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Screening of Aflatoxin and Non-Aflatoxin Producing Strains of *Aspergillus flavus* in Maize

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ABSTRACT

The present study aims to detect aflatoxin and non-aflatoxin producing ability of *A. flavus* isolates which were recovered from district of Punjab Province (Attock, Chakwal, Jhelum and Rawalpindi). The base of aflatoxin production was characterized using Thin Layer Chromatography and were successfully categorized into toxigenic (aflatoxin producing) and atoxigenic (non-aflatoxin producing) strains. Frequency of aflatoxins production of *A. flavus* isolates varied among the four districts of Punjab Province (Attock, Chakwal, Jhelum and Rawalpindi). Out of 212 *A. flavus* isolates, 200 isolates (94.34%) were found positive with aflatoxin B_1 and B_2 and these were classified as aflatoxin producing strains of *A. flavus* while 12 isolates (5.66%) did not show presence of any aflatoxin and these were classified as non-aflatoxin producing strains of *A. flavus*. However, AFG_1 and AFG_2 were not detected in any sample. The percent incidence of B_1 was highest in Rawalpindi region (95.23%) whereas Attock (95.12%), Chakwal and Jhelum

(93.18%) each. While AFB_2 were recorded as the highest in Rawalpindi region (76.19%) followed by Chakwal and Jhelum (68.18%) and Attock (60.97%).

KEYWORDS: Aflatoxin, TLC, Toxigenic, Atoxigenic, Pothwar

INTRODUCTION

Approximately 25-50% of the total crops are contaminated with mycotoxins of total crops harvested because of the environments in tropical areas; about 80% of the crops are damaged by mycotoxins in tropical regions. Among all mycotoxins, aflatoxins (AF) are the major mycotoxins that effect agricultural crops and public health (Mahmoud et al., 2014). Aflatoxins are produced by the *A. flavus* and *A. parasiticus*. These toxins are named from the fungus *A. flavus*, A from *Aspergillus* and 'fla' from *flavus*. There is diversity of aflatoxins that are categorized as aflatoxin B_1 and aflatoxin B_2 as they produce blue fluorescence under Ultra Violet light, aflatoxin G_1 and G_2 as they produce green fluorescence under Ultra Violet light (Richard, 2007).

Under most of the conditions of storage, processing of seeds and feeds made from contaminated seeds, aflatoxins are durable. It is very heat stable. Among aflatoxins, Aflatoxin B_1 is the most carcinogenic and diverse array of compounds. The toxic effects include acute hepatitis, immunosuppression, and hepatocellular carcinoma for human and animals (Richard et al., 2003). Atoxigenic are the isolates which are not able to produce aflatoxin are common within communities of *A. flavus* (Donner et al., 2010). Depending upon the concentration of dose intake, aflatoxins induce three types of effects; (1) lethal, when administered acutely in high amounts (2) histological abnormalities, when intake is sub-acutely in smaller amounts and (3) tumor formation, when administration is chronic (Riba et al., 2008; Qazi & Fayyaz, 2006). In addition to being carcinogens and toxins, aflatoxins are well known as mutagens and immunosuppressants (Yu et al., 2005).

There are major problems in maize, cotton, groundnuts and other nuts with aflatoxin contamination due to infections by *Aspergillus* species, especially *A. flavus* (Lewis et al., 2005). Maize contamination with aflatoxin results from growth of *A. flavus* in maize grains (Reddy and Reddy, 2011). *A. flavus* is frequently found

in crop debris and soil which is the main source for primary inoculum causing infections in maize (Horn, 2007). *A. flavus* isolates of differ in the production of aflatoxin, some of them producing numerous amounts while others are not producing (Atehnkeng et al., 2008). During the development of crop, contamination of aflatoxin can occur either when the crop is damaged by the microorganisms or stressed by drought and heat and exposure to high temperature and moisture after maturation either in storage conditions or before harvest (Bhatnagar et al., 2003). For supply of safe food and feed, in many countries, contamination of aflatoxin in maize grain is heavily monitored and regulated for the consumption human and animal feed (Food and Agriculture Organization (FAO), 2018). Various factors that affect the aflatoxin production include temperature, humidity, growth, culture medium, pH, carbon source, light, aeration and storage conditions. The optimal temperature for the growth of aflatoxins requires 24-30 ^{o}C (Anthony et al., 2012).

Contaminated crops are destroyed in developed countries (Razzaghi-Abyanehet al., 2008), but in the case of their entry into the food chain, they impart serious health effects on animals and contaminate their products such as meat and milk; consequently, these toxins provide health threats to humans also (Khlangwiset & Wu, 2010). These issues are even worse in developing countries like Pakistan, where no regulatory system exists for aflatoxin monitoring. For this reason, untested food-items become one of the basic causes of foodborne outbreaks in these countries. For instance, outbreaks due to aflatoxin contamination, such as those which occurred in 1977, 1980 and 1988 in USA, India and Kenya, respectively, are all examples of such food- related calamities (Kensler et al., 2011; Probst and Bandyopadhyay, 20014). Consequently, it can be evaluated that millions of dollars are lost annually as a consequence of the chaos that aflatoxins cause, because of their severe impacts on agriculture, animals and humans (Upadhaya et al., 2010).

Achakzai & Bazai, (2015) described that according to Pakistan Agricultural Research Council (PARC), food contamination issue is the major issue over the past years due to which many countries rejected a significant number of food items. The main reason for rejection of food items is contamination of mycotoxins in 35

cases. For the evaluation of aflatoxin production, various methods like UV based evaluation method, instrumental analysis, culture based method, serological and Thin Layer Chromatography are commomly used (Orsi et al., 2000; Castellari et al., 2010; Giorni et al., 2008). Present study is targeted to examine production of aflatoxin B_1, B_2, G_1 and G_2 in *A. flavus* isolated from maize kernels by using Thin Layer Chromatography.

