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Original Research Article

Surveillance of Aflatoxigenic Aspergilli and Aflatoxin Contamination in Edible Oils from Karachi: A Molecular and Quantitative Approach

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Abstract

The presence of secondary metabolite, particularly aflatoxin produced by fungi, poses economic challenges in food and feed industries. This study aimed to identify aflatoxigenic Aspergillus species inedible oil and quantify total aflatoxins levels. We collected thirty sample of edible oil from various local market in Karachi and tested them for presence of *Aspergillus flavus* and total aflatoxin levels. Out of thirty, four samples tested positive for *Aspergillus flavus* using culture-based methods, which were subsequently confirmed by amplification of partial transcribed (ITS) region. Aflatoxin production was assessed using colorimetric methods, revealing that all *A. flavus* positive isolates (JB2, MGM4, LMM1, and RLM2) exhibited a plum red change upon exposure to ammonium hydroxide vapors on Sabouraud Dextrose Agar. Notably, JB2 also demonstrated color change on Yeast extract Sucrose and Coconut Agar medium, and exhibited blue fluorescence under UV light on coconut Agar medium. Aflatoxins were extracted from edible oil sample using solvent extraction method and quantified using a VICAM fluorometer. Among samples one exhibited total aflatoxin levels between 20-30ppb, five samples had levels below 20ppb, while 24 samples were negative for aflatoxin. These finding underscore the need of continued monitoring of aflatoxin contamination in edible oil.

Keywords: Aflatoxins, *A. flavus*, Fluorometer, Molecular and morphological characterization.

1. INTRODUCTION

Food quality and safety are the major concern of human and animal health in the developing countries primarily due to inadequate pre- and post-harvesting strategies, poor storage condition, and lack of skilled personnel etc (WHO, 2015; Yu, 2012). Timely identification, detection, and verification of contaminates and their sources are critical factors in ensuring food safety. According to food and agriculture organization (FAO), it is estimated that over 25% of all food and feed materials are contaminated with fungi, which produced secondary metabolites are known as mycotoxins (Haugland *et al*., 2002). Amongst the all mycotoxins, aflatoxins (AFs) pose a serious threat to living organism. Aflatoxin (AFs) and other fungal toxins have emerged as a major hazard to food security and agricultural production due to their adverse effects on both human and animal, as well as the considerably economic losses they incur globally (Karunarathna *et al.*, 2019). Twenty fungal bio transformant of aflatoxigenic contaminats have been identified, with AFB1, AFG1, AFB2, AFG2, AFM1 and AFM2 frequently identified in edible foodstuffs The International Agency for Research on Cancer has classified aflatoxins as potential human carcinogen. Approximately 20µg /Kg of total aflatoxins is considered an acceptable value for food and animal feed, while the tolerable levels in milk are set between 0.05-0.5µg /Kg. In contrast, The European Union (EU) has established a much lower limit of 4 µg/Kg for total AF in food (Janik *et al*., 2020).

The extraction of edible oil from oilseeds ranks second position in global food product tra. The Quality of oil has direct impact on public health, as it is frequently consumed place of animal fat. The conditions under which oilseed crops are harvested along with extraction techniques packaging, transportation, and storage can lead to contamination by fungi and mycotoxin. Oilseeds crops are primarily cultivated during pre and post monsoon season, where the hot and high humid environment promote the growth of fungi and molds, such as *Aspergillus flavus* and *Aspergillus*

parasiticus, which in turn produce of aflatoxins (Cotty & Jaime-Garcia, 2007). The toxicity of aflatoxin poses significant challenge for farmers, traders, and consumers due to serious health implication and considerable financial loss. Additionally, Edible oil may become contaminated with harmful mycotoxins because fungi can produce lipase; allowing their spores to survive in the anaerobic environment of oil (Odoh *et al*., 2017).

Aflatoxigenic species of *Aspergillus* are the significant source of aflatoxin contamination in diet and forage. The section *flavi*, of *Aspergillus* includes approximately twenty-two closely related linked species, with Aspergillus *flavus*, and *Aspergillus parasiticus* are commonly identified as toxic contaminants in various food and feedstuff. Worldwide occurrences of aflatoxins contamination predominantly attributed to *A. flavus*, in contrast to *Aspergillus parasiticus*. Storage and post-harvesting operation are significantly impacted by notorious infection caused by *A. flavus*. this infection adversely affects seed germination and leads to seed deterioration. *A. flavus* produces excessive amounts of mycotoxins including AFB1, AFB2, cyclopiazonic acid, and kojic acid

In this study, we employed a combination of microbial, molecular, and analytical techniques for the identification of *Aspergillus flavus* and the quantification of AFs in edible oil.

