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Isolation and Identification of Aflatoxins from Maize (*Zea mays. L*) and their Biocontrol

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Maize (*Zea mays*) is supreme major agricultural commodity throughout worldwide after rice and wheat from the point of views of production, consumption and trade. FAO estimated that 25% worlds agricultural crop is annually affected with mycotoxins contaminations. Climatic alterations effect maize production in unevenly 70 percent of maize cultivating areas. In Pakistan, economically agriculture has the principal zone from point of view from labor and population employment. The growth of agriculture sector in Pakistan is 2.67%, greater from 0.58% achieved last year. Maize yield is 6.0% have 7.236 million tonnes. It contributes in national GDP is 0.6% and in agriculture the value addition is 2.9%. In 2019-20 the cultivation of maize was 1,413 thousand hectares with 2.9% increase from last year 1,374 thousand hectares. Contamination of commodities by mycotoxins is the worldwide issue. Mycotoxin is the chief health threats for humans and animals that cause important economic damages in both developed and developing countries and also economic losses to both crop growers and traders. Among numerous mycotoxins, aflatoxins, ochratoxin A, trichothecenes (deoxynivalenoland T-2 toxin), zearalenone, and fumonisins have established greatly attention due to the high incidence and severe health effects in humans and animals. This study aimed to evaluate aflatoxin contamination in maize seeds collected from multiple districts in Punjab, Pakistan, including Faisalabad, Kahror Pakka, Jhang, Chiniot, Lodhran, Sargodha, Gojra, Sahiwal, Arifwala, Narowal, Mailsi, and Shakargarh. Seed samples were obtained from seed shops, growers, and research institutes, covering a diverse range of maize genotypes. The seeds were surface-sterilized using 0.1% sodium hypochlorite (NaOCl) solution and rinsed with sterile distilled water. Fungal isolation was performed using Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), and Coconut Agar Medium (CAM). Preliminary findings indicate the widespread occurrence of Aspergillus flavus and Aspergillus parasiticus, the primary producers of aflatoxins, in the collected maize samples. These results highlight the need for continuous monitoring and development of effective management strategies to mitigate mycotoxin contamination and ensure food safety.

Keywords: Mycotoxins, ochratoxin A, trichothecenes (deoxynivalenoland T-2 toxin), zearalenone, fumonisins, aflatoxins and sodium hypochlorite.

INTRODUCTION

Maize (*Zea mays*) is major agricultural commodity throughout worldwide after rice and wheat from the point of views of production, consumption and trade (CDC, 2004). FAO estimated that 25% worlds agricultural crop is annually affected with mycotoxins contaminations (Smith *et al*., 2016). Maize is cultivated at all over places throughout the world (Araus *et al*., 2012). Maize grain production has increased about 2 percent every year since 1930s keen on the 21st, by the development of this credited improved abiotic and biotic anxiety easiness by the developments mainly through genetically about 50-60% (Duvick 2005; Araus *et al*., 2012; Tollenaar & Lee 2006) and field activities remain liable in the residue (Araus *et al*., 2012; Tollenaar & Lee 2006). This is significant to state that the management and breeding interrelates to everyone, there is no aspect that higher the yield himself (Duvick 2005).

Globally climatic changes may reason and improve abiotic anxieties for example drought conditions that unpleasantly stimulus the crops and decrease production. Climatic alterations effect maize production in unevenly 70 percent of maize cultivating areas (Ray *et al*., 2015) therefore establishing a significant food safety concern. (Ray *et al*., 2015) distinguished that the raised atmosphere carbon dioxide ranks remain expected toward counterbalance produce

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decrease owing toward deteriorations and higher temperature in moisture content of soil. Yet, scarcity lack, the yield of maize is not estimated toward rise through increasing carbon dioxide (Leakey *et al*., 2006). Hence, emerging weather tough genotypes of crop remains imperious toward confirm worldwide food safety (Mickelbart *et al*., 2015). Maize is range tropical to southern and northern temperate regions has encouraged (Gore *et al*., 2009) the propose in coming time in maize development determination need plus germplasm throughout whole world to get benefit from genetic data connected with climatic variations. Food security is the main issue now a days facing the whole world and in a number of studies various methods discussed and developed for safe food commodities (Nielsen *et al*., 2009). According to World Health Organization (WHO) and Food and Agricultural Organization (FAO) the presence of many fungal toxins found with in agricultural commodities. Once mycotoxins contaminate the food, they cannot damage or destroyed through regular cooking methods. Though many advance cooking methods have been developed for safe the food products (Cusato *et al*., 2013; Lockis *et al*., 2011). In Pakistan economically agriculture has the principal zone from point of view from labor and population employment which depends indirectly or directly connected with this sector. Though, from past some years the agriculture support is decreased 19.3%. Agriculture sector performance improved from past year and too good performance from further segments (GOP, 2020). The growth of agriculture sector in Pakistan is 2.67%, greater from 0.58% achieved last year. Maize yield is 6.0% have 7.236mollion tonnes (GOP, 2020).

In Pakistan, the third most important cereal crop is maize next to rice and wheat. It contributes in national GDP is 0.6% and in agriculture the value addition is 2.9%. In 2019-20 the cultivation of maize was 1,413 thousand hectares with 2.9% increase from last year 1,374 thousand hectares. The maize production increased 6.0% as 7.236 millions tones as associated previous years yield is 6.826 millions tones. This increase in yield has due to rise in maize cultivated area and convenience of seeds of better-quality varieties (GOP, 2020). Contamination of commodities by mycotoxins is the worldwide issue. Mycotoxin is the chief health threats for humans and animals that cause important economic damages in both developed and developing countries and also economic losses to both crop growers and traders. Among numerous mycotoxins, aflatoxins, ochratoxin A, trichothecenes (deoxynivalenoland T-2 toxin), zearalenone, and fumonisins have established greatly attention due to the high incidence and severe health effects in humans and animals (Bhat *et al*., 2010). Aflatoxin, existence single of the greatest abundant mycotoxins in daily food merchandises is of highest status owing to its hepatotoxic, mutagenic and carcinogenic producing characteristics (Bennett & Klich, 2003) with the widespread financial losses caused by aflatoxin impurity. Food and animal feed contaminated by mycotoxins, aflatoxin is the main food poison that harms humans and animals body mechanism (Boutrif, 1998). Range of poisonousness (toxicity) differs in aflatoxins kinds such as Aflatoxin B1 greater than Aflatoxin G1 greater than Aflatoxin B2 greater than Aflatoxin G2 (Jaimez *et al*., 2000). In 1974, in Gujrat and Rajasthan states in India by using food contaminated with aflatoxins which causes an epidemic of hepatitis results in 106 deaths (Krishna machari *et al*., 1975). This epidemic is lasted for the time of two month and were conformed in ethnic peoples those were major main diet is maize having aflatoxins (Bhatt & Krishna machari, 1978; Krishna machari *et al*., 1975). Additional epidemic of the aflatoxin contamination found in both in humans and animals like dogs, stated from India in northwest region during 1974 (Bhatt & Krishnamachari, 1978; Reddy & Raghavender, 2007). An foremost aflatoxin introduction epidemic were later recognized from kenya during the year of 1981 (Ngindu *et al*., 1982). From 2004 many aflatoxicosis epidemics were identified throughout the world, causing 500 severe infections and 200 deaths Centers for Disease Control and Prevention (Azziz-Baumgartner *et al*., 2005; CDC, 2004). During 1981 presence of the aflatoxins identified as Initial identification from food in affected regions (Ngindu *et al*., 1982). Hence, in maize the natural occurrence of aflatoxins contamination has important consequences on both global trade and human health. Aflatoxins principally formed through fungus *Aspergillus parasiticus* and *Aspergillus flavus*, and effect various essential foods like maize, groundnuts and tree nuts. Aflatoxins are most dominant in significant harvests from subtropical to tropical regions in the world. About five billion peoples throughout the world are exposed by not controllable aflatoxins from their food (Strosnider *et al*., 2006). Severe aflatoxin causes hepatitis, stunted growth in children, jaundice and gastrointestinal damages through high illness (CDC, 2004). Continued chronic revelation is supposed to raise the danger for hepatocellular carcinoma (Marcel & Wild, 1995; Julia, 2005). In several portions of the world especially in Asia and sub-Saharan Africa, peoples are showing both high status of aflatoxin and hepatitis B virus (HBV) infection which has been exposed to significantly rise liver cancer threat (Qian *et al*., 1994). Mostly, *A. flavus* originates in interaction through crops earlier harvest, though it leftovers accompanying with the crop through harvest and storage (Lillehoj, 1987). Aflatoxins are also formed in different cereal, nuts and oilseeds species (Lancaster *et al*., 1961; Reddy *et al*., 2010; Iqbal *et al*., 2014). Due to inappropriate storage conditions the aflatoxin contamination occurs in barley or wheat (Jacobsen, 2008). From milk aflatoxins are commonly from 1 to 6 percent into the whole of feeds (Jacobsen, 2008). Aflatoxin infection in human beings resulting from the ingestion of foods polluted with aflatoxins like egg and products of meat, milk and produces of milk (Bennett & Klich, 2003). Agriculture crops like cocoa beans, linseeds, sunflower seeds and seeds of melon are

