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Evaluate the effect of Tropical Climatic Variations of Aflatoxin Occurrence in Basmati Rice

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Abstract

A wide variety of local crops and food products contain aflatoxins (AFs), which are food pollutants. The species of Aspergillus flavus and A. parasiticus create them under favorable conditions, such as high temperature and moisture, and they are extremely poisonous and carcinogenic. The distribution of aflatoxins (AFs) contamination in food commodities varies throughout the year due to specific temperatures and humidity in each month. Therefore, the present study was designed to assess humidity & temperature effects of each month's Aflatoxins occurrences in rice. In this regard, 120 basmati rice samples were collected from various rice vendors during a different period in 2021 and quantified the AFs contamination using high pressure liquid chromatography with fluorescence detection and Kobra Cell™ Derivatization. About 72% of samples were found contaminated with Aflatoxins, ranging from 0.36–6.35 μgkg^{-1} and an average of 1.31 μgkg^{-1}. Furthermore, in 95% samples contain AFs levels lower than the maximum tolerable limit (MTL = 4 μgkg^{-1}) as suggested by European Union. Furthermore, 67% samples contained AFs levels ranging from 0.36–4.0 μgkg^{-1}. However, only 5% samples exhibit AFs contamination ranging between 4.0–6.35 μgkg^{-1}. Moreover, as per United States (FDA and FAO) and Pakistan (PSQCA) standards, all the samples were below than MTL of 20 μgkg^{-1} as. During the entire study, AFs contamination in basmati rice seems to differ monthly due to climatic variations. During the month of July, samples were found highly contaminated (5.15 μgkg^{-1}), August (6.35 μgkg^{-1}) and September (4.84 μgkg^{-1}), as a highly humid condition in these months. It was concluded that in Pakistani rice was found safe for human consumption as the level of AFs is within limits and could be exported to other countries. However, the AFs contamination is highly influenced by climatic conditions.

Keywords: Aflatoxins, Basmati rice, Climatic conditions, HPLC

1. INTRODUCTION

Food safety has developed into a very significant problem around the world and the likely effects of climate variation on food quality and yield are currently gaining significant consideration by scientists. The staple food such as rice, wheat and maize contaminated with moulds has gained considerable attention due to their chronic and acute effect in humans and animals (Awuchi, et al., 2020).

In the world the most spent staple food (Rice) Oryza sativa Linn is the second most-produced cereal with corn, and wheat being the first. In wet (monsoon) or kharif season basmati rice is cultivated in Pakistan. However, during the post-harvest process, the total yield of rice as per economy and production comes a slight bit the damage in Pakistan. Rice is normally cultivated in a climate favorable for toxigenic fungi infections, eventually possible production of mycotoxins occurrence (Asghar, Iqbal, Ahmed, Shamsuddin, & Khan, 2016). Fungal damage to food causes major worldwide monetary losses. In case of improper storage conditions and favorable environment for fungal attack, rice kernel can be the best substrate for mycotoxin producing fungi (Mannaa & Kim, 2017). The hygroscopic nature of rice is also a vital element that supports the formation and expansion of fungal species. However, the if dehydration is delayed and moisture levels are allowed to rise above permissible limit, post-harvest contamination can happen, (Bradford, et al., 2020). In Pakistan, the predominant sub–tropical environments such as humidity, temperature and the proximity to Arabian ocean also play an active part. The rice produced in Pakistan mostly in a sub-tropical environment & climate with 60-65% of the yearly rain focused from June month to August in rainy season (monsoon) spells (Ali, Zhang, & Yue, 2020). These metrological conditions support the fungal attack and the subsequent contamination of crops with mycotoxins.

Aspergillus, Penicillium and Fusarium are the mycotoxigenic fungi that commonly attack on foodstuffs. These poisonous are hepatotoxic, mutagenic, immune-suppressive and teratogenic toxic fungi that are responsible for reproductive and fetal toxicity. In agriculture, the most concerned species amongst Aspergillus, is A. flavus and A. parasiticus and leading saprotrophs with a partial parasitic ability (Iqbal, 2021).