2. METHODOLOGY

2.1. Sample collection and fungal enumeration

From March 2022 to August 2022, thirty samples collected from different local markets of Karachi in screwed cap sterile bottles, properly labeled, and kept at room temperature until use. To prepare homogenized dilutions ranging from 10^{-1} to 10^{-10} 1mL from each of the vegetable oil sample was mixed with 9 ml peptone water (1.5% peptone) (Harley & P Harley, 2002). Well-mixed 100µL samples from dilution tubes (10^{-3} to 10^{-5}) were spread on Sabouraud Dextrose Agar (SDA) plates containing (ampicillin 100 µg /mL and chloramphenicol 25 µg /mL) to acquire isolated fungal colonies (Atlas, 2010). Plates were incubated at 30°C for seven days. Pure and isolated colonies of aflatoxigenic species were achieved by spreading the spores of the suspected *A. flavus* (yellow-green) on antibiotics containing Potato dextrose Agar (PDA) plates and incubated for up to 7 days.

2.2. Determination of aflatoxin production by culture-based methods

Two cultural method including color change technique and UV light exposure methods have been used for early detection of aflatoxins production and aflatoxigenic *Aspergilli* (Abbas *et al*., 2004). A single pure colony of aflatoxigenic species was inoculated on the surface of Coconut Agar Media (CAM) plates at 30°C for 5-7 days then plates were exposed to UV light to observe fluorescence**.** The aflatoxin production initially identified by ammonium hydroxide vapor method (AMVH). The suspected pure colonies of aflatoxigenic species have been inoculated on SDA, Czapeck Dox Agar (CZA), CAM, and Yeast Extract Sucrose (YES) and incubated at 30^0C for 7 days. The bore was made on SDA, CZA, YES and CAM media plates of aflatoxigenic species of *Aspergilli* and put the plates in inverted position to the lid having few drops of 31 % ammonium hydroxide (Khalid *et al*., 2018; Saito & Machida, 1999).

2.3. DNA Extraction and amplification of the desired genes

Spores of *A. falvus* and *A.niger* were scratched from the surface of SDA agar plates and transferred to Sabouraud Dextrose Broth (SDB) containing ampicillin (100 µg/ml) and chloramphenicol (25 µg/ml) and incubated at 30^0C for 48hours at 200rpm (Atlas, 2010).

Approximately 50mg mycelia was placed in sterile mortar then 650 µL lysis buffer (Tris-HCl, pH 8.0, 100 mM; Sodium EDTA, pH 8.0, 50 mM; SDS (w/v) 1%, and RNase A, 10.0 mg/ml) added and ground with the help of sterile pestle until homogenized mixture obtained (Feng *et al*., 2010). 500 µL homogenized mixture was transferred to fresh 1.5mL Eppendorf tube and centrifuged for 3 minutes at x13000g at room temperature. 500 µL supernatant was transferred to fresh 1.5mL Eppendorf tube then 100 µL neutralization buffer (Potassium-acetate solution 3M, pH 5.5) was added and inverted many times then centrifuged x13000*g* for three minutes at room temperature. 500 µL supernatant pipetted out and transferred to fresh 1.5mL Eppendorf tube then 500 µL isopropanol added and centrifuged at x13000g for 2 minutes at room temperature. Supernatant discarded and 750 µL ice cold 70% ethanol was added to the DNA pellet then centrifuged at x13000g for 30 seconds at room temperature. Supernatant was discarded and DNA pellet was air dried. DNA pellet dissolved in 50 µL sterile water and stored at -20°C after until further use.

ITS region, *aflR* and *aflS* genes were amplified using forward and reverse primers (Table 1). A 50 µL reaction was prepared; 25 µL PCR mix (Dreamtaq Green PCR master mix 2X, K1081 Thermo Fisher Scientific), 0.5-1 µL primers, 1-2 µL (0.1-1 µg) template DNA and the remaining volume prepared by using nuclease-free water. The thermal profile for PCR reaction is shown in Figure 1. The PCR products were observed by gel electrophoresis (1%). PCR products of samples JB2, MGM4, LMM1, and RLM2 for ITS gene were purified by using GeneJet PCR Purification Kit (K0701) according to manufacturer protocol and sent for sanger sequencing to Eurofins MWG|Operon for the identification of aflatoxigenic species of *Aspergillus*. Sequencing results were analyzed using Bioedit Sequence Alignment Editor (7.2.6.1, Ibis Biosciences. USA). The nucleotide sequences for ITS region were compared with the sequences in the databases using the

Basic Local Alignment Search Tool (BLAST) provided by the National Centre for Biotechnology Information website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently, the obtained sequences were submitted to GeneBank database. This approach ensured a precise analysis of nucleotide sequences and accurate comparison with existing sequences in the database.