infrequently polluted (Bankole *et al*., 2010). Due to severe effects of aflatoxins on food and animal feed in 2008 kept on quick alert system from European Union (European Commission, 2009), and latterly B1 aflatoxin categorized in group 1 poison in human beings through International Agency for Research on Cancer (IARC, 2002; Min *et al*., 2011).

Aflatoxin toxicity of liver is a serious concern (Iqbal *et al*., 2014; IARC, 2002). The aflatoxin toxicity expressions are regulated through factors like species, sex, nutrition status, and age of septic animal (Williams *et al*., 2004). Acute aflatoxicosis indications comprises on liver haemorrhagic necrosis, profound lethargy and oedema though results of acute aflatoxicosis in the form of growth retardation, cancer and immune suppression (Cotty & Jaime Garcia, 2007; Gong *et al*., 2004; Williams *et al*., 2004). Aflatoxin are extremely poisonous products formed through numerous species of aspergillus (McKean *et al*. 2006; Klich, 2007). Numerous going on aflatoxins and supreme poisonous, B1 aflatoxin (AFB1), is the genotoxic categorized through International Agency for Research on Cancer (IARC) the 1A group as humans beings poison (IARC, 2002). Continuing disclosure of minute aflatoxin quantities might results in destruction of immune system and cancerous effects has connected with development obstacle. Digestion of higher quantities, occasionally above one ppm which caused severe signs such as liver necrosis, hepatitis, and death (Cardwell & Henry, 2006). In many states, aflatoxins quantities in feed and food are severely controlled (Payne & Yu, 2010). Liver organ are particularly targeted by aflatoxin (Abdel Wahhab *et al*., 2007). The liver hepatotoxicity primary signs are fever, anorexia and malaise monitored by hepatitis, stomach aching and queasiness though acute poisoning rare and exceptional (Etzel, 2002). In chronic toxicity of aflatoxins observed carcinogenic effects and immunosuppressive (Qian *et al*., 2014). Aflatoxin hepatocarcinogenicity is mostly due to oxidative damage and lipid peroxidation to DNA (Verma, 2004). For long time use of aflatoxin can cause liver cancer, impaired children growth and weak immune system (Eaton & Groopman, 1993). In many worlds developing countries the population infected with epidemics of AIDS, tuberculosis, malaria, and other diseases by using maize infected with aflatoxins (Williams *et al*., 2005). Aflatoxins are the group of mycotoxins formed via *Aspergillus flavus* and *Aspergillus parasiticus*. Around four carefully associated aflatoxins G1, B1, G2 and B2 (Diener *et al*.,1987). Poisonous anxieties of *Aspergillus flavus* form chiefly B2 and B1, whereas *Aspergillus parasiticus* results completely four aflatoxins (Diener *et al*., 1987). Aflatoxin impurity in maize is an important issuse throughout the whole world for the reason that aflatoxins exist effective hepatotoxins and carcinogens. In 2003 in Italy aflatoxin M1 and metabolites of AFB1 was detected from milk (Piva *et al*., 2006; Giorni *et al*., 2007). Following its detection practically 60 years before, aflatoxin infection of key principal, financially significant crops concerned universal care (Wu, 2015). Aflatoxin pollution neither simply intimidates community well-being nevertheless furthermore inhibits profession plus financial prospects since field creativities at what time harvests beat patience edges (Kraemer *et al*., 2016; Dzirasah, 2015). The exposure of aflatoxin starts with in uterus in unborn childs and all over the lifecycle (Kumi *et al*., 2015; Lamplugh *et al*., 1988). Aflatoxins are formed by *Aspergillus* fungi (Frisvad *et al*., 2019). *Aspergillus flavus*, is maximum aflatoxins generating type world widely and may grouped into 2 different structures, are S and L structures (Klich, 2007; Cotty, 1989). In the S structure production of frequent tiny sclerotia (average diameter is less than 400 micrometer), rare conidia and steadily greater aflatoxin B ranks (Cotty, 1989). In the L structure production of smaller number, bigger sclerotia (average diameter is more than 400 micrometer), several conidia and inconstant intensities of aflatoxins B. The L structure genotype has deficiency capacity towards making aflatoxins that is atoxigenic owing near losses, transposals, faults of several aflatoxin bio-synthesis genetic factor (Adhikari *et al*., 2016). Throughout globe, numerous extractions similar to *Aspergillus flavus* S structure identified by nearly making numerous quantities both of aflatoxin G and B (Singh & Cotty, 2019; Probst *et al*., 2014). S morphotype fungus creating both of aflatoxin G and B knowns nameless toxin S_{BG} (Atehnkeng *et al*., 2008; Cardwell, 2006; Probst *et al*., 2014; Donner *et al*., 2009). The contamination of aflatoxin in corn owed by aflatoxins producing fungi *Aspergillus flavus* in corn cobs (Diener *et al*., 1987). These fungi usually originate on topsoil and remainders of pre-harvested agriculture crops, that play a vital cause of the prime causal agent of disease in corn infection (Horn, 2007; Jaime-Garcia & Cotty, 2004). The contamination with aflatoxins occurs during crop growth stages, when damage occurs likely by insects or drought or heat stress after or on maturity bare towards higher temperature, greater moisture earlier cutting of crops and stored conditions. Aflatoxins also effect children development and growth and badly affect the immune system of peoples. Contamination of aflatoxin in maize grains intended for the consumption of human food and feed in various states regulate the harmless food supply and animal feed (FAO, 2004). The monitoring controlling measures in Africa greatly unsuccessful (Strosnider *et al*., 2006). In crops contaminated with aflatoxins including maize strategies developed for effective control to avoid from financial losses (Robens & Brown, 2004). In a past study the production of aflatoxin reduced in invitro circumstances before toxigenic isolates inoculation, inoculated with atoxigenic isolates (Brown *et al*., 1991). To control biologically aflatoxin contamination in field conditions in maize (Dorner & Cole, 1999; Brown *et al*., 1991), in cotton and in peanut. The aspergillus specie is an important industrially class of microorganisms disseminated world widely. Aspergillus niger commonly identified as harmless

position according to united states of drug authority and food (USFDA) (Schuster *et al*., 2002). Though, around one or more species have adverse effects and cause infections in maize, peanut, grape, coffee, garlic, onion, besides additional vegetables also fruits (Rooney-Latham *et al*., 2008; Lorbeer *et al*., 2000). Furthermore, aspergillus subdivision nigri forms fungal toxins like fumonisins and ochratoxins on maize, peanut, and grape crops (Frisvad *et al*., 2007). There is complex interactions among toxigenic and aflatoxigenic creating fungus also combine through additional aspects govern range in crops aflatoxins concentration (Cotty & Jaime-Garcia, 2007). The genotypes of toxigenic *Aspergillus flavus* knowns as the valuable biocontrol ingredients formulations in controlling crop contamination in that areas from which this fungus detected (Atehnkeng *et al*., 2008; Cotty & Jaime-Garcia, 2007). The extreme aflatoxin reduction due to displacement in atoxigenic fungus in crops atmosphere via placement of prudently particular toxigenic *Aspergillus flavus* genetic makeup. It is demonstrated from many crops growing areas in United States, Kenya, Nigeria, Gambia, Italy, and Senegal (Dorner, 2010; Cotty & Jaime-Garcia, 2007; Doster *et al*., 2014). These practices are extremely price active from decreasing aflatoxin infection, aflatoxin shortening associated infections, plus accumulative contact towards native and universal superior trading (Mehl *et al*., 2012; Wu and Khlangwiset, 2010).

