

Occurrence of aflatoxins in edible vegetable seeds and oil samples available in retail markets and estimation of dietary intake in consumers

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Abstract: Aflatoxins (AFs) are secondary metabolites and toxic to humans as well as animals. The environmental conditions, conventional agricultural practices, and illiteracy are the main factors which favored the production of AFs in food and feed. In current research 744 samples of vegetable seeds and oils (soybean, sunflower, canola, olive, corn, and mustard) were collected for the presence of aflatoxin B₁ (AFB₁) and total AFs. Liquid-liquid extraction was employed for the extraction of AFs from seeds and oil samples. Reverse phase high performance liquid chromatography equipped with fluorescence detector was used. The results have shown that 92 (56.7%) samples of imported and 108 (57%) samples of local edible seeds were observed to be contaminated with AFs. All samples of edible seeds have AFB₁ levels greater than the proposed limit of European Union (EU, 2 µg/kg) and 12 (7.40%) samples of imported seeds and 14 (7.40%) samples of local seeds were found in the range \geq 50 µg/kg. About 78 (43.3%) samples of imported edible oil and 103 (48.3%) sample of local edible oil were observed to be positive with AFs. Furthermore, 16 (8.88%) and 6 (3.33%) samples of imported vegetable oil have levels of total AFs in a range (21 - 50 µg/kg) and greater than 50 µg/kg, respectively. The findings have indicated significant difference of AFs levels between imported and local vegetable oil samples ($t = 22.274$ and $p = 0.000$) at $\alpha = 0.05$ and a significant difference of AFs levels were found between vegetable seeds and oil samples ($t = -17.75$, $p = 0.000$) at $\alpha = 0.05$. The highest dietary intake was found in local sunflower oil sample (0.90 µg/kg/day) in female individuals (16-22 age group). The results have shown considerably high levels of AFB₁ and total AFs in seeds and oil samples and emphasis the need to observe the levels of these toxic substances in food and feed on regular basis.

Keywords: AFB₁; AFs; vegetable seeds; vegetable oils; dietary intake.

Highlight for review.

Total 743 samples of vegetable seeds & oil were investigated for AFB₁ and total AFs

About 56.7% of imported & 57% samples of local edible seeds were found positive

The maximum averages of total AFs in local samples of soyabean seeds was 36.37 ± 6.10 µg/kg

About 43.3% samples of imported & 48.3% of local samples of edible oil were found positive

The maximum mean of total AFs 25.61 ± 7.50 µg/kg was found in local samples of soybean oil

The highest dietary intake (0.90 µg/kg/day) was found in local samples of sunflower oil samples



1. Introduction

In the current century, edible vegetable oils are preferred during frying of food or used in food handling industries over animal oils due to health issues. Important human nutrition's such as energy, vitamins (vitamins A, D and E), fat soluble and essential fatty acids are supplied by oils [1]. Consumers used edible oil in daily life because it has functions in preventing arteriosclerosis and reducing blood lipid [2-3]. Increasing awareness and having more health benefits of using vegetable oils have boosted its demand and consumption worldwide, especially in developed countries [4]. Worldwide, the highly popular oils are maize oil, olive oil, peanut oil and sunflower, and the consumption rate of these oils is rising. Food and Agriculture Organization [5] has estimated the total utilization of oil and fats worldwide in 2020/21 was 244.8 million tons. China is the leading producer of oilseeds with 2.63 million tons, followed by Pakistan and Malaysia, with 55.8 thousand tones and 20.2 thousand tons, respectively [6]. According to a report, from 2001 through 2011, the projected mean global per capita consumption of vegetable oils was 10.71 kg, which is 1.24 kg higher than vegetable oil consumption from the previous decade [7]. The per capita edible oil consumption of Pakistan is about 17 kg, and in 2017 the total consumption of oil and fats was about 4.41 million tons. Pakistan has imported about 2.7 million tons of edible oil in 2015, which rises to 3.05 million tons in 2017 [8]. The increasing trend in the utilization of vegetable oil has attracted the attention of regulatory authorities and agencies to have a check on its safety and quality [9]. The processing of oil such as during production, packaging, transportation and storage could cause contamination, furthermore with innovation in industrial processing, traditional agricultural practices, environmental pollution and climatic conditions may cause new toxic residues in edible oils [10].