MATERIALS AND METHODS

Preparation of Sample

The 212 *A. flavus* isolates were tested for the production of aflatoxins. *A. flavus* were isolated from maize kernels which were collected from four districts of Pothwar region, Pakistan (in-continuity with Seerat et al., 2022). For the screening of aflatoxin producing and non-aflatoxin producing strains of *A. flavus*, thin Layer Chromatography was used.

Chemicals and standards

The mixed standards of AFB_1, AFB_2, AFG_1 and AFG_2 were purchased from Romer Labs, Austria. The mixture consists of 2.02µg AFB_1, 0.508 µg AFB_2, 2.01 µg AFG_1 and 0.508 µg AFG_2 in 3 ml Acetonitrile. A series of working standards (1-32 ng ml-1) For AFB_1 and AFG_1 and 0.25-8 ng ml-1 for AFG_1 and AFG_2 were prepared. Organic solvents were of analytical grade and were purchased from Fisher-USA.

Aflatoxins analysis

Aflatoxins analysis was carried out according to the protocol of (Probst et al., 2014). Further, 20 g of undamaged kernels was autoclaved at 121 °C for 60 min. Kernels were inoculated with 100 µl of conidial suspension and incubated for 7 days at 31°C in the dark. After incubation, kernels were blended in 50 ml of 80% methanol in a laboratory blender. The homogenized mixture was filtered through Whatman filter paper and the filtrate was spotted directly onto thin-layer chromatography (TLC) plates (Figure 1) together with aflatoxin standards comprising a mixture of aflatoxin B_1, B_2, G_1 and G_2. Plates was developed in ethyl

ether–methanol–water with the ratio of (96:3:1), then air-dried TLC plates and aflatoxins were visualized under 365-nm Ultra Violet light 365nm.

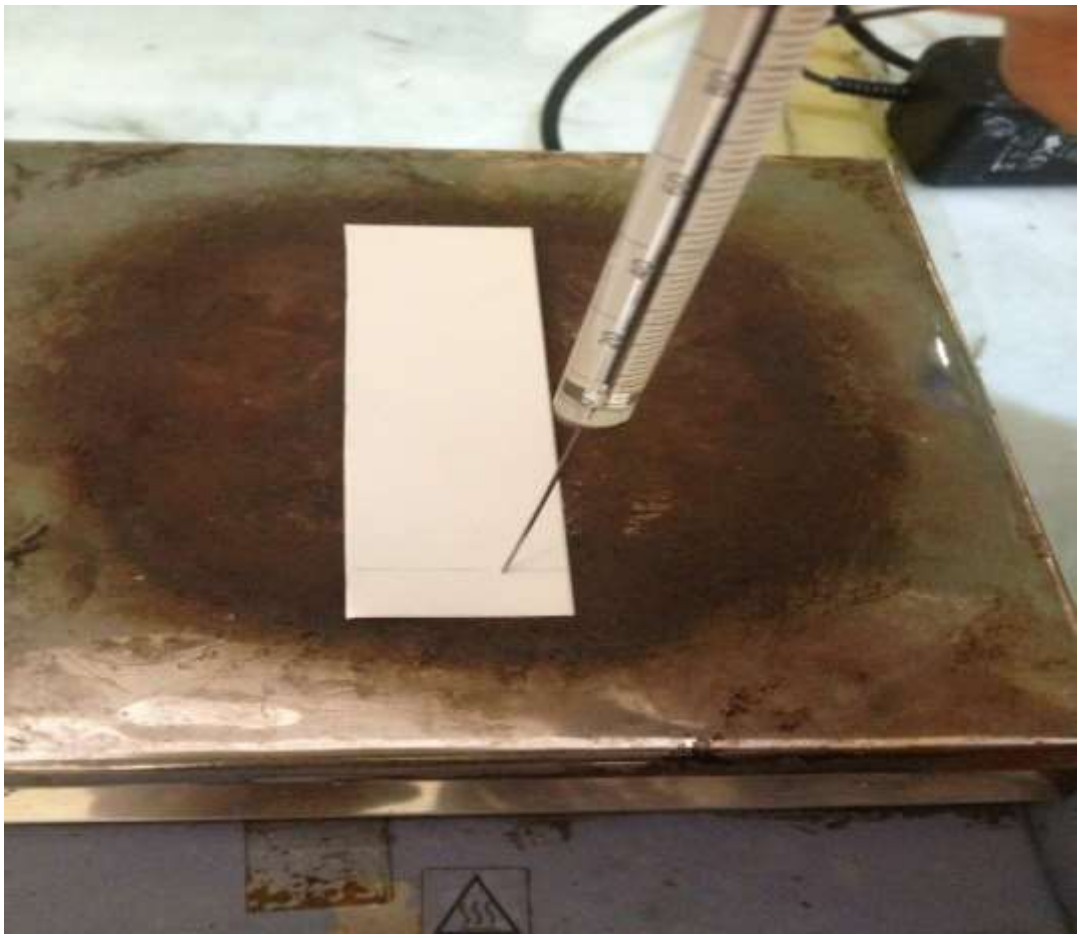

Figure 1: Inoculation of aflatoxins on TLC plate

RESULTS AND DISCUSSION

In the present study, level of aflatoxins production in *A. flavus* isolates was different among the four districts of Punjab Province (Attock, Chakwal, Jhelum and Rawalpindi). Out of 212 *A. flavus* isolates, 200 isolates were found positive with aflatoxin and these were recognized and characterized as aflatoxin producing strains of *A. flavus* (Figure 2) while 12 isolates did not show presence of any aflatoxin and these were recognized and characterized as non-aflatoxin producing strains of *A. flavus*. In accordance with present study, Khatoon et al. (2012) evaluated contamination of aflatoxins from maize grains collected from seven maize producing areas in Pakistan. They concluded that maize samples collected

from Punjab and Khyber Pakhtunkhwa were (57%) contaminated with aflatoxins. Muhammad et al. (2012) examined 40 maize samples for aflatoxin contamination in Pakistan and 34 out of 40 samples were contaminated with aflatoxin.