Among mycotoxins aflatoxins (AFs) are fairly dispersed group and are infected with various agricultural and food commodities, produced by Aspergillus species. AFs are able to produce an active carcinogen, mutagenic, neoplastic and teratogenic actions in humans and animals (Pickova, Ostry, Toman, & Malir, 2021). AFG1, AFG_2

AFB_1 and AFB_2 are most important Aflatoxins where, Aflatoxin B_1 is highest toxic among all and it is recognized as a Class-1 carcinogen declared by the International Agency for Research on Cancer (IARC) based on the confirmation of its carcinogenicity (Ostry, Malir, Toman, & Grosse, 2017). Malnutrition, together with the prolonged ingestion of Aflatoxins, also effect immunosuppression, compromised growth & further illnesses (Khan, Ghazali, Mahyudin, & Samsudin, 2021). According to the quantity consumed, the occurrence of ingestion and the age of a person can be associated with necrosis of liver, encephalopathy, cirrhosis and enhanced vulnerability to hepatitis-B (Benkerroum, 2020).

In literature, the effects of environmental conditions are reported on the AFs production. For instance, (Phan, et al., 2021) described that the optimal temperature for the Aflatoxins production can differ from 24 ^oC to 30 ^oC based on substrate & strain variety. (Asghar, Iqbal, Ahmed, & Khan, 2014) described that the optimal temperature for AFs production on rice grains was 28^oC, with significant AFs production still appearing at 32^oC. However, the temperatures above 32^oC noticeably decline the AFs production however the fungal growth was increased. In addition, the decline in AFs production at temperatures above 32–35^oC has been studied earlier (Mannaa & Kim, 2017). However, the above-mentioned study didn't provide the distribution of AFs contamination w.r.t. humidity/ temperature per month. Thus, the present study was to calculate the AFs contamination in all 12 months with respect to climatic conditions (relative humidity & temperature) in Pakistan.

2. MATERIALS AND METHODS

2.1 Chemical and reagents

The separate vials, AF-B_1, AF-B_2, AF-G_1 and AF-G_2 in acetonitrile were acquired from Biopure (Austria) in a concentration of 2.0, 0.50, 2.0, 0.51 μgmL^{-1}, respectively. The calibration standards in series of the concentration 0.10 to 40.0 ng/ml were being prepared from the readily available stock solution and stored in amber vials at -20 ^oC. The pH 7.4 from tablets of Phosphate buffered saline (PBS) acquired from the Oxide Ltd. (Hampshire, United Kingdom). Acetonitrile (HPLC Grade), Methanol, KBr & HNO_3 were being purchased from Merck (Germany).

2.2 Apparatus & Equipment

The Hitachi Elite LaChrom High-Performance LC system with Pump L-2130, L-2200 Autosampler. L-2200 auto-sampler & L-2200 fluorescence detector (Germany)was used in this analysis. The separation of each Aflatoxin was achieved using a RP-18 (5 mm, 250 × 4.0 mm^2) column (LiChroCART1 100 Å) was being procured from Merck (Germany). The derivatization of Aflatoxin B_1 and Aflatoxin G_1 was achieved by an electrochemical bromine derivatization cell (Kobra Cell™) acquired from R-Biopharm (Scotland). The immunoaffinity columns (IACs) (cat. no. COIAC1001) AflaStar™ was being used to clean-up of rice samples by Romer Labs. (Austria). The blending of samples was done by an Explosion-proof blender (8018) purchased from Ebarch Corporation (USA).

2.3 Samples collection

One hundred twenty (120) samples of the same brand of basmati rice (10 samples of each month) were collected from the super markets of Karachi-Pakistan from January to December 2021. The sampling was conducted during the tropical & sub-tropical climatic conditions, with average annual rainfall (174 mm), relative humidity (>49%) and temperature (24.5–33.8^oC) respectively ("Pakistan Metrological Department," 2022). About 0.5–1 kg of each sample of rice was collected and the sampling method was based on the EU Commission Regulation No. 401/2006 of 23-Feb-2006 (Commission, 2006). It is understood that Aflatoxins are heterogeneously distributed and occurred in pockets form with high concentrations in whole food commodities. Therefore, the collected samples were first well-homogenized and accurately verified for their homogeneity as stated by ISO 13528:2017 (Annexure B: Homogeneity and stability checks of samples) (Anastasopoulos, 2017). The rice sample stored in clear zip lock bags at -20^oC till AFs analysis.