Aflatoxins are extremely toxic metabolites primarily found to be formed by the common fungus *Aspergillus flavus* (Sargeant *et al*., 1961) and late originate to be formed by other fungal species (Codner *et al*., 1963). First time aflatoxins were found from peanut meal that results in greater numbers of deaths of farmhouse animals (Allcroft & Carnaghan, 1963). These toxins not only found in peanut but also found toxicity effects in other agricultural commodities (Allcroft & Carnaghan, 1963). Simple aflatoxins are the mixture of various fluorescent components, from which four isolated (B1, B2, G1 and J2) in pure state having varying degrees of ducklings. Aflatoxin G1 and B1 are derivatives of G2 and B2 (Hartley *et al*., 1963). Among natural toxins the aflatoxin exist as minor toxins recognized towards extremely poisonous plus supreme carcinogenic (IARC, 2002). Aflatoxins formed through numerous species of group *Aspergillus Parasiticus*, *Aspergillus flavus*, *Aspergillus nomius*, *Aspergillus pseudotamarii* and *Aspergillus bombycis* isolates outer from class like *Aspergillus ochraceoroseus* from group circumdati, *Emericella* Venezuelensis, and Emericella *astellata* (Cary & Ehrlich, 2006). Aspergillus flavus and Aspergillus parasiticus are main aflatoxin forming species and these toxins are frequently establishing in foodstuffs and feed and are found in wide range in stored agricultural products. Though altogether species of aspergillus not capable in formation of aflatoxin. There are various approaches for screening the capacity of aflatoxins producing species. Aflatoxins tested by ELISA, chromatographic methods (Yang *et al*., 2004). Intended for that aim several mediums uses such as Yeast sucrose extract, Reddy media also expected medium having wheat, peanut, rice, date, malt, coconut and kernel palm extracts (Ahmed & Robinson, 1999). Aflatoxin are secondary metabolites and highly toxic derivatives of polyketides formed from fungal species like *Aspergillus* parasiticus, Aspergillus *flavus* (Payne & Brown, 1998) and *Aspergillus nomius*. Typically, crops like cereals corn, walnut peanut, cotton, tree nuts and wheat are infected by these fungi species (Severns *et al*., 2003), and serious threats for humans and animal's health by producing teratogenicity, hepatotoxicity and immunotoxicity (Amaike & Keller, 2011). Main aflatoxin is B1, G1, G2 and B2 that cause toxin in figure over mucous, respirational and cutaneous routs (Romani, 2011). Out of 20 known aflatoxins four AFB1, AFB2, AFG1, and AFG2 (Inam *et al*., 2007), though AFB1 and AFB2 produce AFM1 and AFM2 is hydroxyl-lated toxins (Hussain & Anwar, 2008). From eighteen various aflatoxin six namely Aflatoxin B2, Aflatoxin B1, Aflatoxin G2, Aflatoxin G1, Aflatoxin M2 and Aflatoxin M1 are most dangerous (Dors *et al*., 2011). When inhaled, ingested, or absorbed through the bodies of human beings and animals, produce the carcinogenic, teratogenic, mutagenic, and hepatotoxic effects (Stroka & Anklam, 2002). Mostly agricultural soils are the widespread inhabitants of *Aspergillus flavus* and from several species of fungi are two causing ear rot in maize (corn, *Zea mays L.*) (White, 1999). Aflatoxins are mostly occurring mycotoxin and offensive contaminants of significant agricultural supplies such as corn, groundnuts, Brazil nuts, pistachio, oilseeds such as cottonseed and copra (Idris *et al*., 2010). Areas where maize and peanut are used as staple food, aflatoxin can cause acute and chronic threats to decrease income populations. Foods contaminated with high concentration of aflatoxins leads to failure of liver in 1-2 weeks are called acute aflatoxicosis. These aflatoxisosis may cause cancer and immune destruction and at acute levels might cause death (Hsieh, 1988). Aflatoxins are chemically derivatives of difuranocoumarin. In difuranocoumarin a group of bifuran in coumarin nucleus is linked on one side, whereas Aflatoxins and Aflatoxins B series pentanone ring is linked on other side (Nakai *et al*., 2008). Biosynthetic way of aflatoxins contains eighteen enzymatic stages conversion of acetyl- CoA, and 25 genes coding (Yabe & Nakajima, 2004). Genes contains 70kb mycological genetic orders and controlled via monitoring genetic factor aflatoxin R (Yabe & Nakajima, 2004). Metabolism network complex with aflatoxin bio-synthesis (Yabe *et al*., 2003). Hydroxy versicolorone are transformed by cytosol mono oxygenase and cofactor NADPH in versiconal hemi acetalacetate (VHA) (Yabe *et al*., 2003). Several genes and complex enzymes with formation of dihydrosterigmatocystin (DHST), sterigmatocystin (ST), these the originator of aflatoxin. Interactions between plant pathogens studied by molecular markers like green

fluorescent protein (Prasher *et al*., 1992) which separated in victoria aequorea. The Green Fluorescent Protein genes effectively introduced with in Muscodor albus, Fusarium equiseti and Undifilum oxytropis (Mukherjee *et al*., 2010) plus use for studying different expression of proteins and mycotoxins production. Many crops are diseased with *Aspergillus parasiticus* and *Aspergillus flavus* during growing stages, in storage and in processing. In corn, tree nuts and cottonseed the *Aspergillus flavus* is principal contaminant while in peanuts *Aspergillus parasiticus* is largely found. The *Aspergillus flavus* contains conidia, mycelium or sclerotia and grow temperature between from 12-48ºc (Hedayati *et al*., 2007). Aflatoxin B2 and Aflatoxin B1 formed from *Aspergillus flavus* although G1 Aflatoxin, G2 Aflatoxin, Aflatoxin M1, B1 Aflatoxin and B2 Aflatoxin formed from *Aspergillus parasiticus* which results in a numbers of conidia spread by air and infect crops like cotton (Lee *et al*., 1986). Evenly these fungi may settle declining fruit debris and rye crop and of peanut (Griffin & Garren, 1976). Currently decrease the aflatoxin contamination of maize improvements in field performs avoid the post-harvest plus the pre-harvest mycological contaminants (Munkvold, 2003) and also introducing the resistant and agronomically friendly cultivars through conservative breeding (Menkir *et al*., 2006; Munkvold *et al*., 2003) and molecular methods (Duvick, 2001). Endotoxin transgene Bacillus thuringiensis (BT) in maize used for insect resistance and indirectly decrease the mycotoxin contamination. Chemical use of pesticides in some conditions results in increase the growth of Aspergillus mycotoxins. During crop growth atoxigenic biocontrol goods are applied in a specific formulation that results in active control (Mehl *et al*., 2012). On grains favorable fungal spore replicate, colonize additional carbon-based material at ground plus connected during development of preserved crop (Bandyopadhyay *et al*., 2016; Mehl *et al*., 2012).

MATERIALS AND METHODS

***Collection of seed batches*:** A survey was conducted for sample collection of maize from various places of Punjab, Pakistan (Faisalabad, Kahror pakka, Jhang, Chiniot, Lodhran, Sargodha, Gojra, Sahiwal, Arifwala, Narowal, Mailsi and Shakargarh). From various seeds shops, from growers recently harvested maize crop and various research institutes, the seed batches having various genome was collected. For the aim of laboratory analysis samples was kept in sampling bags. Agar Plate method was used on each sample of seeds. The external surface of seeds was sterilized through dipping for one minute in sodium hypochlorite (NaOCl) solution of 0.1% formulation and then wash three times with distil water. Various array of media was use like Malt Extract Agar, Potato Dextrose Agar and Coconut Agar Medium (CAM).