Aflatoxins B_1 producing strains

The percent incidence of aflatoxin B_1 (94.34%) was highest as compare to B_2 (66.98%) (Figure 4). In Rawalpindi region, highest percentage was observed (95.23%) followed by Attock (95.12%), Chakwal 93.18% and Jhelum 93.18% region (Figure 5). It was found that even small concentrations of AFB_1 halt mitotic divisions in lung cells of the human embryo. Moreover, about 2-6 mg/day ingestion of aflatoxins can lead to death by causing acute hepatitis (Lanyasunya et al., 2005).

Similar to our study, Shah et al. (2010) reported AFB_1 contamination in maize samples collected from areas of Swat district. They revealed the occurrence of AFB_1 in 83.33% maize samples. Similarly, Sabahat et al. (2010) found high ratio of aflatoxins 80%, 87% and 90% collected from central Punjab region. Waliyar et al. (2003) reported high level of contamination of AFB_1 from the maize collected from super markets and retail shops in Andhra Pradesh, Hyderabad, India. Rahimi et al. (2016) also reported highest amount of aflatoxin B_1 in 10 species of *A. flavus*.

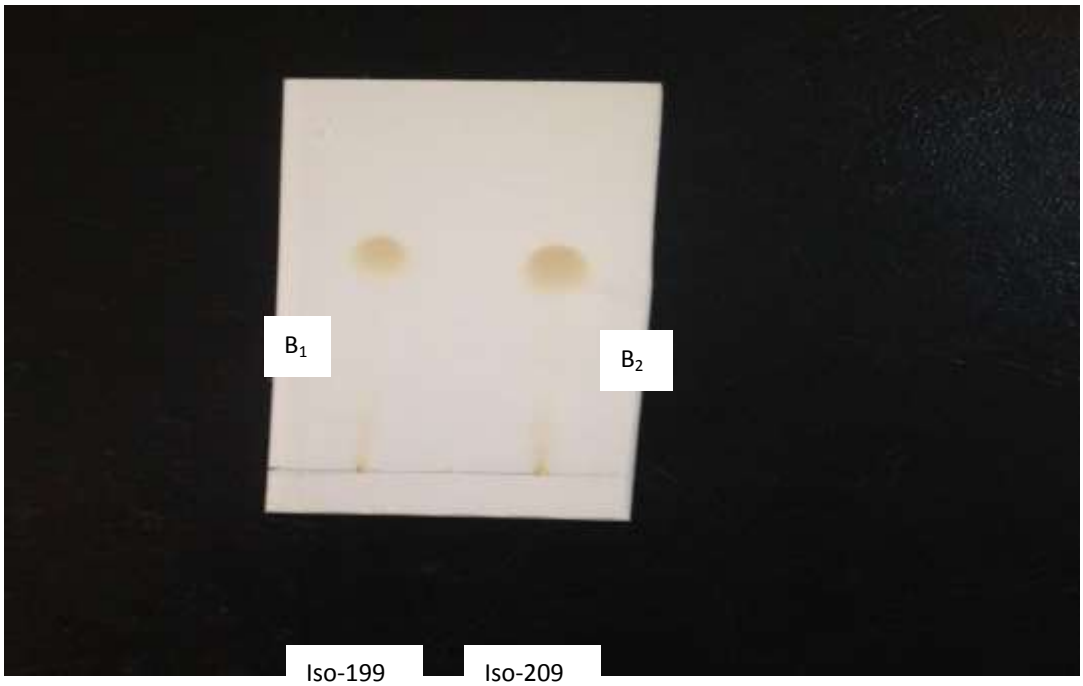

Figure 2: TLC plate showing aflatoxin B_1 and B_2

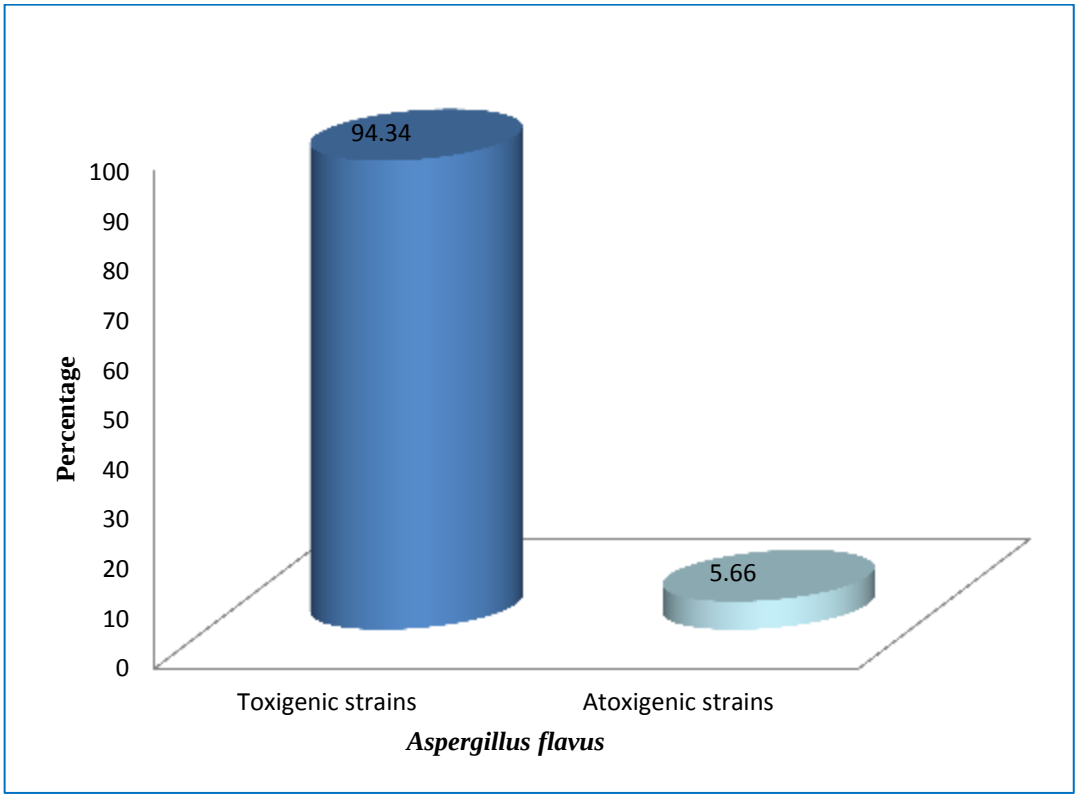

Figure 3: Overall percentage of toxigenic (aflatoxin producing) and atoxigenic (non-aflatoxin producing) strains of *A. flavus*

