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Insect as major carrier of aflatoxins and mycotoxin in foods: A review

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Abstract
Stored grains are predominant food all over the world, so the contagious effects on grains cannot be ignored. This study will help to examine the incidence and transmission of mycotoxic fungi in grains through insect pests. Majorly *Sitotroga cerealella* (Olivier, Lepidoptera, Gelechiidae), *Tribolium castaneum* (Herbst, Coleoptera, Tenebrionidae), and *Rhyzopertha dominica* (Fabricius, Coleoptera, Bostrichidae) are involved. Stored grain insects enormously preferred to contaminate the stored commodities through their feeding preference, their body fragments, and chemical excretion and gave massive contamination to a storage system. Both coleopteran and lepidopteran pests contributed to significant losses in grain commodities. Grains get affected by decreasing their nutritious level and cannot maintain the standard quality of the food according to markets. These pests also involved to cause serious health hazard diseases in terms of producing aflatoxins, mycotoxins fumonisins, and ochratoxin in stored commodities which causes severe chronic diseases. Flour manufacturing areas are at risk and in danger of contamination with mycotoxins. In the atmosphere of storage areas, insects are highly attracted to the odors emitted from deteriorating food such as damaged grains, which is an important clue for host location by insects. Adult populations of stored grain insects collectively move towards the odor of aflatoxin-producing fungi in grains and play a vital role in contaminating them. Therefore, major cereal crops are badly affected due to the occurrence of mycotoxin and aflatoxins-producing fungi and enhancing the durability of stored grain insects. This study concluded possible transmission factors of mycotoxic fungi by stored grain insects which could disturb the salubrious state of human beings and animals through carcinogenic diseases.

Keywords: Stored grain insects, toxigenic fungi, aflatoxins, fumonisins, olfactory cues

Introduction
Grains and their products provide the major source of protein, starch, carbohydrates, vitamins, amino acids, dietary fibers phytochemicals, etc. Particularly dietary fiber from wheat and its products reduced the risk of cancer, diabetes, and cardio- vascular diseases (Shewry & Hey, 2015) [37]. Stored grain insects deteriorate the food commodities in terms of reduction of the germ portion of the seed, and increase the heat and humidity levels during boring into the kernel while feeding and growing. This kind of infestation enhanced the contamination of fungi in seeds such as mycotoxins in storage (Tripathi, 2018) [41]. Global yield losses in wheat grains become rapidly enhanced from 10 to 25% due to insect pests. Grains and their products are at potential risk of chronic diseases due to the infestation of mycotoxigenic fungi on them (Tournas & Niazi, 2018) [40]. A storage fungus such as *A. flavus* requires 40-45 °C optimum temperature for its growth. Fungi increased the rate of temperature in grains which results in decay and discoloration in seeds and grains (Kumar, 2017) [23]. For example, wheat flour samples contaminated with various mycotoxic fungi belonging to the genera *Aspergillus*, *Penicillium*, and Fusarium were isolated. Growth of *Aspergillus* spp was most prominent in wheat flour with a percentage of 21.3% followed by 15.84% of *Penicillium* and 12.23% of *Fusarium* spp (Al-Defiery & Merjan, 2015) [4].
Associations between insects and fungi were studied first time in detail in 1940 (Ozburn & Gibson, 1940) [33]. Losses in stored commodities are influenced by amalgamate attack *T. castaneum* and *Sitophilus oryzae* L. and *A.flavus* within stored maize grain samples. Combination of *T. castaneum*, level of moisture, *A.flavus* infection, aflatoxin concentration, and dust production were recorded more in insects and *A.flavus* contaminated maize samples rather than the individual maize samples of insects and fungi (Sinha & Sinha, 1992) [38].

Most of the under-develop countries are stored their stored food commodities with improper storage conditions which lead towards the serious attack of insect pest and many other microbial infestation as well. Purpose of this study was to investigate the numerous stored grain insects and fungi in collected stored maize grain sample of Angola. Storage of maize grain samples were done by using small and large container to assess the attack of insect pest in stored maize grains. Result showed the major infestation in maize samples was observed due to stored grain insects; *C. ferrugineus*, *Gnatocerus maxillosus* F., *Liposcelis bostrychophila* L., *O. surinamensis*, *R. dominica*, *S. cerealella*, *S. zeamais* and *T. castaneum*. There was also fungal incidence was recorded in infested samples of maize grain due to stored grain insect pests. Dominant genera of fungi were *Aspergillus, Penicillium* and *Fusarium* spp recorded abundantly. These insects and fungi were correlated to contribute severe contamination in stored maize samples. This study concluded the major stored grain insects were identified from stored maize samples and also enhances the infestation of mycotoxigenic fungi on them (Paim *et al*., 2018) [34].

Grain itself may not be the only source of attraction for insects. Volatile compounds emitted from fungi associated with stored commodities attract *T. castaneum* for its growth and survival in the storage system (Mills, 1983) [27]. Likewise, fungi and *S. cerealella* were closely associated in stored wheat, and mature and immature stages of *S. cerealella* were involved in the dissemination of *A. glaucus* fungus in wheat grains. It is estimated that a maximum number of adult *S. cerealella* were more attracted to *A. glaucus* L. inoculated wheat grains as compared to un-inoculated wheat grains (Misra, Christensen, & Hodson, 1961) [28]. This review will help to evaluate the ability of insects to carry toxic spores in their life cycle to make the food unacceptable for humans and animals.