2.4 Determination of aflatoxins in rice using HPLC-FLD

The technique adopted for the extraction, and determination were performed as per the scheme presented by (Asghar, et al., 2022). In brief, 10 g of finely powdered rice sample was blended with 50 mL of water: methanol (20:80; v/v) for 2 minutes by using an explosion proof blender. At 6000 rpm the mixture was centrifuge for 5 mins. and supernatant was collected. Two (02) mL of supernatant, diluted with fourteen (14) mL of PBS solution of pH 7.4 and cleaned through an Aflastar IACs column using a spe-12G (Baker) vacuum unit (London, UK) equipped with a Millipore pump (Billerica, USA). The IACs were washed two times using 10 ml Deionized-H_2O and then dried by a vacuum. AFs from the Immuno Affinity Columns were eluted with 1.5 mL methanol followed by 1.5 mL de-ionized water and then injected into the High-Performance LC system.

A validated and accredited method of HPLC chromatography and fluorescence detection (HPLC-FLD) with post-column derivatization was applied for the AFs quantification in the rice samples as described earlier

(Asghar, Iqbal, Ahmed, Khan, et al., 2016). An aliquot of 99 µL of each standard and sample was injected in triplicate into HPLC-FLD system using auto-sampler. Each AFs was isolated using an RP-18 column. Methanol/acetonitrile/water (20:20:60; volume/volume/volume) containing 167 mL/L Nitric Acid (HNO_3) and 119 mg/L KBr was used as a mobile phase (in an isocratic mode) with a flow rate of 1 $mLmin^{-1}$ at 40°C. The operative settings of the fluorescence detector were adjusted at 362 nm and 464 nm for excitation and emission, respectively. The one (01) complete run Chromatography and detection was completed in 15 mins. The concentrations of AFs were calculated using the calibration curve method.

2.5 Meteorological records

The weather pattern like temperature, humidity and precipitation in Karachi-Pakistan (during the collection of rice samples) were acquired from Islamabad, Meteorological Department from January - December 2021 (Pakistan Metrological Department, 2021).

2.6 Method validation

Each rice sample was examined in triplicate to evaluate the accuracy of the developed HPLC method. This method validation was being executed according to the European Official Decision protocol for confirmatory chromatography techniques and decision 657/2002/EC (Community, 2002). However, the validation parameters such as accuracy & precision, detectable (DL) & quantifiable limit (QL), method recovery and uncertainty measurement were also being evaluated (Miller & Miller, 1993).. In brief, the linearity of the methods was assessed in terms of the determination coefficient (R^2). The efficacy of the process was measured by the fortified spiked samples. The toxin-free sample was spiked with 0.5, 2.5 and 5.0 μgkg^{-1} of AFs concentrations and analyzed as per above-described method. The uncertainty measurement (μ_c) for AFs was being calculated as per quantifying uncertainty in analytical measurement (EURACHEM).

3. RESULTS & DISCUSSION

3.1 Method validation

The validation results of the HPLC analysis are presented in **Table 1**. The linearity in terms of R^2 values was in the range of 0.9993−0.9998 as assessed by the calibration curves over the verified range. The QL & DL were found 0.36 and 0.12 μgkg^{-1} (respectively) in the rice sample. The average recovery values were obtained from 94.1−99.2 %. Whereas, the % RSD and uncertainty measurement (μ_c) values were found between 1.25% & 0.15 μgkg^{-1}, respectively.

Table 1. HPLC Method validation for the analysis of aflatoxin AFB_1, AFB_2, AFG_1, and AFG_2 and T. AFs in rice samples.