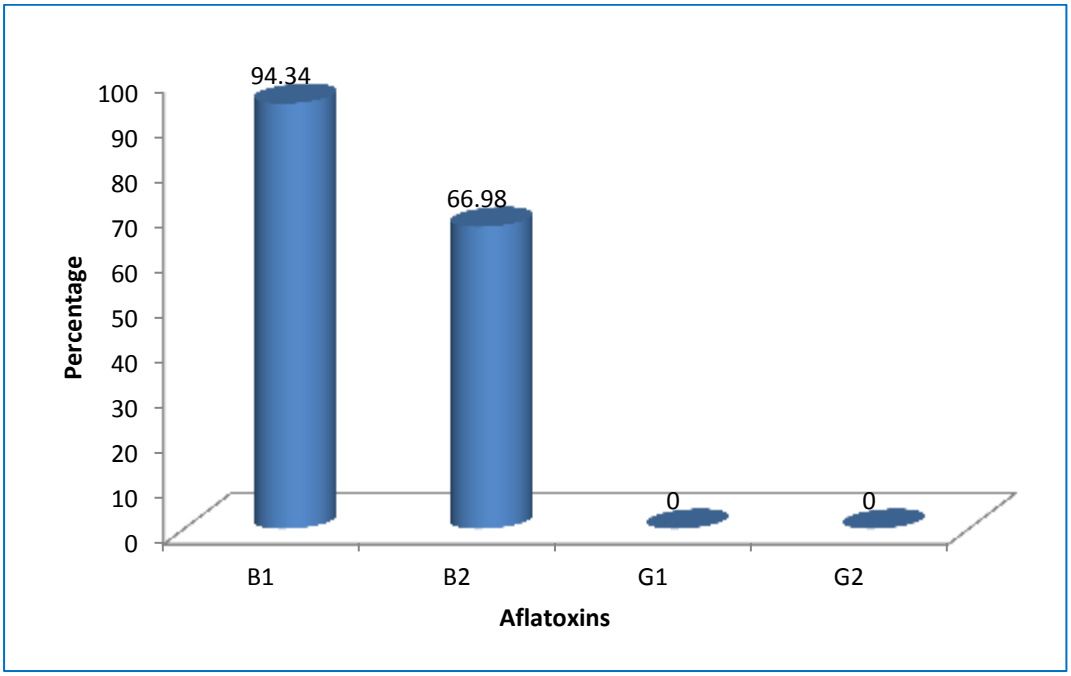

Figure 4: Overall percentage of aflatoxins B_1, B_2, G_1, G_2

Khatoon et al. (2012) reported AFB_1 with contamination level in 27% maize samples by using HP-TLC detection method. According to Iqbal et al.

(2019), aflatoxin B_1 contamination level was 0.04-3.5 µg/kg in maize products like breakfast cereal products. In continuity with this, Garcia-Cela et al. (2019) reported that aflatoxin contamination caused due to poor storage of maize grains. AFB_1 contamination was 56% in *A. flavus*.

Applications of TLC have been reported in areas of food composition, intentional additives, adulterants, contaminants, etc. (Sherma, 2000). Fuch et al. (2010) used TLC and HPTLC to check the ability of *A. flavus* for aflatoxin production. Similarly Klich (2007) used TLC technique for aflatoxin determination. In continuity to this, Lagogianni et al. (2019) tested aflatoxin production by toxigenic *A. flavus* in maize ear rots. They reported AFB_1 severity about 21.9% in maize kernels. Similarly, Choochuay et al. (2018) determined AFB_1 by using rapid method and reported highest level of contamination of AFB_1 in maize grains. Manzoor et al. (2018) determined toxigenic AFB_1 from maize samples by using High-Performance Liquid Chromatography (HPLC) with 61.1% aflatoxin contamination.

