the growth of fungi in food products. Therefore, the weather and climatic conditions are considerably important for AFs production [39]. The fungal attack and levels of AFs in nuts and dried fruit may also vary during seasons. The months of July, August, and September are rainy seasons with a high rain fall levels and thus higher moisture and humidity levels [40-41]. The previous findings have shown that suitable temperature for the growth of various fungal species particularly of *Aspergillus* species ranging between 10.0 to 48.8 °C with 33.8 °C as an optimum temperature [38]. However, there was significant difference of AFs levels in edible seeds from shell and without shell samples ($p < 0.05$).

3.3 Dietary intake estimation

The estimation of dietary intake in edible seeds from the summer and the winter season in male and female individuals are represented in Table 3. The results have shown that maximum dietary intake of AFs was assessed to be 4.38 $\mu\text{g}/\text{day}/\text{kg}$ of the body mass (BW) in males without shell of pumpkin seeds from the winter season and the maximum amount of 6.3 $\mu\text{g}/\text{day}/\text{kg}$ of the BW was found in female individuals in the winter season in pumpkin seeds. The minimum dietary intake was documented in without shell melon seeds, i.e., 1.04 $\mu\text{g}/\text{day}/\text{kg}$ BW in the winter period in males and from the summer season 0.59 $\mu\text{g}/\text{day}/\text{kg}$ BW in the males. In earlier findings, Heshmati et al. [42] have confirmed that the dietary intake in dates, dried mulberries, figs, and apricots was 0.12, 0.04, 0.04 and 0.06 ng/kg/bw/day, respectively. Williams et al. [43] have documented lower levels of dietary exposure compared the findings of current results i.e., levels of AFs was 11.4 to 158.6 ng/kg/day in Swaziland, 3.5 to 14.8 ng/kg/day in Kenya, 38.6 to 183.7 ng/kg/day in Mozambique, 16.5 ng/kg/day in Transkei (South Africa), 4 to 115 ng/kg/day in Gambia, 11.7 to 2027 ng/kg/day from China, 6.5 to 53 ng/kg/day from Thailand, 2.7 ng/kg/day in USA. Sugita-Konishi et al. [44], from Japan have stated that the exposure for AFB₁ was ranging from 0.908 ng/kg BW/day

to 0.909 ng/kg BW/day in one to six years children and 0.288 ng/kg BW/day to 0.289 ng/kg BW/day in adults of age more than 20 years, much lower than the findings of current results.

Table 3. Estimation of dietary intake for AFs in edible seeds in local population from Punjab, Pakistan.

Seasons	Type	Melon seeds		Watermelon seeds		Pumpkin seeds		Cantaloupe seeds	
		Shelled	Without shell	Shelled	Without shell	Shelled	Without shell	Shelled	Without shell
	Consumption g/day	20	5	10	20	15	10	15	10
Winter	AFs mean level ($\mu\text{g}/\text{kg}$)	13.5	14.9	11.1	13.5	20.9	18.5	13.5	14.5
	Dietary Intake (male) $\mu\text{g}/\text{kg}/\text{day}$	3.78	1.04	1.55	3.78	2.59	4.38	2.83	2.03
	Dietary Intake (female) $\mu\text{g}/\text{kg}/\text{day}$	5.4	1.50	2.20	5.40	3.70	6.30	4.10	2.90
Summer	AFs mean level $\mu\text{g}/\text{kg}$	11.8	12.20	8.40	11.60	17.30	15	10.50	11.90
	Dietary Intake (male) $\mu\text{g}/\text{kg}/\text{day}$	1.65	0.85	0.59	1.62	1.21	2.10	1.47	0.83
	Dietary Intake (female) $\mu\text{g}/\text{kg}/\text{day}$	2.40	1.20	0.80	2.30	1.70	3.00	2.10	1.20

Average Weight female=55, Average age=27.4. Average Weight male=71.5, Average age=29.8.

3.4 The vitamin E levels in edible seeds

The variation of vitamin E levels in edible seeds from the winter and the summer season is represented in Table 4. The maximum average amount of total tocopherol content was found in melon seeds i.e. 19.5 ± 4.90 mg/100g and the lowest total tocopherol content was observed in water-melon seeds 3.85 ± 3.60 mg/100g from the winter season. However, the highest level of total tocopherol 14.5 ± 5.50 mg/100g was found in melon seeds and lowest mean level of 2.96 ± 5.60 mg/100g was found in watermelon seeds from summer season. The findings have revealed the significant difference of variation in levels of vitamin E from winter and summer seasons ($\alpha = 0.05$), except watermelon seeds, which shown nonsignificant difference ($\alpha = 0.05$). The individual and total levels of vitamin E content in winter and summer seasons is represented in Figure 4. The study has observed a negative correlation (Pearson correlation -0.370, and shown significant difference between vitamin E and total AFs levels at $\alpha = 0.01$) between AFs amount in different edible seeds and vitamin E content from winter and summer seasons. In our previous study Iqbal [19], have shown significant difference ($p < 0.05$) of vitamin E contents in different rice varieties and a negative correlation ($r = -0.62$) was found between AFs concentration and vitamin E levels, in agreement with present findings. Previous studies have observed that the antioxidant properties of selenium and retinol, ascorbic acid and tocopherol not only safeguard the membrane from the harmful effect of mycotoxins but induce or boost the liver function to detoxify mycotoxins levels [45]. Furthermore, the extracts from certain medicinal herbs and plant could possibly defend them from ochratoxin A, aflatoxin B1, and fumonisin B1 [46-48]. More comprehensive work to establish a relationship between AFs in edible seeds and variation in vitamin E content would be recommended.