***Medias to identify the fungal toxins*:** For identification of fungal toxins amongst whole isolated fungal population was grown on Potato Dextrose Agar, Malt Extract Agar and coconut agar media (CAM) according to Dyer & Mc Cammon (1994) and Davis *et al*. (1987) methods was prepared after minute alterations.

***Separation and identification of storage fungi from maize*:** Sample was superficially sterilized by using 01% sodium hypochlorite for about 02 minutes and then wash numerous time with distill water. Afterward disinfection of surface, 10 grains of every sample was cited on different poured agar medium plates with the help of forceps beneath laminar flow air cabinet and for 7 days incubated at 25ºC. The emerging fungal colonies was counted and their occurrence of percentage frequency was calculated via formulae defined from Ghiasian *et al*. (2011). Recognition of isolated fungus in sample of maize were established on structural study. Species of fungi was recognized rendering to the macroscopic structures e.g. color of colony, diameter of colony, quality and tiny appearances as conidia and conidiophore (Okereke *et al*., 2007; Haggag *et al*., 2006).

***Aflatoxigenic fungi detection*:** For thew detection of aflatoxigenic fungus from whole identified & isolated fungal florae by using different medium. After preparation of media, the fungi species was injected centrally into the petri dishes and then incubate 25-28°C temperature for the duration of 7 days in dark conditions. During the time of incubation the petri plates was inspected for production of aflatoxin according to Heenan *et al*. (1998). The opposite side of every petri plate was detected underneath longwave ultraviolet (UV) rays (365nm) for distinctive blue fluorescence formed by aflatoxins. Occurrence and nonexistence of fluorescence circle the species of fungi were counted as positive and negative for production of toxin.

***Extraction of aflatoxins from aspergillus flavus isolates*:** From this experimentation hereditarily varied sample of *Aspergillus flavus* was verified from the capacity form aflatoxins. Mycotoxins was take out through solvent removal methods such defined Yazdani *et al*. (2010) through various changes. For removal of toxin *Aspergillus flavus* samples was grownup on media. After incubation, the margins of colonies were scratched together with neighboring regions into huge test tube holding 10 ml chloroform. At room temperature (25ºC) the suspension was incubated for 30 minutes and disturbed after each five minutes by using vortex stirrer. Extract were cleaned over filter paper. After dehydrating, remainder re-suspended through methanol 1ml solution plus a-septically cleaned by means of 0.2 micrometer filter syringe. Filtrate were retained at 04ºC & examined through HPLC.

***Analytical techniques for detection of mycotoxins*:** Numerous analytical methods were used for the detection of mycotoxins. Methods normally used are mostly founded continuously high performance liquid chromatography, thin-layer chromatography, or gas chromatography (Gilbert & Anklam, 2002). From last years, liquid chromatography

attached with mass spectrometry has convert more common due to development of immediate determination of various mycotoxins classes. Due to fast screening methods, immuno-chemical techniques were the benefits because in this no need of need any cleaning phase and commercially presented for maximum mycotoxins detection. Additional developing choices are the immunesensors, which deal a profitable alternate for the use of immunechemical techniques (Krska *et al*., 2008).

***Mycotoxins detoxification methods*:** Detoxification approaches was randomly separated into chemical, physical, and micro-biological procedures decontaminate through modifying, abolishing, else fascinating fungal toxins consequently to decrease else eradicate toxic properties. One auspicious method was the use of high-affinity hydrated sodium calcium alumino silicates to fix aflatoxin in foods and feeds (Ronald & Norred, 1999). Though, each treatment was its particular limits, meanwhile treated product was unaffected and harmless by using chemicals and nutritious standards of treated products was not being changed.

Isolation of aflatoxins species associated with maize seeds

***Agar plate method*:** Three to four externally surface disinfected seeds was placed on each sterilized petri plate poured on potato Dextrose Agar (PDA) media plate with the help of forceps in disinfected conditions and incubated petri plates at 24ºC for one-week duration. Entirely all the seed batches were isolated by using agar plate technique.

Purification and preservation of aflatoxins

***Hyphal tip technique*:** Water agar poured into sterile petri plates and permitted to freeze. In sterilized flask from fluctuating mycelium made a conidial deferment by 5ml purified water. In petri plates water agar was poured with the assistance of micro pipette then transfer the 5µl of conidial deferment. By immunized needles was L shaped spread the conidial deferment over the medium. At 25ºC about twelve to sixteen hours inoculated plates was incubated, and then below stereomicroscopy visual clarifications done. On inoculated plates numerous conidial developments was detected, single spore development was detected with the assistance of short magnification microscope. Aseptically picked single spore of conidia and was shifted on petri plates poured with PDA. Single spore isolation was confirmed the pure isolates aflatoxin.

***Microscopy*:** The purified aflatoxin isolates were identified and observed by numerous morphological characters that is shape, spore color, structures of fruiting bodies and aflatoxin septation by subsequent morphological criteria.

***Blotter paper method*:** With the help of forceps three to four surface sterilized seeds was placed on petri plates of 10cm diameter contained three layers of moisturized blotter papers. At equivalent distance seeds was placed along middle rings. From each seed sample total 40 seeds were used for identification and isolation of aflatoxins. At 20ºC plates was incubated for the period of one week on frequent photoperiod (12 hours' day & 12 hours' night). To observe the growth of aflatoxins on the petri plates below stereomicroscope every day and moisture level preserved with distilled water. Chromatographic methods established on physical linking between mobile phase & stationary phase. Among two phases constituents are dispersed like stationary phase and mobile phase separated. Regularly a fluid in mobile phase enters laterally or by motionless single bed liquid or solid. Presently super-critical liquids, liquids and gas use like mobile phase plus names are derivative by countryside mobile phase through chromatographic techniques: super critical fluid chromatography, liquid chromatography, and gas chromatography respectively. Samples was liquefied in mobile phase that was examined and as useful on stationary phase. The various constituents of analytes transportable with different speeds that was results in various divisions in between mobile phase and stationary phase. Generally maximum used chromatographic techniques for aflatoxins detection from thin layer chromatographic technique, high performance liquid chromatographic technique, and gas chromatographic techniques.

***Thin Layer Chromatographic Technique (TLC)*:** It is used as one of the greatest broadly separation method for detection of aflatoxins. TLC comprises on stationary phase completed from silica or alumina or cellulose immobilize on sluggish material like glass or plastic named matrix and mobile phase consist on water mixture, methanol, and aceto nitrile laterally on which samples stimulated through stationary phase. By using the TLC technique, a benefit in many kinds of mycotoxins be able to be identified in a particular sample test. For determination and separation of the organic compounds, use the high performance liquid chromatographic technique is supreme common. Nearly 80% organic compounds are detected in the world by using high performance liquid chromatographic techniques (HPLC). In high performance liquid chromatographic technique use of stationary phase kept on a plastic or glass tube. In mobile phase an aqueous or organic solvent flows by a solid adsorbent. Generally, samples that was analyzed are inoculated in stationary phase and analytes passed through stationary phase by mobile phase by using higher pressure transported through a pump. By physical and chemical relations with mobile and stationary phases, the analytes disseminated inversely in stationary phase. Within short time the HPLC procedure affords precise and fast results for detection of aflatoxins.

***Spectroscopic methods*:** There are two spectroscopic methods using in aflatoxins recognition and analysis 1st) Fluorescence spectrophotometry technique and 2nd) Frontier infrared spectroscopic technique.

***Fluorescence spectrophotometry technique*:** The absorption procedure is monitored via light release having various wavelength in fluorescence spectrophotometry technique. In examination and classification of molecules fluorescence is supreme significant at accurate wavelength in grains which

produce energy used for analysis aflatoxin. Less than five minutes 5-5000ppb aflatoxins counted by fluorometric method.