The contamination of food and food products with AFs is a global food safety concern [4]. AFs are recognized as dangerous and toxic natural compounds. The subclass of AFs, e.g., AFB₁, is recognized as the most toxic and carcinogenic [11-13]. The fungi like *Aspergillus flavus*, *Aspergillus nomius*, and *Aspergillus parasiticus* are the primary producer of aflatoxins [14]. The fungi can contaminate various food and food products, e.g., vegetables, fruits, cereals, spices, and cattle feed [15-16]. Considering its toxicity, AFB₁ has been categorized as group 1 carcinogen by the International Agency for Research on Cancer [17], and it mainly affects the liver [18]. Geographically, Pakistan is placed in the list of tropical countries of the world, and therefore the climatic conditions may be encouraging for fungal production [19]. In tropical countries, high temperature and humidity levels are conducive for the growth of aflatoxigenic fungi. These toxic compounds easily transferred from seeds to edible oils [20]. Furthermore, during the pre-harvest and post-harvest stages, primarily due to inadequate storage conditions, are mainly responsible for contaminating food products due to fungi [21-22]. In tropical countries, factors like high humidity and high-temperature conditions are considered vital for the growth of aflatoxigenic fungi [23-24]. The high levels of AFs in food or vegetables could be transferred to the final edible oil foodstuffs [20]. There were numerous surveys worldwide that account for the contamination of AFs in vegetable oils [4, 20, 25-28]. In Pakistan, no previous reports have conducted for the occurrence of AFs in edible oils. However, high incidence of AFs was present in feed samples (cereals products) [29] and recently in animal feed [30]. In previous study, high incidence of AFs i.e., 180 (43.4%) of samples of edible seeds from the winter season and 122 (33.4%) samples from the summer season were found positive [31].

Therefore, the study has designed to investigate the levels of AFB₁ and total AFs in edible vegetable oils, to compare the levels with EU recommended limits, and to estimate the dietary intake evaluation in local population. The findings of the current research will help to understand the toxicity of AFs in vegetable oils, to generate data about the incidence, and help to implement strict regulations in Pakistan.

2. Materials and methods

2.1. Sample collection

Three fifty-one (351) samples of edible seeds and 393 samples of edible oilseeds (sunflower, soybean, canola, olive, corn & mustard) were gathered from markets, superstores, and farmers from the central cities of Punjab, Pakistan during May 2019 to August 2019. The imported samples were named those imported from other countries, and local were produced locally. These imported sunflower seeds samples were collected from 5 different brands, 7 different brands for soybean, 8 different brands of canola, olive and corn samples and 4 brands for mustard samples main cities and subsequently the oil was extracted (soxhlet apparatus) from these samples and labeled accordingly (Lahore, Faisalabad, Gojira and Islamabad) of Punjab Pakistan as shown in Fig. 1. The sample size was maintained from 1 kg to 5kg, each. A simple random methodology (each portion or lot has equal chance to be included) was used for collecting edible seed samples from farmers, market, and superstores. The gross samples were taken by hands and then homogenized and proper labelling was done. The size of each brand must ensure to be greater than $n = 20$, to be assumed to represent normal distribution. All samples were stored in polythene zip bags or airtight plastic bottles. All the samples of seeds and oil were stored at temperature (25–30 °C) in dark.

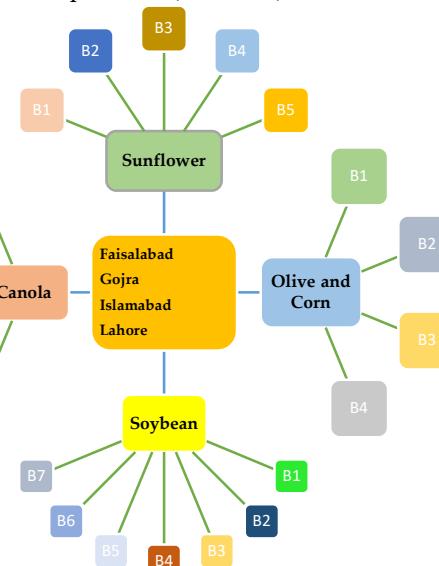


Fig 1: Edible seeds and oils samples distribution with respect to different brands

2.2. Chemicals and reagents

The standards of AFB₁, AFG₁ (2 µg/mL in acetonitrile) and AFB₂ and AFG₂ (0.5 µg/mL in acetonitrile), methanol, acetonitrile (HPLC grade), hexane, sodium chloride, chloroform, anhydrous sodium sulfate, dichloromethane, HCl and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich, Steinheim, Germany. Furthermore, double-distilled water (Millipore, Bedford, MA, USA) was used throughout the analysis.

2.3. Extraction of aflatoxins from seed samples

The extraction of AFs in edible seed samples was performed following the methods [32-33]. The sample 25 g was mixed in 125 mL of methanol: water (55:45 v/v), 100 mL hexane, and 2 g of NaCl and homogenized using an orbital shaker for 15 min. Then Whatman No.1 filter paper was used to filter the solution. After filtration, the filtrate was left for 30 min to form two phases. The lower 25 mL of aqueous methanol phase was transferred into separating funnel, and 10 mL of chloroform was added process is repeated 3 times in separating funnel. Two layers formed, and the layer having chloroform was drained in a 250 mL beaker using anhydrous sodium sulfate. Then, the final solution was evaporated over a water bath near dryness.

2.4. Extraction of aflatoxins from oil samples

The method for AFs extraction in edible oil samples was carried out, as explained by AOAC Official method 2013.05 [34]. With some modifications, 50 mL of oil sample was mixed in 250 ml of methanol-water (55-45 v/v) with the addition of 50 mL of 0.1N HCl and centrifuged at a speed of 4500 RPM. The mixture was filtered with Whitman filter paper, and 50 ml of the filtrate was subjected to the separator and mixed with 50 mL of 10% NaCl and 50 mL of hexane and shaken vigorously for 30 s. The aqueous lower layer was drained into another separator, and 3-25 mL of dichloromethane was added and shake vigorously and allow it to stand for 5 min. Then the dichloromethane layer was collected and evaporated on a water bath to dryness.