Table 1: Primer Sequence of ITS region, *aflR* and *aflS* gene

Primer	Primer sequence	Reference
ITS1-Forward	TCCTCCGCTTATTGATATGC	(Zarrin & Erfaninejad, 2016)
ITS4-Reverse	TCCGTAGGTGAACCTGCGG	
AflR1-Forward	AGGCCACTAAACCCGAGTA	(Gallo *et al*., 2012)
AflR1-Reverse	AAGCTCCGGGATAGCTGTA	
AflS-Forward	TGAATCCGTACCCTTTGAGG	(Gallo *et al*., 2012)
AflS-Reverse	GGAATGGGATGGAGATGAGA	

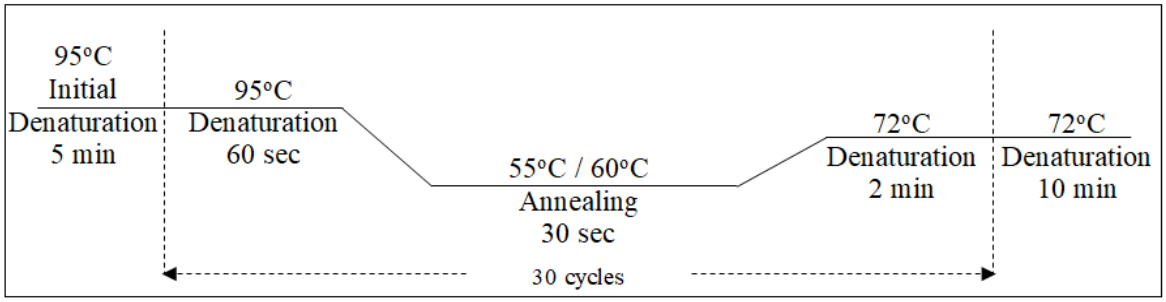


Figure 1: ITS, *aflR* and *aflS* PCR reaction map. The annealing temperature for ITS region was 55°C while for *aflR* and *aflS genes* it was 60°C

2.4. Aflatest fluorometer procedure for edible oil

Mycotoxin calibration standards was used for calibration of the fluorometer (Vicam, 1999). Yellow calibration standard reading was adjusted in the range of 22 ± 2, while, readings for purified water, mixture of methanol and water (60:40), and blank (1ml methanol and 1ml developer) was read 0ppb respectively in calibrated Fluorometer.

For the aflatoxin extraction from oil samples, 25g oil sample and 5g sodium chloride along with a 125 ml mixture of methanol and water (60: 40) were blended in a blender jar at high speed for one minute. The extract containing the mixture was poured into fluted filter paper, and the resulting filtrate was collected in a clean flask. Subsequently, 20mL of the extracted filtrate was transferred to a flask and diluted with 20mL of purified water. This diluted extract was then filtered through a 1.5 µm glass microfiber filter into clean flask.

To analyze the filtered and diluted extract (5 mL, equivalent to 0.5 g sample equivalent), it was passed entirely through an Afla test column, which consisted of Sepharose bound with an antibody against total aflatoxin. The passage of the extract through the column was controlled at the rate of 1-2 drops per second until air began to flow through the column. Following that, purified water (10mL) was passed through the column twice at the same controlled rate until air flow was observed.

A cuvette (VICAM part # 34000) was positioned under the column, and 1mL of methanol was added to a glass syringe barrel. The sample was eluted from the column at a controlled rate of 1-2 drops per second as 1ml of eluate in the cuvette. Subsequently, 1mL of Afla test developer was added to the eluted sample in cuvette. The contents of the cuvette were mixed thoroughly and placed in a calibrated fluorometer. After one minute, the concentration of aflatoxin was determined by reading the results from the fluorometer.

3. RESULTS

3.1. Aflatoxin detection by culturing method

The color change technique proved to be a valuable method for the early identification of aflatoxin-producing species of *Aspergilli*. In this experiment, the aflatoxigenic *Aspergillus* colonies were exposed to ammonium hydroxide vapors (AMHV) and color change is observed downside the plates. Among the four toxigenic isolates, only one plate, sample JB2 exhibited a notable color change from yellow to vibrant plum color, while the remaining showed minimal change on the SDA plate Figure 2(a-h). Furthermore, the Sample JB2 showed positive results on both YES media plate and CAM medium plate, indicating its aflatoxin-producing capabilities. On the other hand, other samples did not exhibit any color change on YES and CAM plates. Especially, sample JB2 also exhibited a positive response with blue fluorescence on the CAM plate under UV light, while the other samples did not display any response on CAM media plates. As a negative control, A. *niger* was used and showed no significant color change or fluorescence on any of plates.