Mycotoxins	Correlation Coefficient (R^2)	DL (μgkg^{-1})	QL (μgkg^{-1})	% Recovery	RSD (%) (n=20)	Uncertainty Measurement (μgkg^{-1})
AFB_1	0.9998	0.05	0.15	96.3–99.2	1.25	0.12
AFB_2	0.9993	0.04	0.12	95.5–96.5	1.14	0.10
AFG_1	0.9997	0.09	0.36	94.1–97.2	1.26	0.09
AFG_2	0.9995	0.07	0.21	96.1–99.0	0.98	0.11
T. AFs	0.9997	0.12	0.36	95.6–99.3	1.25	0.15

RSD = relative standard deviation; QL = limit of quantifiable, limit; DL = Detectable limit; HPLC = High-performance liquid chromatography.

3.2 AFs contamination level in rice samples

The concentrations of T. AFs in basmati rice samples collected from Karachi – Pakistan in 2021 are presented in **Table 2**. T. AFs were detected in 86 (72%) samples out of 120 tested samples. The contamination range was found between 0.36 and 6.35 μgkg^{-1} with a mean value of 1.31 μgkg^{-1}. Overall, in 28% (34) samples, the AFs concentration was quantified below than DL value of 0.12 μgkg^{-1}. In 67% (80) samples, the AFs level was found between the range of 0.36 & 4 μgkg^{-1}. However, the Aflatoxins contamination level in 5% (6) samples were found between the ranges of 4 to 6.35 μgkg^{-1}. The achieved results showed that 95% (114) of tested rice samples were fit for human consumption (linked with AFs Presence) as per the European Union regulation (4 μgkg^{-1}) (Commission, 2010). Whereas, all rice samples were suitable for human utilization according to the USA (FDA & FAO) guideline (20 μgkg^{-1}) (Food & Administration, 2000).

Table 2. Determination of total aflatoxin contamination in rice obtained from Karachi – Pakistan during 2021 as per standards.

TN (n) [a]	% Positive Sample	Number of samples in a concentration range, µgkg^{-1}			Average	Range
		Not Found [b]	0.36 to 4 [c]	4 to 6.35 [d]		
120	86 (72)	34 (28%)	80 (67%)	6 (5%)	1.31 ± 0.23	0.36-6.35

[a]Total number of samples, [b] Not found within the detectable limit (0.12 µgkg^{-1}), [c] Below EU limit, [d] Below USA limit (FDA and FAO)

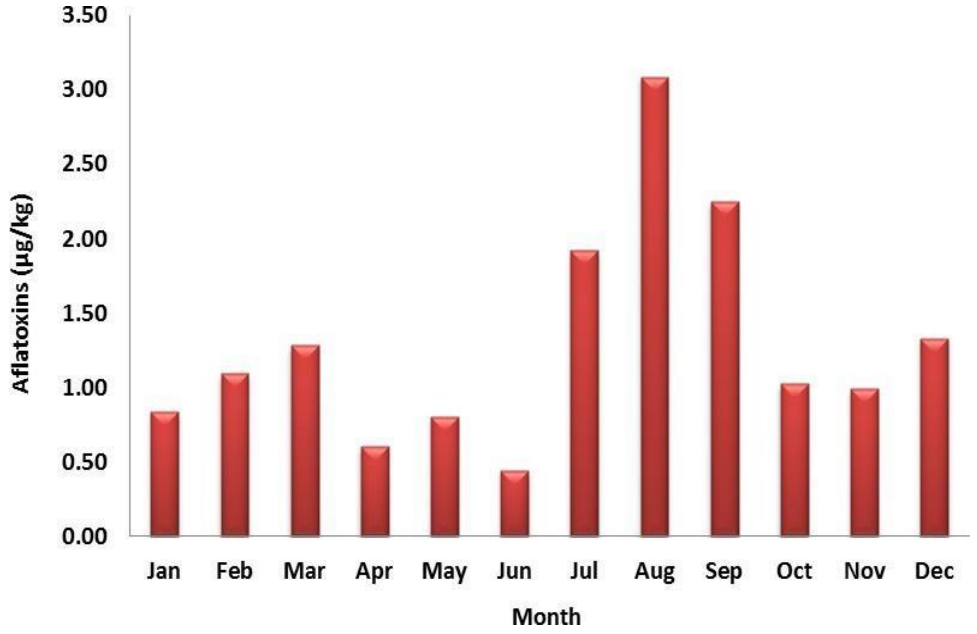
3.3 Effect of climatic conditions on AFs contamination