The derivatization of both seed and oil samples were carried out using 100 μ L of TFA in dried oil or seed samples and vortex for 30 s. Then the sample was left for 5 min in a dark place. Finally, 400 μ L mixture of acetonitrile-water (1:9 v/v) was added to the vials, and 20 μ L solution was subjected for HPLC analysis.

2.5. HPLC conditions

The research was conducted to investigate the incidence of AFs on HPLC instrument (Shimadzu, Model- LC-10A, Kyoto, Japan) with a C₁₈ column (250 mm x 4.6 mm, 5 μ m) (Discovery, HS, Bellefonte, PA, USA) equipped with a fluorescence detector (Model RF-530). The polar isocratic reverse mobile phase consisted of the composition of acetonitrile; water; acetic acid (50; 40; 10 v/v/v) at a flow rate of 1 mL/min. The emission wavelength (440 nm) and excitation wavelengths (365 nm) of the fluorescence detector were set before the analysis.

2.6. Dietary intake estimations

The dietary intake analysis was performed following the method depicted by Iqbal et al. [35], the estimated daily intake (EDI) is calculated as,

$$\text{Dietary intake } (\mu\text{g/kg/day}) = \frac{\text{Consumption of oil (mL)} \times \text{Mean levels of total AFs } (\mu\text{g/kg})}{\text{Average weight (kg) of individuals}}$$

The intake data was obtained by giving a food frequency questionnaire (available in supplementary material) to 645 participants, and 509 have responded and returned their information about utilizing oil in different cooking food items. The questionnaire was filled by viewing every aspect of oil consumption, dietary supplements, and utilization. The bodyweight of the participants was 61 \pm 11 kg. The written consent from each participant was taken and assured them their information was not made public. The all-ethical guidelines have been adopted during questionnaire filling. The participants belong to consumers and their dieting habits, and seasoning effect was not considered, which might affect the results.

2.7. Statistical analysis

The findings of the current research were analyzed statistically and presented as mean \pm standard deviations. The calibration curves of seven-points were constructed for each AFs using simple linear regression/correlation analysis, and coefficient of determination and straight-line equations were calculated. The significant difference among the levels of AFs in imported and local samples and edible seeds and oil samples were verified applying paired t-test ($\alpha = 0.05$) SPSS (IBM, USA). The method was evaluated in terms of linearity, reproducibility, repeatability, recovery analysis, detection limits (LOD), and the limit of quantification (LOQ). Three known concentrations of AFB1 and aflatoxin G1 (AFG1) (1, 6, and 10 μ g/kg) and aflatoxin B2 (AFB2), and AFG2 (1, 4, and 8 μ g/kg) were added in a mixture of samples of edible oils (all five oils samples with equal volume), which have detection levels of all four AFs $<$ LOD.