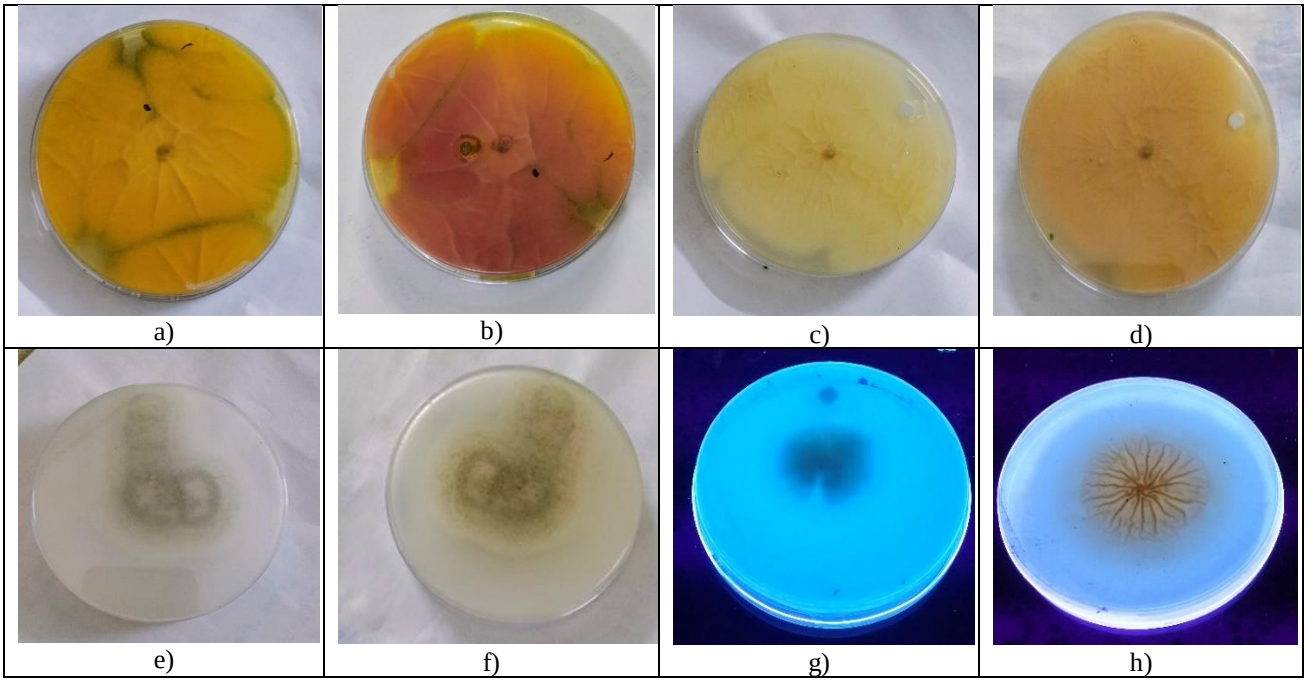
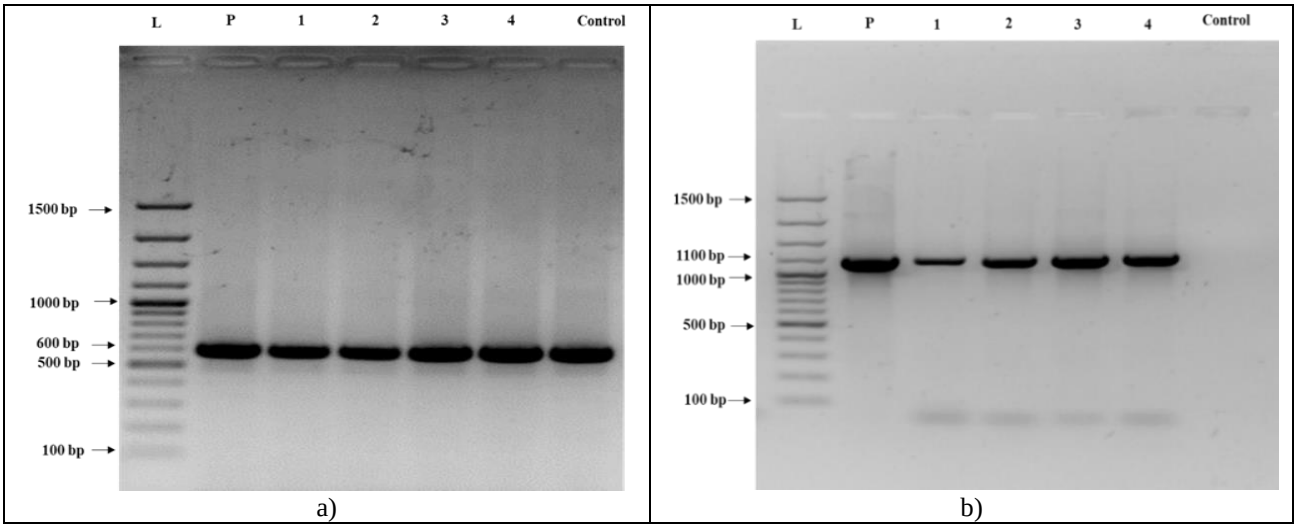
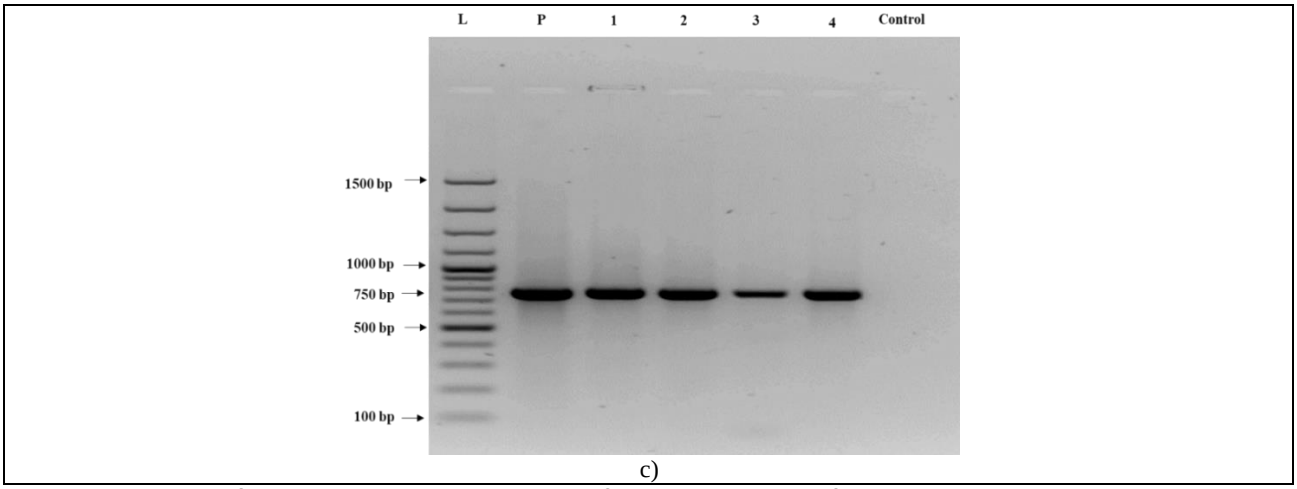