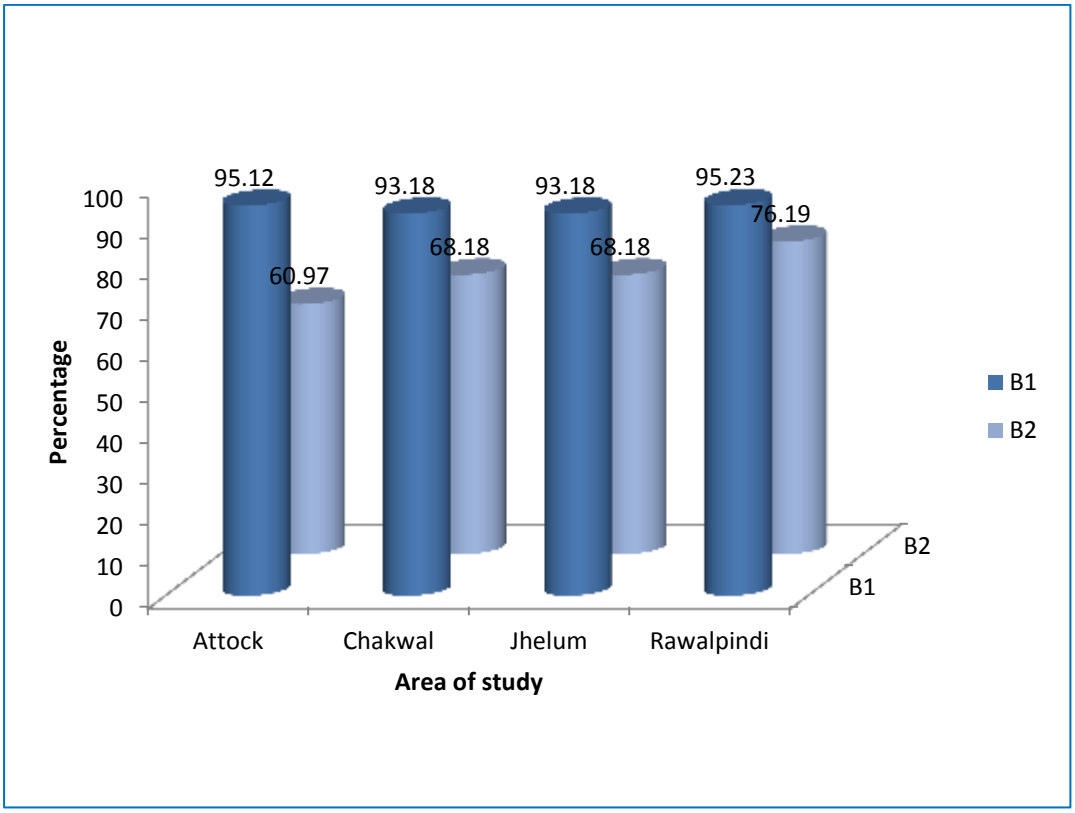

Figure 5: Percentage of aflatoxins B_1, B_2 in pothwar region

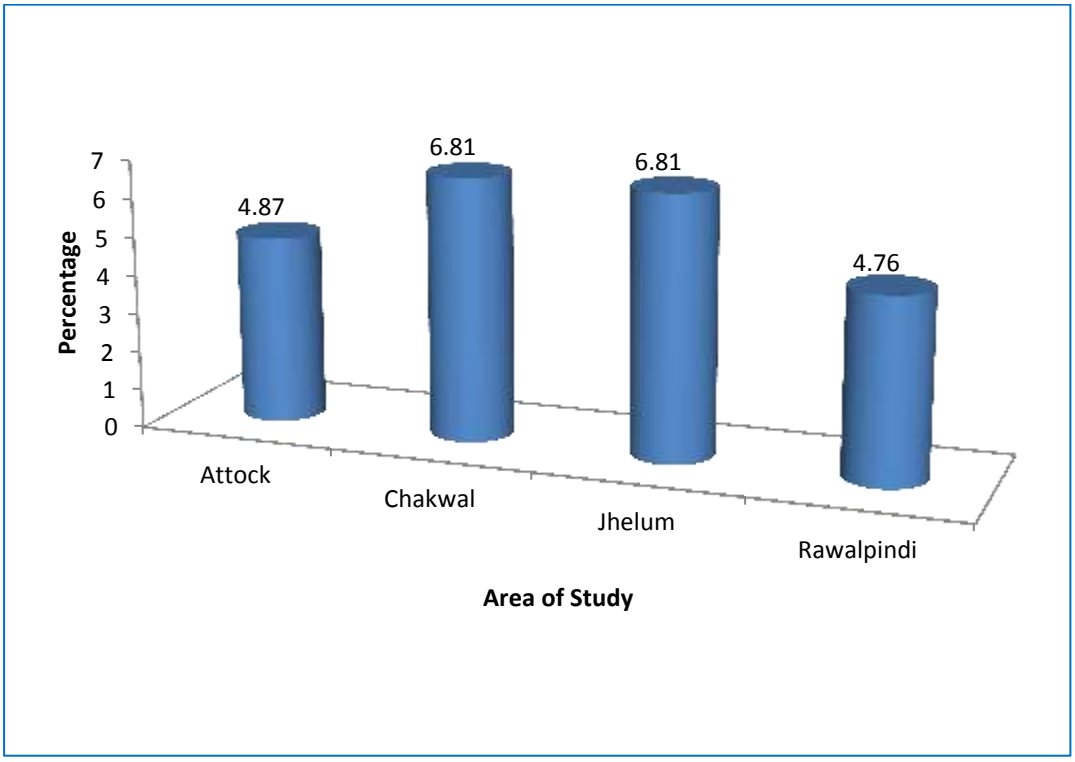

Figure 6: Percentage of non-aflatoxin producing strains of *A. flavus* in pothwar region.