3. Results and discussion

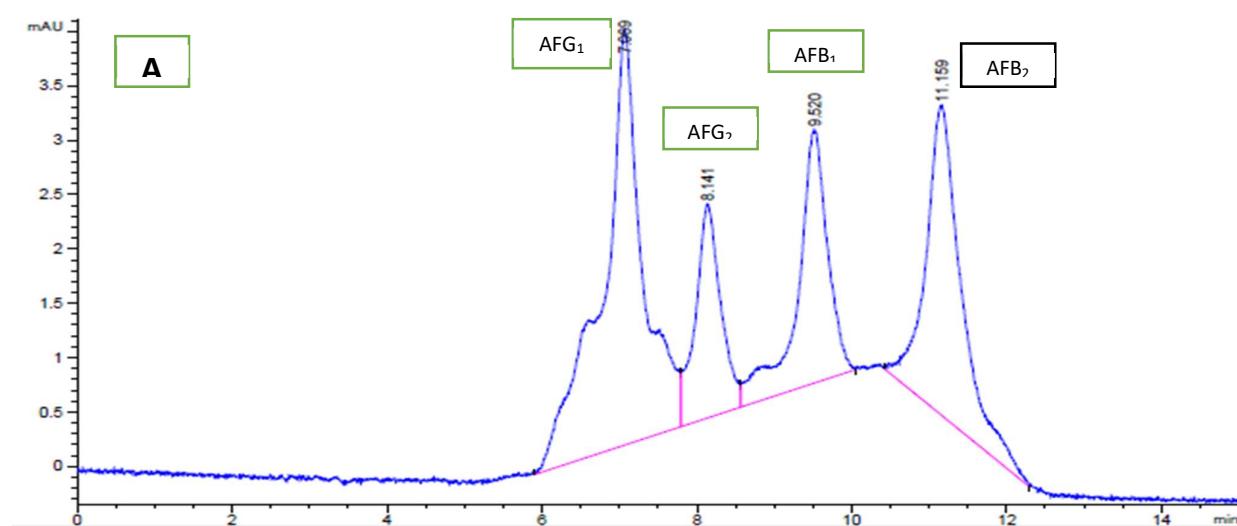
3.1. Quality control parameters

The mean recovery values (triplicate each) ranged from 74.5 to 96.5%, with the relative standard deviation (RSD) varied from 9 to 21.5%, as shown in Table 1. The calibration curve (seven points) was constructed to confirm the linearity of each AFs i.e., for AFB_1 and AFG_1 (1, 10, 20, 60, 80, 100, 140 $\mu\text{g}/\text{kg}$) and AFB_2 and AFG_2 (1, 4, 8, 16, 20, 25 and 30 $\mu\text{g}/\text{kg}$). The curves were linear, with a coefficient of determination (R^2) ≥ 0.99 . The detection limits (LOD) of AFB_1 and AFG_1 were 0.08 $\mu\text{g}/\text{kg}$, and LOQ was 0.24 $\mu\text{g}/\text{kg}$. However, the LOD and LOQ for AFG_2 and AFB_2 were 0.07 and 0.21 $\mu\text{g}/\text{kg}$, respectively. The repeatability and reproducibility have also been calculated. In the previous study, the linearity range for AFB_1 and AFG_1 was 1 to 80 $\mu\text{g}/\text{kg}$ and 0.5 to 12 $\mu\text{g}/\text{L}$ for AFB_2 and AFG_2 , in agreement to current study. The LOD and LOQ were 0.04 and 0.12 $\mu\text{g}/\text{kg}$ for AFB_1 and AFG_1 and 0.6 and 0.18 $\mu\text{g}/\text{kg}$ for AFG_2 and AFB_2 , respectively [36]. The peaks of individual AFs are separated quite efficiently. The standard chromatogram showing the retention time of four individual AFs (Fig 2a); The natural incidence of four AFs in sunflower sample (Fig 2b); and the presence of individual AFs in mustard seed sample (Fig 2c) are represented.

Table 1. Validation parameters of aflatoxins in edible seeds and oil samples.

Mycotoxins	Fortified level ($\mu\text{g}/\text{kg}$)	Recovery %	RSD %	Retention time (min)	R^2	LOD ($\mu\text{g}/\text{kg}$)	LOQ ($\mu\text{g}/\text{kg}$)	Precision	
								Repeatability (n = 5) RSD%	Reproducibility (n = 5) RSD%
AFG_1	1	84.5	9.0	7.074 ± 0.017	0.9910	0.08	0.24	14	11
	6	91.4	13.5						
	10	90.5	14.0						
AFB_1	1	82.5	12.0	9.530 ± 0.026	0.9920	0.08	0.24	18	12
	6	94.5	10.0						
	10	96.5	21.5						
AFG_2	1	74.5	18.5	8.150 ± 0.021	0.9980	0.07	0.21	19	10
	4	89.4	11.5						
	8	91.5	16.5						
AFB_2	1	78.5	12.5	11.175 ± 0.037	0.9985	0.07	0.21	13	14
	4	94.6	11.1						
	8	90.5	14.0						

RSD = relative standard deviation, LOD = limit of detection , LOQ = limit of quantification; R^2 = coefficient of determination; Repeatability and reproducibility are given as mean percent RSD (%).