Figure 2: Aflatoxin production determination by the culturing method: a) SDA before AMHV exposure, b) SDA after AMHV exposure, c) YES before AMHV exposure, d) YES after AMHV exposure, e) CAM before AMHV exposure, f) CAM after AMHV exposure, g) CAM after UV exposure, h) *A. niger* on CAM after UV exposure

3.2. Molecular detection of aflatoxigenic Aspergilli

A total of thirty edible oil samples were collected and analyzed for the presence of aflatoxigenic *Aspergilli*. From these samples, only four showed positive growth on SDA and PDA plates, indicating the presence of aflatoxin-producing *Aspergilli*. These four positive samples were selected for further analysis, including DNA extraction and amplification of specific genetic regions. The ITS region and aflatoxigenic genes *aflR* an *aflS* were amplified by using the conventional PCR method. The PCR results for ITS region showed a product size of 600 base pair(bp), while the amplicon sizes *aflR* an *aflS* genes were 1100 bp and 750 bp, respectively Figure 3(a, b, c). The PCR reactions confirmed that all the samples that displayed growth on the media plates were indeed aflatoxigenic isolates, as indicated by the positive PCR results. To further characterize these isolates, the sequences of *A. flavus* JB2, MGM4, RLM2, and LMM1 were submitted to the GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). The provided accession number for these sequences are numbers are OQ674091, OQ674089, OQ674090, and OQ674092 respectively. These findings demonstrate the presence of aflatoxigenic *Aspergilli* in edible oil samples, as confirmed by both the growth on culture plates and the positive amplification of specific genetic markers. The submission of the sequences to the GenBank database allows for further reference and comparative analysis of these isolates.





c)

Figure 3: PCR amplification: a) ITS region (600bp), b) *aflR* gene (1100bp), c) *aflS* gene (750bp). Ladder 100bp, P= positive control, 1-4(Aflatoxigenic sample's DNA), control= negative control (*A.niger*)

3.3. Fluorometric estimation of aflatoxin

The results of the fluorometric analysis are summarized in Table 2, that displays the variable values of total aflatoxin in different oil samples. Out of thirty samples only one sample (3.33%) exhibited a total aflatoxin concentration between 20-30 ppb. While five samples (16.6%) had a total aflatoxin concentration below 20ppb, while the remaining twenty-four samples (80%) tested negative for aflatoxins.