***Frontier infrared spectroscopic technique*:** Frontier infrared spectroscopic technique is reliant on modifications in the molecular vibrations. In molecules bonds vibrations to be able for measuring. Temporarily the bond strength, bond length, and atomic size vary significantly from single molecule to another molecule, the degree of ultraviolet radiations fascination was fluctuating from individual bond to additional bond in mode of vibration.

RESULTS

In mandate to evaluate the extraction efficiency, the samples of maize was spiked with various quantities of aflatoxins B1, G1, B2, G2 and *ochratoxin A.* Spikey samples of maize was analyzed and extracted by HPLC. Data showed that the percentage of recovery aflatoxins in samples of maize was ≥ 84%. Whereas the percentage for ochratoxin recovery was ≥ 85%. Relative standard deviation (RSD) morals exhibited that advanced methods is accurate. The used solvents presented good moral performance to trap aflatoxins in samples of corn. Authenticated techniques were additional use to evaluate aflatoxins plus ochratoxin A matters from collected samples of maize. Data exposed that contamination of B1 aflatoxin and B2 aflatoxin were originate as 97.3 percent and 78.7 percent from samples of maize respectively. Although AFG2, AFG1 plus ochratoxin A remained no identified from experienced samples. Our from whole seventy five sample, twenty seven sample was originate to comprise aflatoxin B1 overhead perimeter of twenty micrograms per kilogram as usual fixed through US Drug and Food Administration (USFDA) though thirty one sample was ranks of AFBI more then European Union constitutional limits that is 02 micrograms per kilogram of B1 aflatoxin and 04 micrograms per kilogram of entire mycotoxins (aflatoxin). Though, between entirely samples that was tested, twenty one sample were kept in check B2 aflatoxin quantities over restrictions set from European Union.

Maximum B1 aflatoxin infection was detected from samples that gained from arid (rain fed) zone. The AFB1 level was highly identified from samples that taken from district Attock with an normal quantity of 240.6 µg/kg. This was monitored by samples taken from Chakwal with malicious AFB1 quantity of 148.7 micrograms per kilogram. Though, from districts Jhelum, Rawalpindi, and Khushab from arid region, AFB1 levels were noted as 29.0, 5.0 and 77.7 micrograms per kilogram respectively, while B2 aflatoxin identified from twenty-two sample by usual quantity 2.0 micrograms per kilogram. It was demonstrated from the consequences that from 25 contaminated samples from arid region, 36%and 28% samples contained AFB2 and AFB1 correspondingly within the quantity level of 2-16 µg/kg; 8% samples containing AFB1 in level of 16-20 micrograms per kilogram whereas further than twenty micrograms per kilogram of B1 aflatoxin were establish from 48% of the samples.

Table 1. Frequency (%) of the associated fungal isolates with region seed samples.

Sr.	City	Fungus Isolates associated with seed samples	Frequency
1	Faisalabad	Aspergillus	10.21
		Penicillium	8.11
		Fusarium	5.01
		Alternaria	3.01
2	Jhang	Aspergillus	11.02
		Penicillium	7.25
		Fusarium	5.12
3	Kahror Paka	Aspergillus	9.02
		Penicillium	7.34
		Alternaria	6.04
4	Chiniot	Aspergillus	9.01
		Penicillium	5.06
5	Lodhran	Aspergillus	7.01
6	Sargodha	Aspergillus	11.04
		Penicillium	6.07
		Fusarium	4.03
7	Gojra	Aspergillus	8.08
		Penicillium	4.78
8	Sahiwal	Aspergillus	6.04
		Penicillium	4.50
		Fusarium	1.25
9	Arifwala	Aspergillus	5.06
		Penicillium	3.67
		Fusarium	2.24
10	Narowal	Aspergillus	5.62
		Penicillium	4.09
11	Mailsi	Aspergillus	7.90
		Fusarium	4.01
12	Jharanwala	Aspergillus	9.07
		Penicillium	6.04
		Fusarium	2.34

Ten samples was found to be infected with AFB1. Maximum AFB1 contented with average quantity of 104.3 micrograms per kilogram were perceived from seed batches gained throughout Rahim Yar Khan. Though, average quantity of B1 aflatoxin that is 12.2, 17.5, 14.8 and 29.0 micro grams per kilogram was documented sample batches gained Multan, Lodhran, Bhawalnagar and Bhawalpur respectively. 24 samples were create infected with B2 aflatoxin, then its mean quantity was under the limited level that is, 1.3 µg/kg. The data showed that 64% and 28% samples delimited AFB1 and AFB2 in the average quantity level of 4-16 micro grams per kilogram. Merely four percent of sample exhibited toxin stages array of 16-20 micro grams per kilogram, whereas thirty two sample comprising B1 aflatoxin increase permitted parameters of twenty micro grams per kilogram. Though, aflatoxin contented in samples of maize was originate to be lesser than southern and arid. Amongst five districts,

maximum AFB1contamination was originate in samples gained from district Sahiwal that is, 116.2 µg/kg. Though, average AFB1 quantity were observed 20.3, 1.2, 1.8, and 0.7µg/kg from gained samples from Sialkot, Lahore, Chiniot, and Faisalabad respectively. Average B2 aflatoxin value 1.1 micro grams per kilogram. The results show that 2 percent sample delimited B1 and B2 aflatoxin in variety of 2-16 micro grams per kilogram whereas twenty-eight percent samples were containing B1 aflatoxin range over twenty micro grams per kilogram.

Furthermore, infection level of aflatoxins in various cultivars of corn samples were studied. Total 10 cultivars of maize was composed throughout the three agro-ecological districts of Punjab, Pakistan. Amongst them, supreme AFB1 and AFB2 contamination was originate in Hybrid 80Y80, Sadaf, and NK-278, though stages in varieties, Pioneer 32B33, Agaitii 2002, Golden-85, Sahiwal 2002, Afgoyee, FH-810 Hybrid and Moncento 979 was in the variety of 64.1-333.4 micro grams per kilogram and 1.2-17.2 micro grams per kilogram, respectively. Though, additional cultivars presented reasonable to actual small range of both B1 aflatoxin 0.03-52.0 micro grams per kilogram and B2 0-3.7 micro grams per kilogram. Maximum cultivars which presented maximum toxin level was recorded from arid zone while, numerous cultivars in northern wet area exhibited decreased level of toxin production.

Findings showed there is straight affiliation among production of aflatoxin and moisture contents in maize grains. Highest moisture ratio in grains of maize was tested in samples with highest yield of aflatoxins. Correspondingly, in southern and northern irrigated zones, lower level of aflatoxins in maize grains was detected with reducing moisture contents. Fungal data shown 18 fungus ssp. belonging 6 classes was insulated from collected seed sample of maize infected via aflatoxins then recognized on foundations from morphological & cultural types, thru the use of stereoscopic microscope. Results showed that the greatest main genera were found such as Aspergillus 57.65 percent tendency existence amongst cultured fungus from maize deposited seed batches. It is denoted that 7 ssp. of *A. flavus* were maximum predominant ssp. tendency on existence sixety percent, monitored *Aspergillus. Niger* was 45.3%.

This monitored through class Penicillium that produces 24.0% tendency existence amongst whole isolated fungal toxins. Screening of mycoflora that produced aflatoxins isolated through seed batches of maize founded on florescence of fungus colonies on exposuring via UV rays on 365 nanometer, that affords reliable plus simple ways of removing non generating fungal toxins. For determination, extracted fungal florae was full growing on coconut agar media for detect florescence reaction to UV rays. Through this experiment, totally 06 fungus species was extracted in maize poised seed batches have maximum ratio tendency existence of Aspergillus group. Strategy of sampling may be responsible for the dissimilarities in results because the above-mentioned experiments used recently harvested maize seed samples.