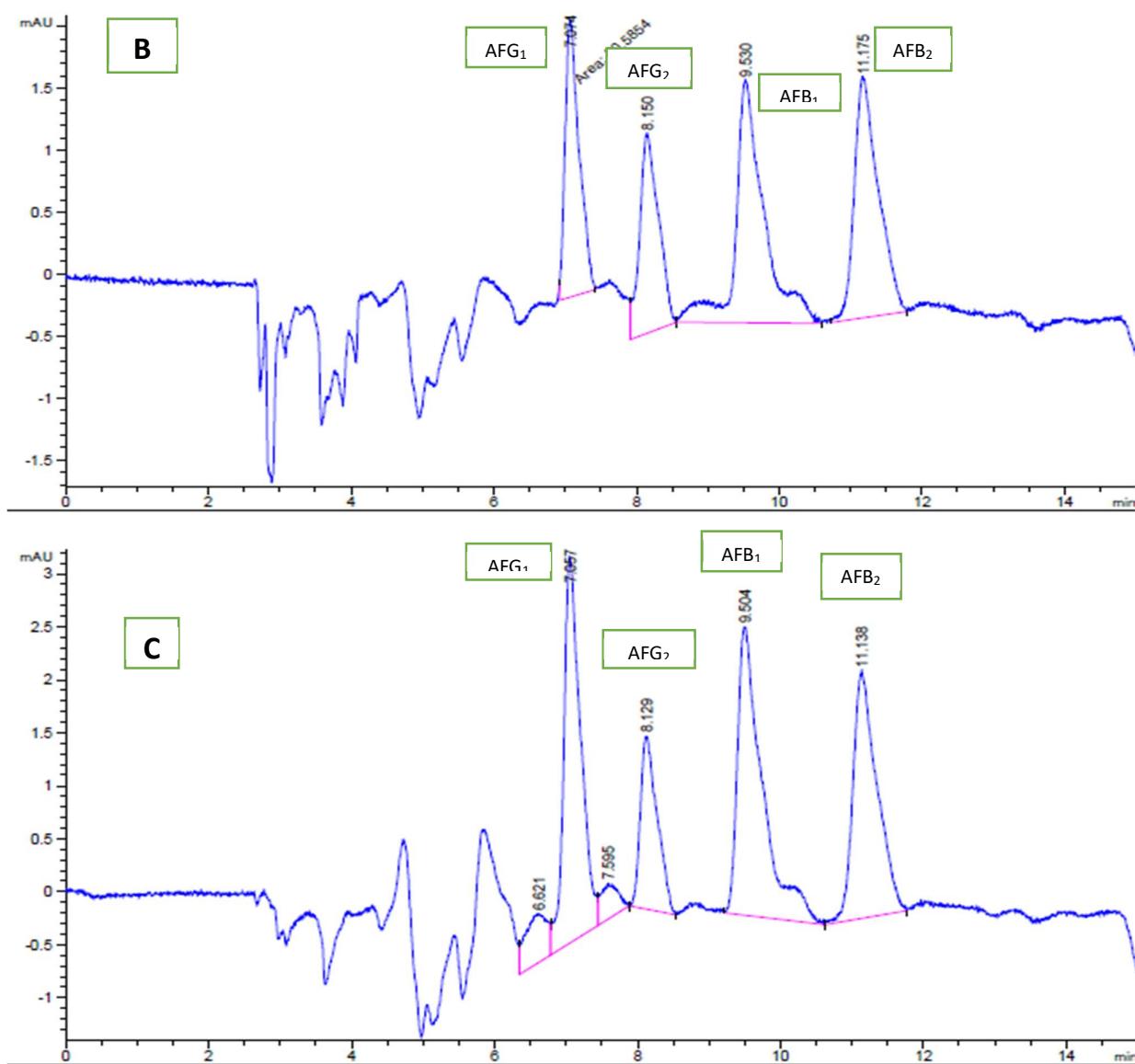


Figure 2. The presence of standard of all flour AFs (A); the natural occurrence of AFs in sunflower sample (B); The presence of AFs in mustard seed sample (C).

3.2. Occurrence of AFs inedible seeds and oil samples

The outcomes have exposed that 92 (56.7%) samples of imported and 108 (57%) samples of local edible seeds were observed to be contaminated with AFs. The maximum average amount of AFB₁ and total AFs in local soybean seeds were 21.01 ± 4.70 and $36.37 \pm 6.10 \mu\text{g/kg}$, respectively. The amount of 13.29 ± 3.50 and $20.42 \pm 5.20 \mu\text{g/kg}$ was found for AFB₁ and total AFs in imported sunflower seed samples, respectively, as shown in Table 2. Furthermore, all edible seeds samples have levels of AFB₁ greater than the proposed limit of the EU (i.e., $2 \mu\text{g/kg}$). On the other hand, 12 (7.40%) samples of imported and 14 (7.40%) samples of local seeds were found in the range $\geq 50 \mu\text{g/kg}$, as shown in Figure 2 (a). About 393 samples of edible oil, of which 180 samples were imported, and 213 samples were local, were investigated for the incidence of AFB₁ and total AFs, as shown in Table 3. The 78 (43.3%) samples of imported and 103 (48.3%) samples of local edible oils were positive with AFs. The maximum average amount of AFB₁ ($14.29 \pm 2.51 \mu\text{g/kg}$) and total AFs ($25.61 \pm 7.50 \mu\text{g/kg}$) were documented in local samples, and the amount of 6.94 ± 1.90 and $12.71 \pm 4.30 \mu\text{g/kg}$ were documented in imported soybean oil samples, respectively. About 46.6% samples of sunflower oil have levels of total AFs in a range of $(21 - 50 \mu\text{g/kg})$ as shown in Fig 3a. Furthermore, 66.6% samples of soybean oil produced locally have concentrations of total AFs in a

range of $\geq 50 \mu\text{g}/\text{kg}$, as shown in Fig 3 (b). There existed a higher level of AFs in locally produced vegetable oil samples compared to imported samples ($t = 22.274$ and $p = 0.009$) at $\alpha = 0.05$. A significant difference in levels of AFs in seeds compared to oil samples were found ($t = -17.75$, $P = 0.0009$) at $\alpha = 0.05$.

Table 2. Occurrence of AFB₁ and total AFs in edible vegetable seed samples from imported and local origin.