Aflatoxins B_2 producing strains

Overall percentage of aflatoxin B_2 was (66.95%) (Figure 4). Aflatoxin B_2 were recorded highest in Rawalpindi (76.19%) followed by Chakwal and Jhelum (68.18%) each and Attock region (60.97%) region (Figure 5). Similar to our study, Zhou et al. (2019) detected aflatoxin B_1 and B_2 by using HPLC. In their work, high contamination level was detected by AFB_1 and AFB_2. In continuity to this, Ouakhssase et al. (2019), detected aflatoxin B_2 by using LC-MS/MS method and HPLC. Similarly, Iram et al. (2016), described aflatoxin contamination from maize grains of ecological zones of Punjab, Pakistan. Their results indicated that contamination of aflatoxin B_1 and B_2 was 78.9% and 97.3%. In continuity to this, Rahimi et al. (2016) reported presence of aflatoxin B_2 in all *A. flavus* species. Rocha et al. (2009) analyzed 200 corn samples for aflatoxin contamination. They concluded that 21 out of 200 (10.5%) were contaminated with AFB_1, 7 out of 200 (3.5%) contaminated with AFB_2 and only 1 out of 200 (0.5%) contaminated with G_1 and G_2.

Non-aflatoxigenic strains of *Aspergillus flavus*

Out of 212 isolates, 12 strains (5.66%) (Figure 3) did not show presence of any aflatoxin and these were classified as non-aflatoxin producing strains of *A. flavus* because they did not show any fluorescence under 365nm UV light. From Chakwal and Jhelum 3(6.81%) strains, Rawalpindi 2(4.76%) and Attock 4(4.87%) strains were observed as non-aflatoxin producing (Figure 6). Similar to our study, Aikore et al. (2019) reported atoxigenic *A. flavus* strains as biocontrol product from maize crop. This study was done in Nigeria where atoxigenic (non-aflatoxin producing) strains were used as biocontrol agents against toxigenic *A. flavus* strains. Hua et al. (2019) also described atoxigenic strains's application against toxigenic strains of *A. flavus* strains to compete each other. They identified aflatoxigenic and non-aflatoxigenic strains of *A. flavus* by using biosynthetic gene clusters and exposure under Ultra Violet light. Lanubile et al. (2017) described application of atoxigenic *A. flavus* strains in maize fields for controlling contamination of aflatoxins. They used defense genes against toxigenic strains of *A. flavus* in maize fields. Similar to our study, Teja et al. (2017) described non-aflatoxigenic strains of *A. flavus* in maize and groundnut samples in India.

Ruiqian et al., (2004) reviewed the biocontrol and degradation of *A. flavus* and aflatoxins, respectively. They demonstrated the significant role of atoxigenic strains of *A. flavus*, *Bacillus subtilis* and *Trichoderma* species in inhibiting the growth and *A. flavus*. Brown et al. (2003) have suggested using non-toxigenic strains of *A. flavus* to outcompete their aflatoxigenic relatives, as well as employing bioengineered resistant crops to prevent aflatoxin infestation. In addition, they have also provided certain useful antagonistic biocontrol approaches as a solution to this predicament of aflatoxins. Antagonistic and biodegradation strategies using harmless and useful microorganisms, isolated from different food matrices, and their enzymes can provide the best way to purge aflatoxins and aflatoxigenic *A. flavus* (Guan, 2011; Velmourougane et al., 2011).

Scherm et al. (2005) described that all the *A. flavus* isolates are not able to produce aflatoxins. Aflatoxigenic strains further encouraged screening for aflatoxin production abilities on the basis of presence of blue and green fluorescence on the undersides of the colonies ultra violet light, while non-aflatoxigenic strains do not

produce fluoresce under UV light.

CONCLUSION

Screening through Thin Layer Chromatography revealed the presence of 94.34% aflatoxin producing strains and 5.66% non-aflatoxin producing strains of *A. flavus*. High percentage of aflatoxin B_1 as compared to aflatoxin B_2 was observed from all regions of Pothwar plateau. Aflatoxin B_1 percentage was highest in Rawalpindi region followed by Attock, Chakwal and Jhelum. Further investigations are required on the molecular identification of aflatoxin producing and non-aflatoxin producing strains of *A. flavus*.

REFERENCES

Achakzai, A. K. K., & Bazai, Z. A. (2015). Occurrence and health hazard status of aflatoxin in human food and animal feed of wheat from Pakistan: A review paper. *Pure and Applied Biology*, *4*(4), 611.

Aikore, M. O., Ortega-Beltran, A., Eruvbetine, D., Atehnkeng, J., Falade, T., Cotty, P. J., & Bandyopadhyay, R. (2019). Performance of broilers fed with maize colonized by either toxigenic or atoxigenic strains of *Aspergillus flavus* with and without an aflatoxin-sequestering agent. *Toxins*, *11*(10), 565.

Anthony, M. H., Francis, D. M., Patrick, N., Berka, G. T. A., & Haruna, O. G. (2012). Aflatoxin contamination in foods and feeds: A special focus on Africa. *Trends in Vital Food and Control Engineering*, *187*.

Atehnkeng, J., Ojiambo, P. S., Donner, M., Ikotun, T., Sikora, R. A., Cotty, P. J., & Bandyopadhyay, R. (2008). Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. *International Journal of Food Microbiology*, *122*(1-2), 74-84.

Bhatnagar, D., Ehrlich, K. C., & Cleveland, T. E. (2003). Molecular genetic analysis and regulation of aflatoxin biosynthesis. *Applied Microbiology and Biotechnology*, *61*(2), 83-93.

Brown, R. L., Zhi-Yuan, C., Menkir, A., & Cleveland, T. E. (2003). Using biotechnology to enhance host resistance to aflatoxin contamination of corn. *African Journal of Biotechnology*, *2*(12), 557-562.

Castellari, C., Marcos Valle, F., Mutti, J., Cardoso, L., & Bartosik, R. (2010). Toxigenic fungi in corn (maize) stored in hermetic plastic bags. *Julius-Kuhn-Archiv*, (425), 501-504.

Choochuay, S., Phakam, J., Jala, P., Maneeboon, T., & Tansakul, N. (2018). Determination of aflatoxin B_1 in feedstuffs without clean-up step by high-performance liquid chromatography. *International Journal of Analytical Chemistry*.