The distribution of AFs content in rice samples with respect to climate conditions of Karachi-Pakistan is presented in **Table 3 and Figure 1**. Five samples in each month during 2021 were collected from Karachi- Pakistan. The AFs contamination level in rice differs between different months due to deviation in climatic situations. A considerable difference in AFs contamination was observed in the all months at $p < 0.05$. In July (5.15 µgkg^{-1}), August (6.35 µgkg^{-1}) and September (4.84 µgkg^{-1}), highly contaminated rice samples were seen as mentioned in **Table 3**, but the level of contamination was noticed within the acceptable range and does not likely hazard to the human health. During these months, the mean temperature/RH was 31.6ºC/77%, 30.5ºC/79% and 31.2ºC/74%, respectively and considered promising conditions for the aflatoxigenic fungi. (Mannaa & Kim, 2017) reported that the environmental situations considerably influence on the AFs production during harvesting and storage.

Table–3: Distribution of total aflatoxins in basmati rice in different months with respect to average temperature and relative humidity in Karachi-Pakistan during 2021[a].

Month	No. of Samples	Positive Samples (%)	Mean (µgkg^{-1})	Range (µgkg$^{-1)}$	T[b] (°C)	RH[c] (%)
Jan	10	8 (80)	0.84** ± 0.14	0.53–1.56	24.5	49
Feb	10	8 (80)	1.09** ± 0.06	0.41–1.85	26.7	50
Mar	10	6 (60)	1.28** ± 0.08	1.65–2.53	30.5	54
Apr	10	4 (40)	0.60* ± 0.16	1.36–1.65	33.3	59
May	10	6 (60)	0.81* ± 0.11	1.17–2.83	33.8	70
Jun	10	8 (80)	0.44* ± 0.04	1.56–2.04	33.4	73
Jul	10	8 (80)	1.92*** ± 0.10	0.48–5.15	31.6	77
Aug	10	10 (100)	2.88*** ± 0.18	1.65–6.35	30.5	79
Sep	10	8 (80)	2.08** ± 0.09	1.42–4.84	31.2	74
Oct	10	4 (40)	0.82** ± 0.13	1.75–2.34	33.1	62
Nov	10	6 (60)	0.99** ± 0.02	0.36–2.34	30.6	51
Dec	10	8 (80)	1.33** ± 0.04	0.52–2.24	26.3	49

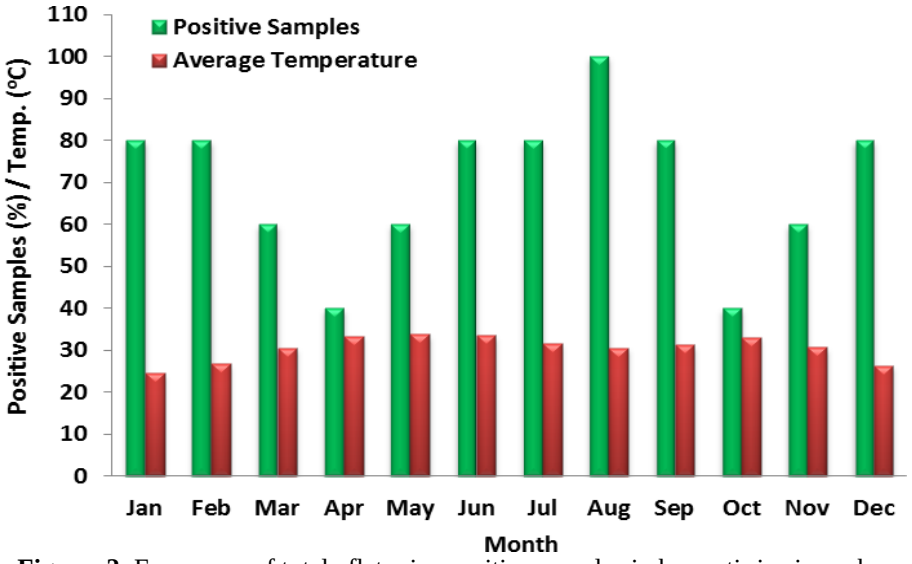
Mean (average) * or ** or *** values along the column are considerably different at $p < 0.05$.
[a] Triplicate analysis, represent as mean ± SD.
[b] Average temperature in Karachi-Pakistan.
[c] Average relative humidity in Karachi-Pakistan.