Sample category	Imported Samples				Local samples			
	Total sample	Positive samples	AFB ₁	Total AFs	Total samples	Positive samples	AFB ₁	Total AFs
	N	N (%)	Mean ($\mu\text{g}/\text{kg}$) \pm S.D.	Mean ($\mu\text{g}/\text{kg}$) \pm S.D.	N	N (%)	Mean ($\mu\text{g}/\text{kg}$) \pm S.D.	Mean ($\mu\text{g}/\text{kg}$) \pm S.D.
Sunflower	25	18 (72)	13.29 \pm 3.5	20.428 \pm 5.2	32	20 (62.5)	14.23 \pm 3.6	20.27 \pm 5.4
Soybean	15	7 (46.6)	10.36 \pm 4.1	18.42 \pm 4.6	12	9 (75)	21.01 \pm 4.7	36.37 \pm 6.1
Canola	28	14 (50)	9.30 \pm 2.4	16.23 \pm 3.7	34	18 (52.9)	13.29 \pm 5.6	20.05 \pm 5.9
Olive	22	8 (36.3)	5.74 \pm 2.5	9.48 \pm 1.6	20	10 (50)	11.24 \pm 2.5	16.50 \pm 5.4
Corn	38	25 (65.7)	10.16 \pm 4.3	16.08 \pm 2.9	45	28 (62.2)	12.80 \pm 5.3	19.92 \pm 3.5
Mustard	34	20 (58.8)	6.41 \pm 2.7	12.95 \pm 3.5	46	23 (50)	8.74 \pm 4.5	13.88 \pm 4.6
Total	162	92 (56.7)			189	108 (57)		

The data in parenthesis represents the percentage of total analysed samples; LOD = limit of detection.

Table 3. Occurrence of AFB₁ and total AFs in vegetable oil samples from imported and local origin.

Sample category	Imported Samples				Local samples			
	Total sample	Positive samples	Mean AFB ₁	Mean AFs	Total samples	Positive samples	Mean of AFB ₁	Mean of AFs
	N	N (%)	($\mu\text{g}/\text{kg}$) \pm S.D.	($\mu\text{g}/\text{kg}$) \pm S.D.	N	N (%)	$\mu\text{g}/\text{kg}$ \pm S.D.	$\mu\text{g}/\text{kg}$ \pm S.D.
Sunflower	20	12 (60)	5.93 \pm 2.3	11.16 \pm 2.9	25	15 (60)	8.7 \pm 3.2	15.1 \pm 4.3
Soybean	18	9 (50)	6.94 \pm 1.9	12.71 \pm 4.3	15	10 (66)	14.29 \pm 2.5	25.61 \pm 7.5
Canola	42	18 (42.8)	4.87 \pm 1.8	9.25 \pm 3.4	50	22 (44)	7.41 \pm 3.4	11.80 \pm 3.5
Olive	20	8 (40)	4.38 \pm 2.4	7.47 \pm 2.4	18	8 (44)	8.51 \pm 3.5	12.78 \pm 5.3
Corn	45	10 (22)	2.43 \pm 1.9	3.98 \pm 1.5	55	20 (36.3)	6.34 \pm 2.8	8.83 \pm 2.8
Mustard	35	21 (60)	5.69 \pm 2.4	11.56 \pm 1.9	50	28 (56)	7.71 \pm 1.8	13.43 \pm 4.6
Total	180	78 (43.3)			213	103 (48.3)		

The data in parenthesis represents the percentage of total analysed samples; LOD = limit of detection.

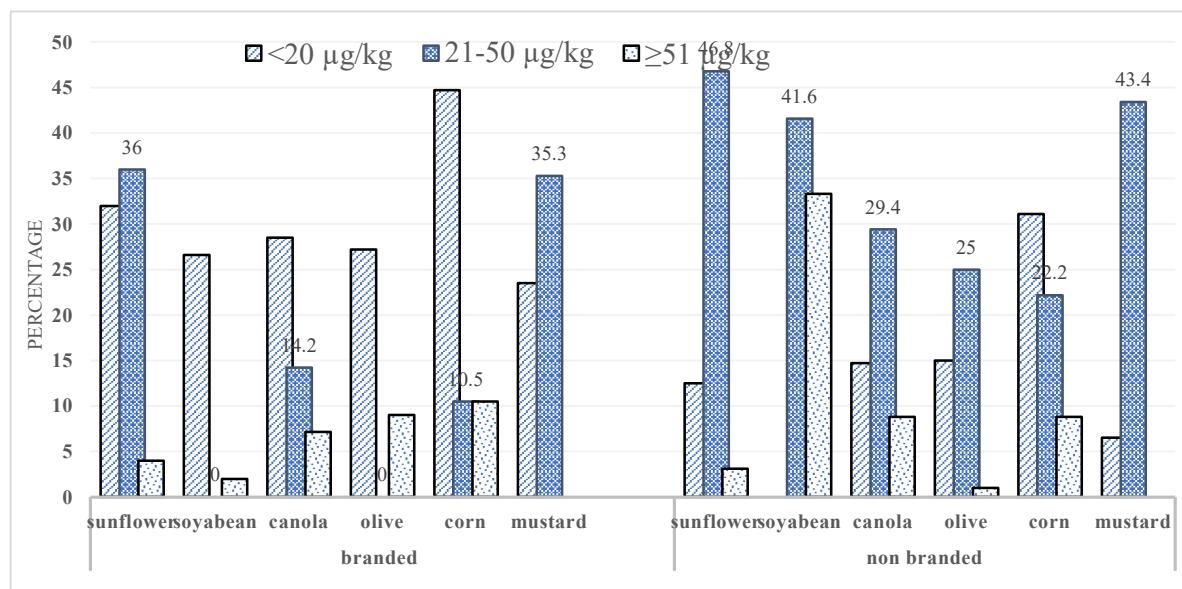


Figure 3. (a): The percentage of samples having total levels of AFs in ($\leq 20 \mu\text{g}/\text{kg}$), (21 -50 $\mu\text{g}/\text{kg}$) and ($\geq 51 \mu\text{g}/\text{kg}$) in imported and local vegetable seed samples.