Table 4. Detection of vitamin E content in edible seeds from Punjab, Pakistan.

Seasons	Seeds Type	Samples	α tocopherol (mg/100g)	γ tocopherol (mg/100g)	δ tocopherol (mg/100g)	Total tocopherol content (mg/100g)
Winter Season	Melon seeds	125	11.5 ± 2.70	4.5 ± 2.30	3.5 ± 1.90	$19.5 \pm 4.90^*$
	Watermelon seeds	74	0.93 ± 1.40	0.01 ± 4.15	2.91 ± 1.05	3.85 ± 3.60 NS
	Pumpkin seeds	135	12.1 ± 2.80	2.0 ± 2.50	8.1 ± 2.10	$22.2 \pm 7.70^*$
	Cantaloupe seeds	80	8.07 ± 1.30	4.03 ± 1.15	3.02 ± 1.10	$15.12 \pm 4.65^*$
Summer Season	Melon seeds	110	9.5 ± 3.75	2.5 ± 2.80	2.5 ± 2.94	$14.5 \pm 5.50^*$

	Watermelon seeds	80	1.04±2.45	0.01±1.15	1.91±1.45	2.96 ± 5.60 ^{NS}
	Pumpkin seeds	110	8.1±3.85	1.50±2.10	4.15±3.15	13.75 ± 6.50 [*]
	Cantaloupe seeds	65	4.05±3.35	2.04±3.10	2.02±3.15	8.11 ± 3.40 [*]

*= The variation in total levels of tocopherols shows significant difference in winter compared to summers seasons samples ($\alpha = 0.05$). ^{NS}= The variation in total levels of tocopherols shows non-significant difference in winter compared to summers seasons samples ($\alpha = 0.05$)

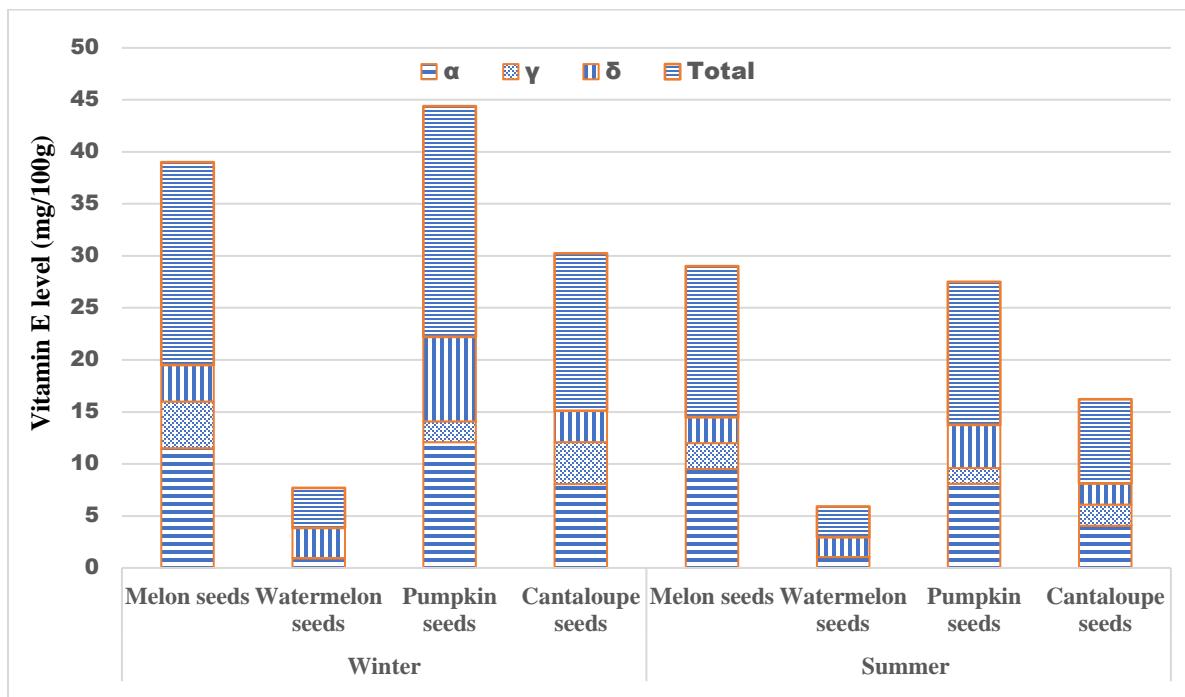


Figure 4: The variation of individual and total tocopherol in edible seed samples from winter and summer seasons.

4. Conclusions

The average amount of total AFs in edible seeds from winter and summer seasons are considerably high as compared to our previous studies. Furthermore, no significant difference of AFs levels was found in winter versus the summer season, however, there exists a significant difference of AFs variation in edible seeds samples taken as shelled versus without shelled samples. The highest dietary intake of 6.30 $\mu\text{g}/\text{kg}/\text{day}$ was calculated in female individuals from pumpkin seeds samples from the winter season. The highest levels of vitamin E $22.2 \pm 7.70 \text{ mg}/100 \text{ g}$ was found in pumpkin seeds from winter samples. There exists a negative correlation between AFs levels and vitamin E contents in edible seeds. The results of present research are informative for farmers, traders, and consumers regarding the health consequences correlated from these toxins. Unfortunately we have purchased these edible seeds available in market as shells or without shells, however, in future we would be more interested to collect samples during field with fruits and then study the effect of pre-harvest fungi or aflatoxins.

Acknowledgements: The authors recognize the financial assistance of Higher Education Commission, Islamabad, IPFP. The analytical facilities offered by NIAB, Faisalabad are highly valued.

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