Table 2: Detection of aflatoxins by the fluorometer

S. #	Sample Location	ID	Aflatoxin concentration (ppb)	S. #	Sample Location	ID	Aflatoxin concentration (ppb)
1.	Jodia Bazar	JB1	0	2.	Jodia Bazar	JB2	27
	JB1						
3.	Jodia Bazar	JB3	0	4.	Jodia Bazar	JB4	0
	JB3						
5.	Jodia Bazar	JB5	0	6.	Jodia Bazar	JB6	0
	JB5				JB6		
7.	Jodia Bazar	JB7	0	8.	Jodia Bazar	JB8	0
	JB7				JB8		
9.	Jodia Bazar	JB9	0	10.	Jodia Bazar	JB10	0
	JB9				JB9		
11.	Fried Item	FIS1	0	12.	Fried Item	FIS2	0
	Stall				Stall		
13.	Fried Item	FIS3C	5.5	14.	Fried Item	FIS4B	8
	Stall				Stal		
15.	Fried Item	FIS5	0	16.	Malir Gosht	MGM1	0
	Stall				Market		
17.	Malir Gosht	MGM2	0	18.	Malir Gosht	MGM3	0
	Market				Market		
19.	Malir Gosht	MGM	4.5	20.	Liaquat	LMM1	11
	Market				Market		
					Malir		
21.	Liaquat	LMM	20	22.	Liaquat	LMM3	0
	Market				Market		
	Malir				Malir		
23.	Liaquat	LMM4	0	24.	Liaquat	LMM5	0
	Market				Market		
	Malir				Malir		
25.	Quaidabad	QB1	0	26.	Quaidabad	QB2	0
27.	Runchor	RLM1	0	28.	Runchor	QLM2	4.9
	Line Market				Line Market		
29.	Water Pump	WPM1	0	30.	Water Pump	WPM2	0
	Market				Market		

3.4. Phylogenetic analysis

Phylogenetic cladogram was constructed using MEGA-X: molecular Evolutionary Genetic Analysis Version 11 to analyze the sequences of *A. flavus* JB2 MGM4, RLM2, and LMM1. The phylogenetic tree (Figure 4) revealed the three distinct cluster (A, B, and C) based on sequence similarities. Cluster A, the largest cluster, consisted of numerous strains within the phylogenetic tree, including *A. flavus* JB2. This indicates the JB2 shares a close genetic relationship with strains in cluster A. Cluster B, on the other hand, contained only the RLM2 strain, suggesting its distinct genetic lineage compared to other isolates. This indicate that RLM2 possesses unique genetic characteristics within the analyze strains. Cluster C consisted of the remaining two strains, MGM4 and LMM1. These strains showed similarity in their genetic sequences and were grouped together witin the cluster C.