Isolated all fungal florae was testify of capability on production of toxic strains via optical recognition ways. Davis *et al*. (1987) stated that technique used coconut agar to aflatoxins recognition. In present methodology, species of aflatoxin producing fungi made blue color rays through opposite way culture below UV cabnit. Likewise, the current study, PDA, MEA and CAM etc. was use for the screening of toxic fungus. Findings revealed between extracted fungal florae, *Aspergillus flavus* exhibited most tough. Florescence of 3 aspergillus types importantly reducing PDA, MEA and CAM medias. Related results was gained through Dyer & Mc Cammon (1994). Yazdani *et al*. (2010) although estimating numerous recognition methods of aspergillus toxic extracts stated aflatoxins formation identified on PDA, MEA and CAM media through occurrence of UV rays circle round groups. In an additional research Nair *et al*. (2014) exhibited the occurrence of the CAM clusters of fungus showed blue color florescence regions associated PDA media. Consequently, statistics shown the CAM agar founded media was costly operative implement in primary detection of toxigenic and non-toxigenic fungus.

In current research, it was correspondingly detected that high florescence below UV was detected in *Aspergillus flavus* separates which was the greatest predominant specie with maximum frequency of existence among Aspergillus genus. High florescence showed the affiliated high aflatoxins production in primary screening procedure. Isolates of samples formed 22.8 to 290.5 micro grams per litter and 7.4 to 48.8 and 7.4-48.8 micro grams per litter of B1 aflatoxin and B2 aflatoxin, correspondingly. Variances in formation of aflatoxin values from extracted samples was owing to genetic factors and environmental. Not a particular single isolate from all the samples were originate to be elaborate in the AFG1 and AFG2 production. These findings are in nearby contract with Abbas *et al*. (2006) who perceived more variances in production of aflatoxin level by extracts of *A. flavus*. In present learning, the production of, aflatoxin level by *A. flavus* isolates were greater then described through Almoammar *et al*. (2013), stated separates of *Aspergillus flavus* was initiate in B1 aflatoxin and B2 aflatoxin production heaving array 0.9 to 7.8 micro grams per litter and 0.1 to 6.4 micro grams per litter, respectively. Significant stages of aspergillus isolated through toxic fungus as developed level prime to the emphasis of preventing the fungi growth by using eco-friendly environmental plant extracts. Use of definite biocontrol agents and plant extracts as a basis of more effective and safer control on the aflatoxigenic fungi growth and aflatoxin production was investigated by numerous writers (Reddy *et al*., 2009). Abundant importance particular in medicinal, aromatic and herbal plants of the anti-fungal actions in contradiction of spoiled food and toxigenic fungus.

Crop plant has higher foundation bio active subordinate metabolites like terpenoids, tannins, flavonoids and alkaloids described as antifungal properties.

Current research extract of 10 aqueous medicinal plants i.e. *Eucalyptus citriodora*, *Ocimum basilicum*, *Trachyspermum ammi*, *Acacia nilotica*, *Azadirachta indica*, *Mentha arvensis*, *Cassia fistula*, *Nigella sativa*, *Allium sativum* and *Foeniculum vulgar* were experienced for their antifungal actions in contradiction isolates of aflatoxigenic fungus *A. flavus*. All experienced plant extracts showed various degree of anti-fungal action against *A. flavus* separates. The extreme anti-fungal action was showed by extract of aqueous of citriodora plant leafs monitored through *T. ammi* plant seed, *A. nilotica* and *O. basilicum* plant leaf abstract. Numerous oxidizing agents, for example sodium hypochlorite, chlorine, potassium permanganate, hydrogen peroxide, sodium perborate and ozone respond with aflatoxin and variation in aflatoxin molecule results in the fluorescence loss. The procedure of these responses are undefined and the response yields persist unknown in maximum cases. Decrease of aflatoxin B2 and B1 with sodium borohydride produced aflatoxin RB2 and RB1, respectively. Hydrogenation of aflatoxin G1 and B1 yields aflatoxin G2 and B2 respectively.

In this study, data revealed that 8 conceivable deprivation yields of AFB1 was gained after action through *E. citriodora*. Amongst, twenty-five percent yields was produced later alteration in lactone band. 07 extracts of *Aspergillus flavus* was recognized with in the samples. In samples 18 and 17 exhibited higher formation of B1 aflatoxin and B2 aflatoxin in array of 204.2 to 290.5 micro gram per litter and 40.6 to 48.8 micro gram per litter, correspondingly. However expressively the aflatoxins production level was low through other isolates of the maize seeds samples that is 22.8 to 79.5 micro gram per litter of B1 aflatoxin and 7.4 to 35.4 micro gram per litter of B2 aflatoxin. Furthermore, sixteen extracts of *Aspergillus flavus* was recognized from collected sample. In these samples level of production of aflatoxins in the range for AFB1 was 62.8-899.8 µg/L and for AFB2 was 40.9-142.8 µg/L.

DISCUSSION

Amongst them, aflatoxins are greatest severe hepatotoxic, carcinogenic, mutagenic and teratogenic subordinate complexes badly upset the human beings and other kind of animal health. Aflatoxins initial recognized as transferable mycotoxin which devastated higher then one lakh poults (Kuhn & Ghannoum, 2003) of turkey early in 1960s (England). Aflatoxigenic fungi have been described as one of the severe contaminants of various plants and plants produces such as maize, rice, cotton seeds, peanut, and spices, and also in milk products (Payne, 1998). While it is significant discovery an applied, low cost and nontoxic technique to avoid fungal infection in feeds and foods. In maize natural manifestation of mycotoxins was premeditated in several areas of biosphere. Amongst aflatoxin is originate to supreme leading ones. The current study covers survey of samples of maize collected from different areas in different agricultural regions of Punjab responsible for aflatoxin and ochratoxin levels from them. Results showed that aflatoxin B2 and B1 infection was originate 97.3 percent and 78.9 percent in poised tasters, correspondingly. Though G1 aflatoxin, G2 aflatoxin was not identified from any single sample. In current study, contents of aflatoxin in corn were maximum described in Shah *et al*. (2010) publication, he described 77.8 percent and 88.9 percent AFs percentage pollution in corn an average rate 14.9 micro gram per kilogram and 16.2 micro gram per kilogram from lower & higher regions of Swat correspondingly. Though, findings of current study showed that contents of aflatoxin in maize was ranged from < Limit of identification to 168.2µg/kg with mean morals 29.1, 102.2 micro gram per kilogram from northern moistened district, southern moistened district correspondingly. Findings was nearby promise to Khatoon *et al*. (2012) and Ahsan *et al*. (2010).

It is observed that relative humidity and temperature are significant environmental elements that distress growth and production of mycotoxins through fungus spores. Corn is undoubtedly crop at higher distress universe as cultivated environments have constant contaminated by mycotoxins like aflatoxins. Correspondingly from Pakistan corn is frequently cultivated throughout humid and warm period, that may favoritisms of fungal occurrence, causes higher fungal toxin development. Variation in climate significantly effects on agronomical practices, not in Pakistan nonetheless throughout whole biosphere. Temperature up rise detected country, especially throughout last rare decades is clear. Moreover, average humidity in corn were originate greater from harmless stored value of 15 percent. While it is stated greater humidity (Kumar *et al*., 2008) heavy rains and late storage rise aflatoxins value. Saleemullah *et al*. (2006) analysed impact on the aflatoxin production in storage situations and originate an optimistic relationship among the aflatoxin level and the moisture content. A research led Liu *et al*. (2006) published parallel results. These findings are also very much in line with the present research.

Results suggested that the toxigenic fungi on various coconut media displayed varying florescene levels. Three types of the Aspergillus genera have been originating to produce noticeable blue florescence which indicates the occurrence of aflatoxins in coconut media. Among these, *Aspergills flavus* presented precise strong florescence reaction in CCA media whereas its florescence reductions gradually in CAM and CMA media respectively. *Aspergillus flavus* was selected for further studies from this preliminary screening of aflatoxigenic fungi because it presented very durable florescence reaction in CCA media to UV radiation and its fraction frequency of incidence in the maize collected

samples was too supreme between entirely isolated mycoflora. Various *Aspergillus flavus* isolates have been verified for their aptitude to aflatoxins production. The HPLC assessed the degree to which toxins formed. Results showed all *A. Flavus* isolates of exhibited an aflatoxigenic outline, that is whole developed B1 aflatoxins, G1 aflatoxins and G2 aflatoxins development not found via some isolates.