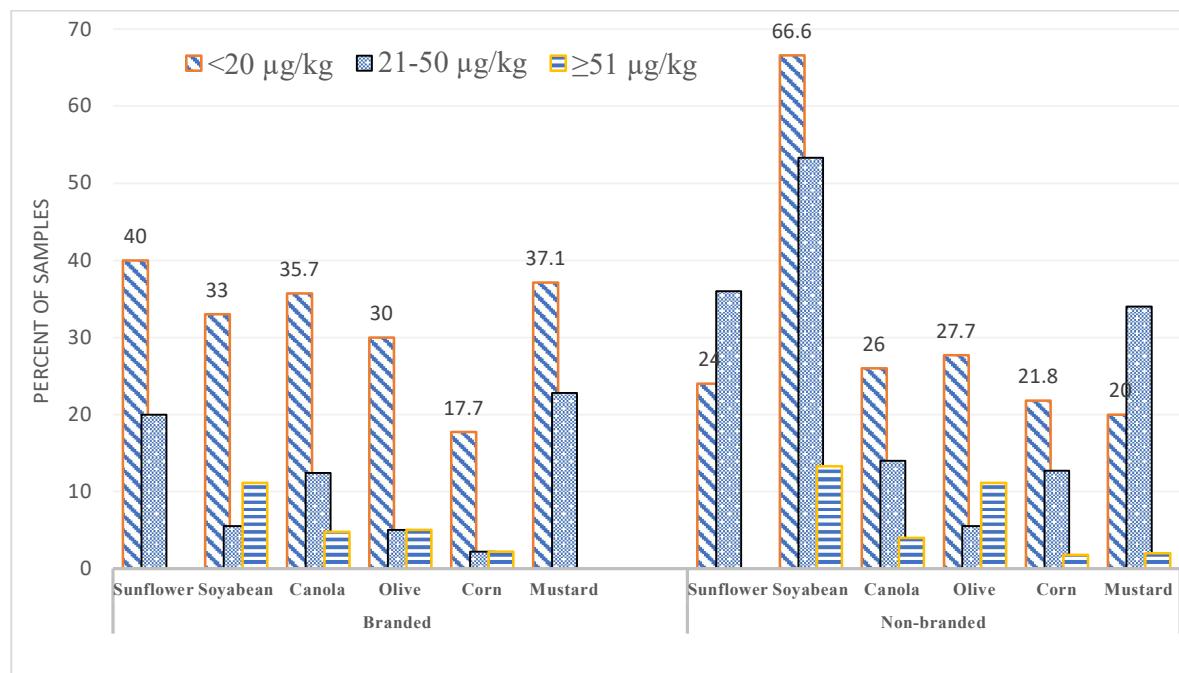


Figure 3. (b): The percentage of samples having total levels of AFs in ($\leq 20 \mu\text{g/kg}$), (21 -50 $\mu\text{g/kg}$) and ($\geq 50 \mu\text{g/kg}$) in imported and local vegetable oil samples.

High incidence and higher levels of AFs than the current study's findings were reported from Iran by Beheshti and Asadi [37]. They observed 111 (64%) out of 173 samples of sunflower and safflower were found to be contaminated with AFB₁ and 103 (83.7%) samples of safflower seeds with a mean of 2.81 to 0.44 ng/g and 8 (16%) samples of sunflowers with a mean of 40.68 ng/g were contaminated with AFB₁. However, only 5 and 2 samples of sunflower and safflower seed were levels higher than the EU limit (2 $\mu\text{g/kg}$), respectively. Similarly, from the same geographical region, i.e., from Sri Lanka, Karunaratne et al. [4] have analyzed 59 vegetable oil samples (43 imported & 16 local) of seven different categories (coconut, palm, olein, sunflower, olive, sesame, soybean and corn oil). They documented that 12 (37.5%) out of 32 coconut samples were observed to be contaminated with AFs. They documented that AFB₁ and total AFs ranged from 2.25 to 72.70 $\mu\text{g/kg}$ and 1.76 to 60.92 $\mu\text{g/kg}$, respectively, and 2 out of 12 oil samples with levels that exceeded the EU's high permitted limit of 2 $\mu\text{g/kg}$, for AFB₁. From Tanzania, Mohammed et al. [38] have studied 40 samples of sunflowers seeds and 21 samples of unrefined oils of sunflowers and found that 6 (15%) samples were discovered to be infected with AFB₁, ranging from LOD to 218 ng/g, comparatively higher than the findings of the present study. Only 3 samples have levels greater than the Tanzanian Bureau of Standards (TBS) and EC/EU permissible limit.