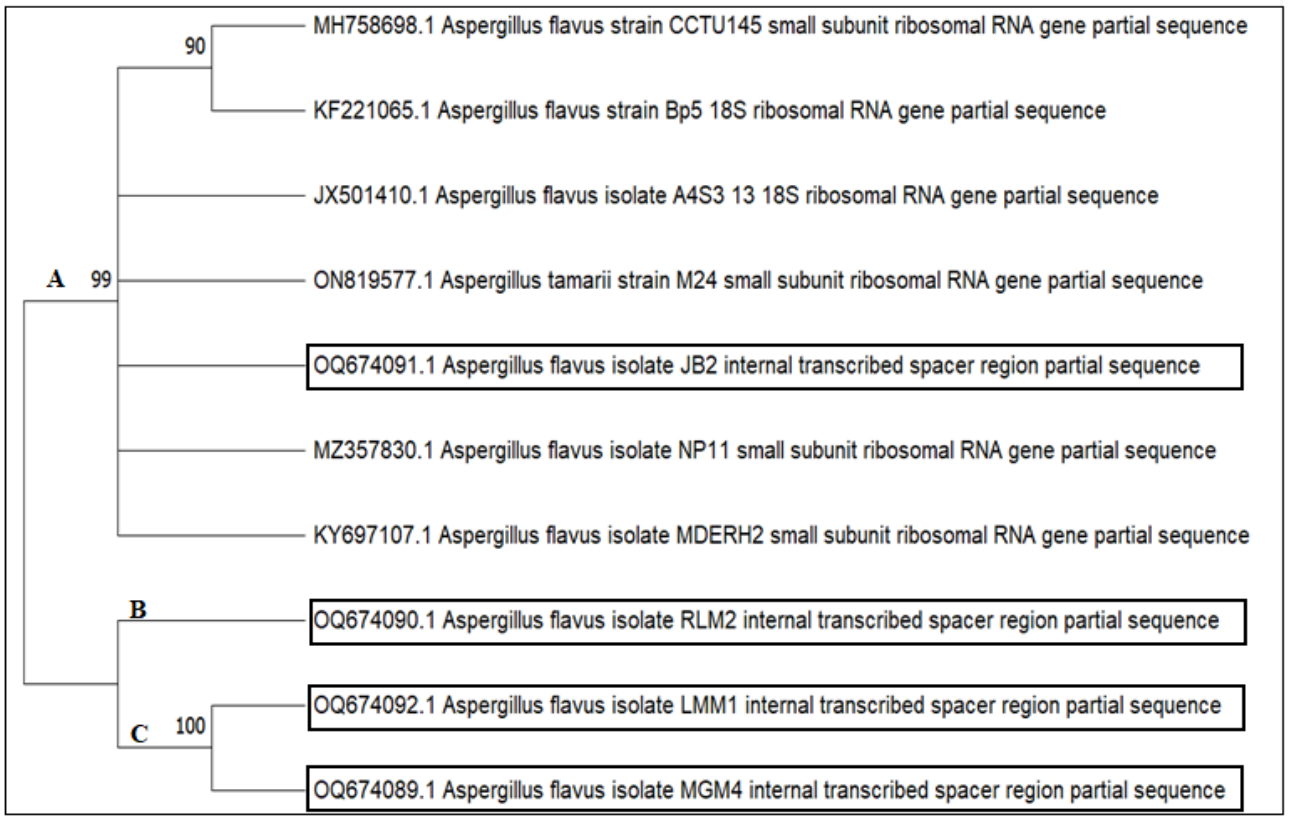


Figure 4: Phylogenetic cladogram of *A. flavus* JB2, MGM4, RLM2 and MLM1

4. DISCUSSION

Conventional procedures for the early and accurate detection of aflatoxigenic *Aspergilli* are most appropriate methods in the diagnostic field but are lengthy and critical. These conventional techniques help in the selection of appropriate methods for finding and identifying of aflatoxigenic *Aspergilli.* Therefore, morphological methods are best way of describing aflatoxigenic *Aspergilli* (Horn *et al*., 2009). In this study different media such as PDA, CZA, YES, CAM and SDA were used which enabled the ideal growth of the fungus and enabled consistent assessment. At the early stage, the white mycelium from the *A. flavus* colonies spread outward and cover the whole surface of media. The while mycelium turns yellow-green and becomes foggy or lumpy conidia when sporulation starts. The color transition initiates from the center of the colony and gradually spreads towards the edges, finally covering the whole surface. These colonies are flat with radial grooves, a fluffy center and a cream color on the reverse (Thathana *et al*., 2017). The appearance of colonies on CZA media of aflatoxigenic species is light yellow to parrot green and yellowish orange to peach on reverse while on YES it has been observed bright yellow on front and reverse too. The morphological observation in this study yielded similar results to the aforementioned studies.

Numerous studies utilized color change technique and have revealed that not all strains of *A. flavus* are capable of producing aflatoxin. To observe color change, several media including PDA, SDA, YES (yeast extract sucrose), CAM (coconut agar media), CZA (Czapeck Dox Agar), and GMS (glucose mineral salts) are utilized in ammonia exposure method. This method offers an easy and cost-effective pilot approach for detecting aflatoxigenic species in contaminated food and feed samples as compared to analytical and molecular techniques. However, the weakness of this technique lies in its lower sensitivity and specificity (Atanda *et al.*, 2011). In this study SDA, YES, CAM, and CZA media have been used to corelate the relation between color

change and aflatoxin production. Anthraquinone, an intermediate produced during the aflatoxin bioproduction, act as pH indicator turns yellow to plum red or red when exposed to a base such as ammonium hydroxide. A research conducted by (Saito & Machida, 1999) exposed that ammonia vapors induced a vibrant color change on CAM and YES, with lower intensity observed on PDA. In our study, all sample displayed a significant to negligible color transition on SDA, while sample JB2 exhibited insignificant color variation on YES and CAM and no transformation on CZA media. After exposing to UV light the colony either exhibited gray or black or displayed blue-green fluorescence, providing initial indication of aflatoxigenic potential, contrary the white colonies indicated a non-aflatoxigenic nature of molds (Abbas *et al*., 2004). The results of the current research for the sample JB2 are consistent with the aforementioned study regarding UV light experiment on CAM while others samples presented no outcomes.

Multiple research projects have confirmed that biosynthesis of aflatoxin is principally regulated by direct participation of pivotal genes, *aflR* and *aflS*. The AflR protein, classified as a zinc cluster Zn (II)2Cys6 Gla4-type regulatory protein, is 47kDa protein. The AflR protein binds to at least 17 genes within the aflatoxin cluster, triggering the enzymatic cascade and initiating the synthesis of various aflatoxins (Okayo *et al.*, 2020). The *aflS* gene, located adjacent to *aflR* gene in the aflatoxin biosynthesis cluster, serves as a crucial transcription enhancer for aflatoxin production. Remarkably, *aflR* also directs the regulation of *aflS*. *aflR* and *aflS* share approximately 737bp intergenic regions in duration of translation. *aflS* acts as co-activator and interacting with *aflR* but does not interact with biosynthetic enzymes in aflatoxin production (Caceres *et al*., 2020).