In mandate to evaluate the extraction efficiency, the samples of maize was spiked with various quantities B1, G1, B2, and G2 & *Ochratoxin A* aflatoxins. Spikey samples of maize was analyzed and extracted by HPLC. Data exposed that the percentage of recovery aflatoxins in samples of maize was ≥ 84%. Whereas the percentage for ochratoxin recovery was ≥ 85%. Relative standard deviation (RSD) morals exhibited that advanced methods is accurate. The used solvents presented good moral performance to trap aflatoxins and *ochratoxin A* in samples of maize. Reddy *et al.* (2009) investigated reduction in the concentration of B1 aflatoxin from deposited rice by using several extracts of plants as biocontrol. *Syzigium aromaticum*, Curcuma longa (L.), Ocimum sanctum (Linn.) and Allium sativum L. plant extracts used which successfully inhibited the growth of *Aspergillus flavus* and AFB1 formation.

Velazhahan *et al*. (2010) estimated for the detoxification of aflatoxin G1 (AFG1) by using several medicinal plants extracts through thin-layer chromatography and enzyme-linked immuno sorbent assay (ELISA). From several extracts of plants, especially extracts of seeds of *Trachyspermum ammi* indicated supreme control of aflatoxin G1. The results showed that T. ammi extract was extra effective than from crude extract, in order to reduce toxin. They concluded T. ammi extract may afford a biologically harmless method to safe livestock or poultry feeds and additional agricultural merchandises from aflatoxins. El-Nagerabi *et al*. (2012) studied Hibiscus sabdariffa (Linn.) calyx extract and Nigella sativa (Linn.) oil effects on B1 aflatoxin production and the growth through *Aspergillus flavus* and *Aspergillus parasiticus*. They conclude that above-mentioned plant extracts can display important metabolic result on aflatoxin biosynthetic pathways for the both Aspergillus species and can also be used as operative biocontrol and non-toxic biopreservatives against aflatoxin contamination in food industry.

In another similar study El-Nagerabi *et al*. (2013) estimate that on aflatoxin production and growth via *Aspergillus parasiticus* and *Aspergillus flavus* have the Invitro inhibitory effect on essential oil, resin, and extracts of leaf of Boswellia flueck. They reached over results *B. sacra* plus essential oil retain biological action in contradiction of metabolic pathways and biological creation of aflatoxin creation through two species of aspergillus. Consequently, the French jasmine powders and Borage antitoxigenic activity were evaluated as a result of Hussein *et al.* (2014) study. The results from the study showed treatment through aforementioned florae, substantial decrease the quantity of Ochratoxin A and Deoxynivalenol fungal toxin were detected from polluted diet of poultry underneath storing circumstances.

Similarly, Kannan & Velazhahan (2014) discovered prospective from approximately native pharmaceutical extracts of plants to the decontamination of aflatoxins. In this study presented between numerous verified plants, Barleria lindl extract of leaves showed supreme decontamination from aflatoxin B2, B1, G2 and G1 at 10 pH while decontamination ratio declined at 7 pH and three times sequence aflatoxin decontamination study by extract of lupulina presented the aflatoxin deterioration happened in ten mins and that ratio was improved through rise in development time.

Also likewise Rajarajan (2014) evaluated antimicrobial outcome of Hyptis suaveolens extracts of leaves in contradiction of *Aspergillus parasiticus* and *Aspergillus flavus*. Study exposed occurrence of starch, volatile oil, proteins, saponins, tannins, fats, glycosides and alkaloids in an initial phytochemical screening which are liable for their inhibitory capability in contradiction of tested fungal species. Nearly totally agriculture merchandises sustenance evolution of *Aspergillus* specie. Initially stated usual aflatoxins occurrence from Brazilian meal peanut and products of peanut from Nigeria, and also from tropical areas in cakes as well as meals from Latin America, Africa and Asia (Wogan, 1965). After studied that toxic influence not limited on certain merchandises in Brazil nevertheless also exist in samples of peanut from thirteen further countries plus Sri Lanka and India.

Muhlemann *et al*. (1997) planed a study for the spreading and level of existence of aflatoxin in cereals, legumes, groundnuts, milk and their products. According to their observations maize, milk and bean samples were severely polluted through aflatoxin. Samples of rice were temperately polluted. Though peanut was supreme widely deliberate on every kind of merchandises, usual infection by aflatoxin was stated from pistachio, and pecans. In 1997 Shetty and Bhat examined rain effected and typical seed batches of maize, poultry feed and sorghum for aflatoxin plus establish aflatoxins was existing throughout entirely rain effected grains batches and also typical in feed samples of poultry.

Jaffar *et al*. (1994) examined sample of pepper, maize aimed at the aflatoxin infection gained by the local marketplaces of Sindh, Punjab and KPK Pakistan provinces. 30% samples of maize by Punjab however 7% samples of maize by KPK was infected from toxin substances like aflatoxins. The aflatoxins array is 30.6 to 65.2 µg/kg was found in maize samples. Aflatoxin infection of pepper wide-ranging up to 32.2-48.1 µg/kg. Alternative findings, aflatoxins substances from samples of nuts as well as cereals, gained at the marketplaces from KPK, Pakistan was examined (Saleemullah *et al*., 2006). Aflatoxins detected in nuts as almond, peanut and walnut, varied up to 5-17 µg/kg and in cereals like wheat, rice and maize reached up to 14-45 µg/kg. Though, it is prominent

range of infection were inside from allowable perimeter (50 µg/kg) from FAO. They similarly calculated consequence of aflatoxins contents in storage seeds time and were originate prolonged stored for time period of 18 months meaningfully improved aflatoxin substances from seed by way of associated to small storage age of 2-3 month.

In Pakistan for economic development agriculture is the primary sector, contributed 21% national GDP and 45% from total labor force involved in agriculture that shows an energetic role in improvements of country economy (GOP, 2010). The maize use in feed, wet milling industry and food is grown at high scale than anticipated. Maize is third greatest significant Pakistan crop afterward rice and wheat and frequently stated as "the cereals king" having the Genus Zea and family Poaceae. Maize is effectively grown from temperate, tropical and subtropical areas in world (Bukhsh *et al*., 2010). Due to C4 plant it explorative photosynthesis harvest with extraordinary hereditary probable (Tariq & Iqbal, 2010). Maize usual productivity revenue was 3940 kg/ha, that greater than entirely grown cereal from country (GOP, 2012). Though almost in all countries provinces maize is cultivated, however Punjab and Khyber Pakhtoon Khuwa (KPK) are the major producers almost 99% maize produced from these two provinces. KPK holding 51 percent of whole part having 31 percent of whole creation and Punjab had 48% maize cultivated part and 69 percent of whole grains of maize yield. A minor quantity 1.0% had formed from Baluchistan and Sindh. Also in Azad Jammu Kashmir (AJ&K) maize is a significant crop, about 0.122 million hector maize cultivated throughout autumn. Correspondingly, a progressively significant and great production part is seasonal maize from Punjab, that is about 10000 hectares plus yields around 71000 tons of corn grain (Tariq & Iqbal, 2010). Particularly in emerging countries cereals importance is widely recognized in food, providing considerable quantities of energy and protein to millions of individuals (FAO, 2011). About an estimate 15% and 10% world's protein and calories provides by cereals respectively (Nuss & Tanumihardjo, 2010). Maize contains high values of nutrients 72% starch, 10% protein, 4.8% oil 5.8% fiber, 1.7% ash, 3.0% sugar, macro and micronutrients such as phosphorus, calcium, sodium, zinc, magnesium, iron, potassium, manganese and copper (Nuss & Tanumihardjo, 2010). For industry it is raw material source where extensively it is used in the corn oil, corn flakes, wax, tanning material for leather industry, dextrose, corn syrup, cosmetics and alcohol preparation. For industrial starch maize is the excellent source. For numerous types of industrialized fermentation foodstuffs like vitamins, enzymes, antibiotics, industrial produces like skin care items, soaps, biodegradable plastics, absorbents and glues corn steep used as important base material (Krishna, 2012).