However, studies documented a high occurrence of positive samples with AFs contamination but with low AFs concentrations. From India, Banu and Muthumary [25] have shown that 10 (43.4%) out of 23 samples of sunflower oil were observed to be contaminated with AFB₁, and all refined oil samples have levels lower than LOD. Even from Europe, i.e., from Italy and Morocco, Ferracane et al. [26] have studied virgin olive oil samples and found that only 3 (10%) out of 30 samples of olive oil was found to be contaminated with AFB₁ along with ochratoxin A; ranging between 0.54 to 2.50 ng/g. From Sudan, Mariod & Idris [39] documented that 54.8% of samples of groundnuts and 14.5% (out of 8) sunflower oil samples were found to be contaminated with AFB₁. Nabizadeh et al. [40] have examined 97 edible oil samples of six categories (olive, sunflower, canola, blend, frying, unrefined olive oil). They observed that 98% of samples had shown levels of AFB₁ lower than LOD and all the positive samples with AFs were within the EU regulations 20 $\mu\text{g/kg}$. In Pakistan, Shar et al. [41] have observed the natural occurrence of AFB₁ in 110

samples of cotton seeds and found the maximum level of AFB₁ in cottonseed cakes, i.e., 89 µg/kg.

Edible oil and groundnuts are considered important cash crops in many parts of the world. However, due to adopting old conventional agricultural practices and illiteracy among farmers, and traders about toxigenic fungi, makes the safety and quality of crops questionable. Edible oils (Olives, sunflower, coconut) are often stored for a long period of time in conditions e.g. contact with the ground, moisture, in jute bags. The extended period promotes the growth of molds, such as all favoring the toxicogenic molds' colonization Yassa et al. [42]. The environmental conditions like drought might affect the crops during preharvest phase and could produce fungi like *Aspergillus*. That is why it becomes unfit for human or animal consumption Okello et al. [43]. With achieving favorable conditions of temperature and humidity, these fungi grow on certain foods and feed products and thus produce secondary metabolites like mycotoxins. High humidity and temperature should be controlled, which is a severe problem in tropical countries, like Pakistan, to avoid aflatoxigenic fungi's growth [20]. Furthermore, the variation in results of AFs in food depends on various factors, like analytical methods used, environmental conditions, crops and harvesting practices. Adopting good harvesting practices, and good storage practices might minimize the presence of aflatoxigenic fungi in food and products.

3.3. Estimation of dietary intake in sunflower oil samples

The estimation of dietary intake of total AFs in sunflowers oil samples in different age groups of males and females' individuals is represented in Table 4. The sunflower oil was the most consumed vegetable oil in Pakistan, and therefore most realistic approach was to estimate dietary intake from this oil. The highest dietary intake was found in a local sunflower oil sample (0.90 µg/kg/day) in female individuals (16-22 age group), followed by the dietary intake of 0.69 µg/kg/day body weight in the male group (16-22 age group). The results have shown that high dietary intake values were found in both male and female individuals of 16-22 years of age group. The local sunflower oil samples have shown the highest dietary intake levels in both male and female individuals. No comparative data to correlate the results of the present study was found. The high dietary intake levels of total AFs consuming sunflower would cause severe effect on the health of consumers, because the country has already insufficient health facilities. However, neglecting the dieting pattern, seasons and traditional eating habits of participants might alter the results of dietary intake assessments.

Table 4. Estimation of dietary intake (µg/kg/day) for total AFs in sunflower oil samples.

Category	Type	Males			Females		
		Age groups			Age groups		
		16-22	23-32	≥33	16-22	23-32	≥33
Imported	Consumption mg/day	2.7	2.2	2.1	2.5	2.1	1.9
	AFs mean level (µg/kg)	11.16	11.16	11.16	11.16	11.16	11.16
	Dietary Intake µg/kg/day	0.49	0.32	0.25	0.57	0.43	0.33
Local	Consumption mg/day	2.8	2.4	2.1	2.9	2.2	2.0
	AFs mean level (µg/kg)	15.1	15.1	15.1	15.1	15.1	15.1
	Dietary Intake µg/kg/day	0.69	0.48	0.33	0.90	0.58	0.45

Exposure of dietary intake = mean contamination level x per capita consumption/average weight.

3. Conclusions

The study documented considerably high AFB₁ and total AFs in vegetable seeds and oil samples originated from imported and locally produced samples. The results might drastically affect the consumption of locally grown vegetable oils. Regular monitoring of food and feed samples and organize workshops for farmers, traders, and exporters might help create awareness about the toxic nature of AFs. Currently, Punjab Food Authority has started to monitor food safety and quality, which is a good initiative. However, liquid-liquid extraction instead of immunoaffinity columns and unavailability of LC-MS analysis are the weak point of the present study.

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