Figure–1: Distribution of total aflatoxins in basmati rice in each month during 2021.

The variation in AFs contamination was observed due to the many environmental aspects such as relative humidity, temperature & storage conditions. Normally, warm and moist conditions are considered to be promising for toxigenic fungal growth and eventually aflatoxins production in crops (Awuchi, et al., 2021). The incidence of Aflatoxins in crops is supposed to be strong encouragement by weather during and after the cultivating time. An environmental variation is probably led to intensification in hot and dry spells and improved risk of AFs contamination in food commodities (Duchenne, Ranghoo-Sanmukhiya, & Neetoo, 2021).

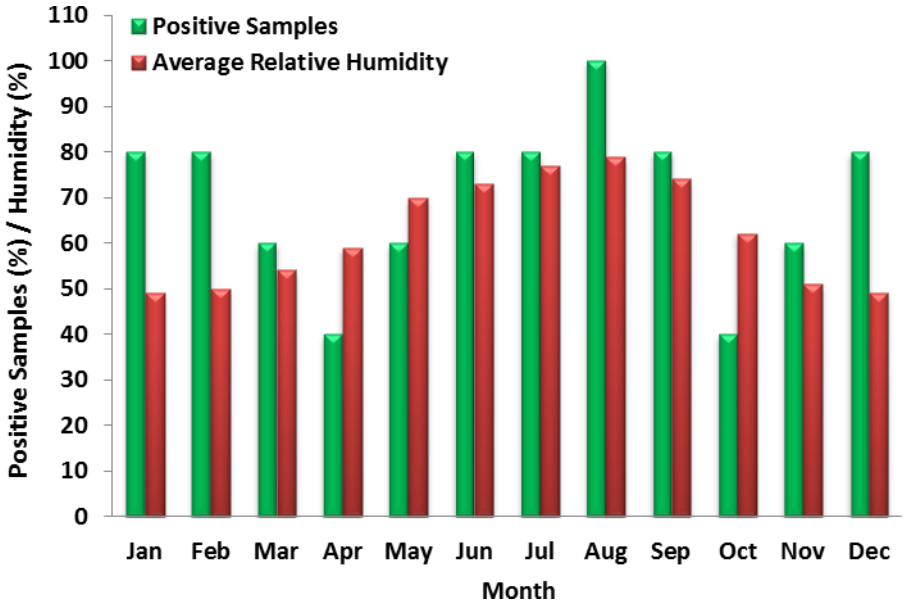
The environmental data such as regular temperature (T) & relative humidity (RH) throughout the investigation was collected from Climate-data. org (Org, 2022). **Figure 2** shows the percentage of AFs contamination in rice month-wise with respect to the average temperature in Karachi, Pakistan during year 2021.



Figure–2: Frequency of total aflatoxins positive samples in basmati rice in each month with connection to the average temperature in Karachi-Pakistan.

All tested samples were positive for AFs in August 2021. During this month, the average temperature was 30.5ºC, which is considered a promising environment for the growth of AFs-producing fungi (Pei, et al., 2021). Our previous study also described that the growth of the *A. parasiticus and production of Aflatoxins in basmati rice is in between temperature ranging from* 25 to 30ºC (Asghar, et al., 2022). The favorable temperatures for the growth of *A. flavus* vary from the lowest of 10 °C to 12.8°C to the highest of 43°C to 48.8°C with an optimal of about 33.8°C as reported by (Ono, et al., 2021). However, (Gallo, Solfrizzo, Epifani, Panzarini, & Perrone, 2016) reported that the AFs production is allowable at 28°C and entirely prevented at 37°C. Achakzai and Bazai in year 2021 reported that the maximum growth of *A. parasiticus* and AFB_1 production was detected at 30ºC (Achakzai, Samiullah, & Bazai, 2021). Similarly, at 30ºC the maximum fungal growth was found (Okereke, 2020). The results of the current observation are in correlation with these earlier studies.