During consequent storage and treating, the nutritional value of harvested grains of maize might be considerably reduced and in poor condition for humans and animal's intake or for industrial use. Around 10-30% of overall world grain yield production is decrease later harvest (Chelkowski, 1993). These damages rise principally owing to inadequate drying, extraordinary comparative humidity and temperature changes, sowing of crops in diseased fields, initial and late cutting and measly stored environments. Corn susceptible towards approximately on that issues get an great chance of fungus contamination that might, apparently improve growth of fungal toxins besides lastly prominent in damaging to the grains by numerous ways (Dubale *et al*., 2014).

The production of mycotoxin is influenced highly by environmental aspects resembling moisture, temperature, oxygen concentration and pH. The toxigenic fungi growth also affects dur to these environmental issues. Temperature, moisture are two variable issues that crucially effected biosynthesis of toxin and fungal propagation (Paterson & Lima, 2010). Occurrence and range of fungal toxin impurity remain narrowly associated with topographical locations plus weather reasons like the harvesting, cultivation, transport, and stocking conditions (Milicevic *et al*., 2010). Presently, more than 400 mycotoxins exist recognized, between them ochratoxins, and aflatoxins have supposed worth owing to their toxic effects on livestock, humans, and poultry (Wild & Montesano, 2009). Biologically aflatoxins (AFAs) active polyketide resulting secondary metabolites that comprise on a group of strictly associated extremely oxygenated bisfuranocoumarin heterocyclic complexes, typically produced by *A. parasiticus* and *A. flavus* (Bhatnagar *et al*., 1992). These complexes have a great acute poisonousness, also mutagenic, immune-suppressive, carcinogenic activities, and teratogenic and are categorized by Global Agency of Investigation on cancer like group one carcinogens (IARC., 2002). Aflatoxins stood first recognized as the convenient toxin that demolished in early 1960s in England higher from 100,000 poults of turkey (turkey X infection) (Kuhn & Ghanoum, 2003). Contamination of aflatoxins on extensive variety affect agricultural merchandises like corn formed foods and corn, coffee beans, groundnuts, spices, rice, dairy products, groundnut and milk (Bankole *et al*., 2005). Maize grains presented complex risks of contamination of aflatoxins such as it offers superb substrate for the infection of mold. Aflatoxins grow in field in maize and throughout storage consequently production of the grains is harmful for use of any living body. Hence, there is expected that, subsequently especially rainfall, climatic conditions, temperature and relative humidity also storage structures differ within the country, by fungi maize contamination results in the consequent growth of aflatoxin might also differ (Kumar *et al*., 2000).

The identified different types of aflatoxins are about 18 most commonly occurs are aflatoxin B1, G1, B2, M1, G2, and M2. AFB2 and AFB1 are naturally formed by *Aspergillus flavus*, however by *Aspergillus parasiticus* AFG2 and AFG1 produce and also AFB2 and AFB1. Four additional aflatoxins M2, M1,

G2A, B2A, which produced in small quantities were successively isolated from *A. parasiticus* and *A. flavus*. A numeral closely associated complexes named as parasiticol, aflatoxicol and aflatoxin GM1 are too formed through *Aspergillus flavus*. Order of severe and protracted poisonousness AFB1 greater than AFG1 greater than AFB2 greater than AFG2 that displays larger strength connected by the cyclo-pentenone band in B sequence compared by six-member lactone band of G sequence. M2 aflatoxin and M1 Aflatoxin are the hepatic hydroxylated made up from AFB2 and AFB1, correspondingly (Creppy, 2002). In milk products and milk AFM1 is produce attained from livestock that consumed feed contaminated with AFB1 (Filazi *et al*., 2010). The existence of aflatoxins remains extra dominant from subtropical to tropical areas in the world due to humid and warm environments. Rate and range of aflatoxins differ evidently from 1 geographical region to other and however also within the seasons. The presence of aflatoxins in food commodities established high range of aflatoxins (Tozzi *et al*., 2003) are important issue for mutually food merchants and users. Tabuc *et al*. (2009) have originate around 30% samples from corn taken in 2002-2004 in south eastern Romania was polluted through aflatoxin B1. Ghiasian *et al*. (2010) presented that 80-58.3% samples of maize was infected with aflatoxins gained from Mazandaran and Kermanshah provinces of Iran, respectively. It is reported that maize and groundnut is the most important source of aflatoxin infection round the globe mainly in Far East, Asia, and South America in late 1990.

Borutova *et al*. (2012) stated results of an experiment a constructive association between AFB2 and AFB1 for the occurrence of various mycotoxins on numerous feedstuffs that is corn, soybean meal, wheat, dried distiller grains, corn gluten meal etc. in 2010 in Asian-Oceania region and determined the incidence of particular fungal toxins throughout some feedstuffs infrequent. Mardani *et al*. (2011) in Iran from Kahskineh food samples by HPLC did not discover AFB1, AFG1, AFG2 and AFB2 to measureable limit excluding single sample that have B1 aflatoxin was 0.64 µg/kg. Basaran & Ozcan (2009) examined B1 aflatoxin concentration found greatest copious was (0.2-36.81 µg/kg) tested in 4 sample holding G1 aflatoxin 0.6 - 20.2 µg/kg between two hundred and seventeen peanuts, hazelnuts and pistachio nut samples from Turkey. Around 87.09 % from all sample was precise less in B1 aflatoxin. Shareef, (2010) establish aflatoxins was the greatest predominant fungal toxins (mycotoxins) 91.1 percent through attentiveness as 179.1 microgram per kilogram, Ochratoxin 127 microgram per kilogram throughout survey of 02 years 2005 to 2007 in various chicken feeds trials at Pakistan. In another study Anjum *et al*. (2011) resulted that B2 aflatoxin 2.16 ± 10.80 to 3.67 ± 39.20 µg/kg from broiler & layer starting provisions collected by 10 various mills of feed in Punjab, Pakistan. In between all, 40 percent sample was found having B2 aflatoxin more than twenty micrograms per kilogram. Bokhari (2002) found that 26.1 percent sample infected through aflatoxins particularly in grains of cereals, oil seed and poultry feed was abundantly polluted from whole sample. B1 aflatoxins were originate maximum often in grains particularly in maize grains. Luttfullah & Hussain (2011), originate extreme occurrence quantity of aflatoxins contamination in walnut without shell (70 %), walnut with shell (40 %), in groundnut with shell (40 %) in an inspection throughout the northern parts from Pakistan & KPK. Lutfullah & Arshad (2012) findings maximum aflatoxins occurrence degree throughout rice (25 %), sorghum (30%), and maize (40 %) in various selling points & marketplaces at various position of Pakistan. In the Pakistan, the incidence rate is highest of *A. flavus* and responsible for AFB1 production in Swat valley in corn (Shah *et al*., 2010).

***Conclusion*:** This study reveals a high prevalence of aflatoxin contamination in maize grains collected from various districts of Punjab, Pakistan. Among the identified toxins, aflatoxin B1 was found at concentrations exceeding international safety limits in several regions, particularly in Sahiwal and Kahror Pakka. The predominance of *Aspergillus flavus* as the causal organism further highlights the significance of post-harvest management and environmental conditions in influencing contamination levels. The findings underscore the urgent need for integrated control strategies, including routine surveillance, farmer education, improved storage facilities, and the promotion of resistant maize varieties. Regulatory enforcement and public awareness are critical to safeguarding consumer health and ensuring food safety across the maize supply chain in Pakistan. Further research should focus on biocontrol interventions and the molecular mechanisms underlying aflatoxin biosynthesis in local strains. Future research should explore the genetic variability of aflatoxin-producing fungi in local ecosystems and assess the efficacy of plant-based antifungal agents under field conditions. Strengthening food safety policies and fostering public-private partnerships will be critical to reducing aflatoxin exposure, safeguarding exports, and improving public health outcomes in Pakistan.

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