Donner, M., Atehnkeng, J., Sikora, R. A., Bandyopadhyay, R., & Cotty, P. J. (2010). Molecular characterization of atoxigenic strains for biological control of aflatoxins in Nigeria. *Food Additives and Contaminants*, *27*(5), 576-590.

Food and Agriculture Organization (FAO). 2018. Integrated management of the fall armyworm on maize**. A** Guide for Farmer Field Schools in Africa.

Fuchs, B., Sub, R., Teuber, K., Eibisch, M., & Schiller, J. (2011). Lipid analysis by thin-layer chromatography - a review of the current state. *Journal of Chromatography A*, *1218*(19), 2754-2774.

Garcia-Cela, E., Kiaitsi, E., Sulyok, M., Krska, R., Medina, A., Petit Damico, I., & Magan, N. (2019). Influence of storage environment on maize grain: CO2 production, dry matter losses and aflatoxins contamination. *Food Additives and Contaminants: Part A*, *36*(1), 175-185.

Giorni, P., Battilani, P., & Magan, N. (2008). Effect of solute and matric potential on in vitro growth and sporulation of strains from a new population of *Aspergillus flavus* isolated in Italy. *Fungal Ecology*, *1*(2-3), 102-106.

Guan, D., Li, P., Zhang, Q., Zhang, W., Zhang, D., & Jiang, J. (2011). An ultra sensitive monoclonal antibody-based competitive enzyme immunoassay for aflatoxin M1 in milk and infant milk products. *Food Chemistry*, *125*(4), 1359-1364.

Horn, B. W. (2007). Biodiversity of *Aspergillus* section Flavi in the United States: a review. *Food Additives and Contaminants*, *24*(10), 1088-1101.

Hua, S. S. T., Parfitt, D. E., Sarreal, S. B. L., & Sidhu, G. (2019). Dual culture of atoxigenic and toxigenic strains of *Aspergillus flavus* to gain insight into

repression of aflatoxin biosynthesis and fungal interaction. *Mycotoxin Research*, *35*(4), 381-389.

Iqbal, N., Hussain, S., Ahmed, Z., Yang, F., Wang, X., Liu, W., ... & Liu, J. (2019). Comparative analysis of maize–soybean strip intercropping systems: a review. *Plant Production Science*, *22*(2), 131-142.

Iram, W., Anjum, T., Iqbal, M., Ghaffar, A., & Abbas, M. (2016). Structural elucidation and toxicity assessment of degraded products of aflatoxin B_1 and B2 by aqueous extracts of *Trachyspermum ammi*. *Frontiers in Microbiology*, *7*, 346.

Kensler, T. W., Roebuck, B. D., Wogan, G. N., & Groopman, J. D. (2011). Aflatoxin: a 50-year odyssey of mechanistic and translational toxicology. *Toxicological Sciences*, *120*(suppl_1), S28-S48.

Khatoon, S., Hanif, N. Q., Tahira, I., Sultana, N., Sultana, K., & Ayub, N. (2012). Natural occurrence of aflatoxins, zearalenone and trichothecenes in maize grown in Pakistan. *Pakistan Journal of Botany*, *44*(1), 231-236.

Khlangwiset, P., & Wu, F. (2010). Costs and efficacy of public health interventions to reduce aflatoxin-induced human disease. *Food Additives and Contaminants*, *27*(7), 998-1014.

Klich, M. A. (2007). *Aspergillus flavus*: the major producer of aflatoxin. *Molecular Plant Pathology*, *8*(6), 713-722.

Lagogianni, C. S., & Tsitsigiannis, D. I. (2019). Effective biopesticides and biostimulants to reduce aflatoxins in maize fields. *Frontiers in Microbiology*, *10*, 2645.

Lanubile, A., Maschietto, V., Borrelli, V. M., Stagnati, L., Logrieco, A. F., & Marocco, A. (2017). Molecular basis of resistance to Fusarium ear rot in maize. *Frontiers in Plant Science*, *8*, 1774.

Lanyasunya, T. P., Wamae, L. W., Musa, H. H., Olowofeso, O., & Lokwaleput, I. K. (2005). The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. *Pakistan Journal of Nutrition*, *4*(3), 162-169.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., ... & Kenya Aflatoxicosis Investigation Group. (2005). Aflatoxin

contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environmental Health Perspectives*, *113*(12), 1763-1767. doi:10.1289/ehp.7998.

Mahmoud, M. A., Ali, H. M., El-Aziz, A. R. M., Al-Othman, M. R., & Al-Wadai, A. S. (2014). Molecular characterization of aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* isolates collected from corn grains. *Genetics and Molecular Research*, *13*, 9352-9370.

Manzoor, M., Farooq, Z., Iqbal, S., Mukhtar, H., & Nawaz, M. (2018). Quantification of aflatoxins in maize samples collected from various parts of the Punjab, Pakistan. *JAPS: Journal of Animal and Plant Sciences*, *28*(6).

Muhammad, D., Chand, N., Khan, S., Sultan, A., & Mushtaq, M. (2012). Hepatoprotective Role of Milk Thistle (*Silybum marianum*) in Meat Type Chicken Fed Aflatoxin B 1 Contaminated Feed. *Pakistan Veterinary Journal*, *32*(3), 443-446.

Orsi, R. B., Corrêa, B., Possi, C. R., Schammass, E. A., Nogueira, J. R., Dias, S. M., & Malozzi, M. A. (2000). Mycoflora and occurrence of fumonisins in freshly harvested and stored hybrid maize. *Journal of Stored Products Research*, *36*(1), 75-87.