The relative humidity is another factor that influenced on the growth of aflatoxigenic moulds and AFs contamination. The AFs contamination in rice as for the usual relative humidity in Karachi, Pakistan during 2021 is presented in **Figure 3.** Maximum relative humidity was observed in August (79%), which favored the production of AFs contamination as shown in **Figure 3**.



Figure–3: Frequency of total aflatoxins positive samples in basmati rice in each month with connection to the average relative humidity in Karachi-Pakistan.

Earlier observations also reported that the environmental conditions are a key factor in the development of *Aspergillus* spices and Aflatoxins production. For instance, (J. Ali, Hussain, & Ullah, 2011) reported from Pakistan that in 40 tested rice samples, 70% of samples were found contaminated with Aflatoxins with average contamination 4.9 μgkg^{-1}. In another report from Pakistan, a highly contaminated sample was observed in August (2010). However, the climatic conditions in March, July and August (2010) looks to be promising environmental situations for the growth of these entities (Firdous, Ejaz, Aman, & Khan, 2012). In the present study, the highly contaminated sample was also detected in August showing 6.35 μgkg^{-1}, as the temperature and relative humidity were most promising concerning other months.

The climatic conditions seem to be similar in different regions of South Asia as in Pakistan, which favour the growth of *Aspergillus* and AFs commodities. (Bandara, Vithanege, & Bean, 1991) reported from Sri Lanka that the contamination of AFB_1 and AFG_1 in rice was found to be 185 and 963 μgkg^{-1}, respectively. The samples collection time, the average temperature and humidity were noted at 27°C and 78%, respectively, which was the highest among the rice cultivation regions in Sri Lanka. In addition, Sri Lanka's climate has tropical and comprises of diverse wet and dry spells. In general, the seaside regions like temperatures are around 28°C, which favor the production of AFs contamination as described by Bandara el at., (1991).

In a report from India, in 1200 rice-tested samples about 67.8% were contaminated with AFB_1 ranging between 01–308 μgkg^{-1} (Reddy, Reddy, & Muralidharan, 2009). In many parts of India, the state has a tropical climate which over most of the inside is a combination of rainy and dry tropical weather. In northern zones, there is a moist tropical environment and along the western seaside lies wet tropical regions (Nematchoua, et al.,

2020). These conditions support the superior development of aflatoxigenic moulds & AFs occurrence as mentioned in this above study.

The present data revealed that the Pakistani rice was contaminated with AFs at low levels and not harmful to human health. However, the occurrence and small detection of AFs specified that there is a necessity for further examinations and consistent observing and execution analysis on daily basis according to the food quality control measure.

In tropical and sub-tropical zones of the world, AFs levels were found high as the climatic conditions favors the condition of fungal growth in food commodities (Perrone, Ferrara, Medina, Pascale, & Magan, 2020). In urban nations, the chances of the AFs production are extremely high due to the temperate settings as the temperature increases up to 30ºC. The variation in climatic environments like temperature, humidity, precipitation and additional aspects may lead to food safety hazards (Banik, et al., 2015).

The effect of climate on the production of AFs can be reduced if hermetic bags are used to rice samples. In our previous research strongly sealed hermetic bags shows significant decrease (>90%) the AFs production. Whereas, after 30 days of incubation, no significant change was observed in the concentration of AFs and it is remain constant for up to 90 days (Asghar, et al., 2022). Additionally, it was suggested that hermetic bags should be used for cereals and grains to prevent from moulds and AFs formation.

4. CONCLUSION

In summary, the AFs contamination in rice samples doesn't simultaneously exist as a possible threat to human health. The climatic conditions completely favor the development of aflatoxigenic moulds and Aflatoxin production. Highly AFs contaminated rice was being found from July to September months. The obtained results indicated that the rice samples must be examined on a routine basis in South Asian countries including Pakistan since the climate situations in these regions support moulds growth and AFs production.

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