In this research, the AflR and AflS primers produced positive PCR results demonstrating better specificity towards the identification aflatoxigenic nature of the pure culture. Compared to morphological studies, PCR based identification of aflatoxigenic fungi offers greater reliability, sensitivity and precision (Alkhursan *et al*., 2021). In this study, PCR analysis targeting the *aflR* and *aflS* genes and yielded 100% positive results for all four samples, both genes are considered regulatory genes for aflatoxin production in the gene cluster at the transcriptional level. This indicates that PCR method is effective for detecting and identifying aflatoxigenic strains with high success rate (Manonmani, 2005). Though, some researches have indicated that the *aflR* gene in the genome of aflatoxigenic species does not play a significant role in aflatoxin production (Liu & Chu, 1998). On the contrary, according to (Paterson, 2006) it was confirmed that the *aflR* gene indeed regulates the bioproduction of sterigmatocystin in various aflatoxigenic *Aspergilli*. The results of the current study were consistent with the aforementioned research, as these strains not only exhibited amplification of *aflR* and *aflS* genes but also produced aflatoxins, as confirmed by cultural and analytical methods. The conventional and molecular identification of isolates further confirmed the fungal strains JB2, MGM4, RLM2, and LLM1 belonged to the species Aspergillus.

Moreover, the sequencing results of the ITS region, which is the highly conserved region. Within species, further confirmed that all the investigated aflatoxigenic species were identified as *A. flavus*. The phylogenetic cladogram offers understandings into the genetic relationship and clustering patterns among the analyzed *A. flavus* strains. It reveals the presence of the distinct genetic lineages represented by different clusters, with JB2 belonging to cluster A, RLM2 forming its own cluster (B), and MGM4 and LMM1 are grouped together in cluster C. These results improve our understanding of genetic diversity and associations among the *A. flavus* strains investigated in this study.

The results obtained from cultural and molecular methods in this study were verified using analytical techniques. These techniques include chromatographic, spectrophotometric, immunological methods to quantify the accurate concentration aflatoxin in different edible commodities. However, these techniques are often time-consuming, require specialized equipment, and can be expensive due to need for specific kits and reagents (Malone *et al*., 2000). In this study, a solvent extraction method was employed with fluorometric detection to quantify the concentration of aflatoxin in oil samples without dilution. The method involved the use of a methanol-water mixture as the solvent system. This solvent system is broadly documented as one of the most effective method for efficient aflatoxin extraction from the matrix.(Cole & Dorner, 1994) highlighted , it is crucial to ensure that the solvent system utilized for aflatoxin extraction should be suitable and compatible with the chosen analytical technique. Table 2 reveals that out of thirty sample only JB2 showed a significantly high level of aflatoxin at 27ppb, which was not significantly higher than the European regulation but also exceeded the FDA allowed value. The other three samples observed aflatoxin level ranging 4.5 – 11ppb, which were significantly lower compared to the FDA limit but still exceeded the limit allowed by European regulation. Table 3 displays the comprehensive results obtained from the various methods employed in this study. Among thirty Analyzed samples, four samples were identified as positive for aflatoxin contamination using cultural method. These four samples used for secondary analysis using the molecular method, and interestingly, the results of the molecular method also confirmed the presence of aflatoxin contamination that had tested positive through the cultural method. This consistency between the cultural and molecular method was measuring and indicated a strong relationship among these techniques. In the final step, all 30 samples subjected to analytical method for further investigation. Notably, the analytical

method detected aflatoxin contamination in two additional samples, FIS3 and FIS4, which had not been detected by either molecular or cultural methods. This outcome suggests that analytical method may have higher sensitivity or capability to detect a broad range of aflatoxin compounds as compared to the other methods.

Table 3: Detection of the aflatoxigenic nature of *Aspergilli* using ammonium hydroxide vapors, colony fluorescence, biosynthesis genes including *aflR*, *aflS* and estimation of aflatoxin

Isolate code	Aflatoxin levels in ppb	Gene-based detection of aflatoxin production		Culture-based detection of aflatoxins				
				Ammonium Hydroxide Vapor				UV Light
		aflR	*aflS*	SDA	YES	CAM	CZK	CAM
JB2	27	+	+	++++	+	+	-	++
MGM4	5	+	+	+	-	-	-	-
LMM1	11	+	+	+	-	-	-	-
RLM2	4.9	+	+	+	-	-	-	-
FIS3	5.5	_	_	_	_	_	_	_
FIS4	8	_	_	_	_	_	_	_

5. CONCLUSIONS

The findings indicate that edible oil is being sold in local Market of Karachi has been found to have been significantly high level of aflatoxin, exceeding EU regulation. Additionally, the oil is contaminated with various fungi, demonstrating inadequate hygiene practices throughout processing, packaging, and marketing stages. Consuming contaminated oil can pose serious health risks to consumers who are unaware of its quality. To address this issue, it is crucial to raise awareness among consumers, agriculturists, dealers, and foreign traders about toxic nature of aflatoxins. Monitoring and improving the conditions for processing, transporting and storing oil seeds and edible oil is necessary, including high quality packaging material and maintaining controlled humidity and temperature to prevent the growth of toxigenic fungi. Additionally, there is a need to enhance the quality control system to ensure the availability of safer edible oil in market.

Conflicts of interest: All authors of this manuscript have declared no conflict of interest.

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