Ouakhssase, A., Chahid, A., Choubbane, H., Aitmazirt, A., & Addi, E. A. (2019). Optimization and validation of a liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of aflatoxins in maize. *Heliyon*, *5*(5), e01565.

Probst, C., Bandyopadhyay, R., & Cotty, P. J. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*, *174*, 113-122.

Probst, C., Bandyopadhyay, R., & Cotty, P. J. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology*, *174*, 113-122.

Qazi, J. I., & Fayyaz, Z. (2006). Aflatoxin contaminated foods and health risk perspective for Pakistani population. *Mycopathologia*, *4*(2), 27-34.

Rahimi, S., Sohrabi, N., Ebrahimi, M. A., Tebyanian, M., Taghyzadeh, M., & Rahimi, S. A. (2016). Studying the effect of aflatoxin genes Aflp and Aflq on *Aspergillus flavus* and *Aspergillus parasiticus* in the cattle feed used in industrial animal husbandries. *Acta Medica Austriaca*, *32*, 2091.

Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Yoshinari, T., Rezaee, M. B., Jaimand, K., Nagasawa, H., & Sakuda, S. (2008). Inhibitory effects of Satureja hortensis L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. *International Journal of Food Microbiology*, *123*(3), 228-233.

Reddy, E. S., Sudhakar, C., & Reddy, N. E. (2011). Aflatoxin contamination in groundnut induced by *Aspergillus flavus* type fungi: a critical review. *International Journal of Applied Biology and Pharmaceutical Technology*, *2*(2), 180-192.

Riba, A., Mokrane, S., Mathieu, F., Lebrihi, A., & Sabaou, N. (2008). Mycoflora and ochratoxin A producing strains of *Aspergillus* in Algerian wheat. *International Journal of Food Microbiology*, *122*(1-2), 85-92.

Richard, J. L. (2007). Some major mycotoxins and their mycotoxicoses—An overview. *International Journal of Food Microbiology*, *119*(1-2), 3-10.

Richard, J. L., Payne, G. A., Desjardins, A. E., Maragos, C., Norred, W. P., & Pestka, J. J. (2003). Mycotoxins: risks in plant, animal and human systems. *CAST Task Force Report*, *139*, 101-103.

Rocha, L. O., Nakai, V. K., Braghini, R., Reis, T. A., Kobashigawa, E., & Corrêa, B. (2009). Mycoflora and co-occurrence of fumonisins and aflatoxins in freshly harvested corn in different regions of Brazil. *International Journal of Molecular Sciences*, *10*(11), 5090-5103.

Ruiqian, L., Qian, Y., Thanaboripat, D., & Thansukon, P. (2004). Biocontrol of *Aspergillus flavus* and aflatoxin production. *Current Applied Science and Technology*, *4*(1), 238-250.

Sabahat, A., Bhatti, I. A., Asi, M. R., Bhatti, H. N., & Sheikh, M. A. (2010). Occurrence of aflatoxins in maize grains from central areas of Punjab, Pakistan. *International Journal of Agriculture and Biology*, *12*(4), 571-575.

Scherm, B., Palomba, M., Serra, D., Marcello, A., & Migheli, Q. (2005). Detection of transcripts of the aflatoxin genes aflD, aflO, and aflP by reverse transcription polymerase chain reaction allows differentiation of aflatoxin-producing and non producing isolates of *Aspergillus flavus* and *Aspergillus parasiticus*. *International Journal of Food Microbiology*, *98*(2), 201-210.

Seerat, W., Akram, A., Qureshi, R., Yaseen, G., Mukhtar, T., & Hanif, N. Q. (2022). Light and scanning electron microscopic characterization of aflatoxins producing *Aspergillus flavus* in the maize crop. *Microscopy Research and Technique*, *85*(8), 2894-2903.

Shah, H. U., Simpson, T. J., Alam, S., Khattak, K. F., & Perveen, S. (2010). Mould incidence and mycotoxin contamination in maize kernels from Swat Valley, North West Frontier Province of Pakistan. *Food and Chemical Toxicology*, *48*(4), 1111-1116.

Sherma, J. (2000). Thin-layer chromatography in food and agricultural analysis. *Journal of Chromatography A*, *880*(1-2), 129-147.

Teja, R. M., Vijay Krishna Kumar, K., Srilakshmi, P., Sudini, H., Kishore Varma, P., & Koteswara Rao, S. R. (2017). Prevalence of *Aspergillus flavus* Infection and Aflatoxin Contamination of Groundnut in Telangana and Andhra Pradesh. *International Journal of Pure and Applied Bioscience*, *5*(5), 1603-1614.

Upadhaya, S. D., Park, M. A., & Ha, J. K. (2010). Mycotoxins and their biotransformation in the rumen: a review. *Asian-Australasian Journal of Animal Sciences*, *23*(9), 1250-1260.

Velmourougane, K., Bhat, R., Gopinandhan, T. N., & Panneerselvam, P. (2011). Management of *Aspergillus ochraceus* and Ochratoxin-A contamination in coffee during on-farm processing through commercial yeast inoculation. *Biological Control*, *57*(3), 215-221.

Waliyar, F., Reddy, S. V., Subramaniam, K., Reddy, T. Y., Devi, K. R., Craufurd, P. Q., & Wheeler, T. R. (2003). Importance of mycotoxins in food and feed in India. *Aspects of Applied Biology*, *68*, 147-154.

Yu, J. H., & Keller, N. (2005). Regulation of secondary metabolism in filamentous fungi. *Annual Review of Phytopathology*, *43*, 437-458.

Zhou, R., Liu, M., Liang, X., Su, M., & Li, R. (2019). Clinical features of aflatoxin B_1-exposed patients with liver cancer and the molecular mechanism of aflatoxin B_1 on liver cancer cells. *Environmental Toxicology and Pharmacology*, *71*, 103225.