Mycotoxin accumulation in storage areas

Cereal grains carry dietary importance to maintain human health. There must be some factors responsible for the deterioration of cereal commodities such as the accretion of mycotoxigenic fungi. There were field fungi (Alternaria, Fusarium, and Cladosporium) associated with cereal grains at their growing stage. Cereal crops like barley, maize, and wheat were highly susceptible to the aflatoxins-producing fungi: *A. flavus, A. restrictus, A. glaucus,* and *A. candidus* (Lacey, 1975) [24].

Cereal grains are carrying dietary importance to maintain human health. There must be some factors responsible in deterioration of cereal commodities such as accretion of mycotoxigenic fungi. Field fungi like Alternaria, Fusarium and Cladosporium are associated with cereal grains at their growing stage. In terms of storing stored grains of barley, maize and wheat were highly susceptible to the aflatoxins producing fungi: *A. flavus, A. restrictus, A. glaucus* and *A. candidus*. However the level of ochratoxin, citrinin and zearalenone were also identified in cereals grains. These fungi were correlated with the increased level of temperature and humidity in storage system which prevail the amount of fungi in cereals commodities. This study provide the indication of mycotoxins become ensure through irregular abiotic factors in field and storage system (Lacey, 1975) [24].

Postharvest losses in storage areas are evaluated as the foremost areas to identify insect and microbe relationships. During the storage period of grains, level of moisture, temperature, humidity, infestation of insect pests, color and weight loss, and aflatoxins were considered important critical factors. For example, a stored grain insect, *S. zeamais* M. is responsible for the transmission of green fungus, *A. flavus* in maize. A study in Ghana reported that maize varieties were highly contaminated by maize weevil to spread and enhance the spores of green fungus. (Bhusal & Khanal, 2019) [10].

Mycotoxins are previously reported in food which is naturally produced by fungi and it is difficult to avoid this contamination from food. Stored grain are highly infested with mycotoxin contaminants either from direct or indirect growth of fungi on them. This study evaluates the various mycotoxins from soybeans, corn, cereals, peanuts and other food commodities during pre and post- harvest stage. Result showed the numerous mycotoxigenic fungi but *Aspergillus*, Fusarium, and *Penicillium* spp were recorded in higher amount. Mycotoxins such as aflatoxins, ochratoxin, trichothecenes, zearalenone, fumonisins and patulin were also evaluated from these isolated fungi. This study concluded the different species of fungi but mycotoxigenic fungi was dominated in all the stored commodities (Alshannaq & Yu, 2017) [5].

Insects such as *S. cerealella* affiliated with some storage fungi in stored wheat. The extensive number of larvae, pupae, adult and frass of grain moth were selected to isolate the *A. amstelodami, A. glaucus A. repens,* and *A. ruber*. Adult's moths were used to check response towards infected or uninfected grain. Rate of *A. glaucus* was identified in maximum amount and associated within almost all stages of insects. In free choice bioassay, adult of *S. cerealella* was attracted towards its favorable fungi: *A. glaucus*+wheat grains which are necessary for its entire life cycle. This study examined the species of *Aspergillus* from mature and immature stages of *S. cerealella* within wheat grains and analyzed the maximum response of this insects towards its preferred fungus inoculated wheat grains (Misra *et al*., 1961) [28].

Mycotoxin producing fungi, *Fusarium* spp was commonly associated pathogen of plants, insects and animals in all over the world. Most severe stored grain insect, *Tribolium* spp was used to isolate *Fusarium* spp in most of the region of Iran. Both *T. castaneum* and *T. confusum* spp were identified in all samples and was the efficient carrier of many isolates of Fusarium species. Specifically two species of Fusarium i.e., *Fusarium keratoplasticum* T.M. and *F. proliferatum* were abundantly isolated from samples of Tribolium species. Results demonstrated that the molecular-based identifications were done to analyze the maximum sample of *F. proliferatum* with in *T. confusum* species. This study primarily indicated the relationship of mycotoxigenic fungi, *Fusarium* spp with Tribolium species (Chehri, 2017) [14].

In the last few years, insect pest attacks have been increased due to unfavorable environmental conditions which enhance post-harvest losses by up to 50%. Stored rice grains are affected in terms of quality due to various microflora which produce numerous mycotoxins. The study was investigated to isolate different fungi from rice grains. Fungi were isolated from stored rice grain samples such as aflatoxin B^1, aflatoxin G^1, ochratoxin-A, terrain, gliotoxin, and fumagillin. This study sums up the idea that rice grains are highly contaminated with numerous mycotoxigenic fungi (Moharram, Yasser, Sayed, Omar, & Idres, 2019) [30].

Insect and mycotoxins contamination on food commodities act as depreciative agents and are associated with production

of off-colors and flavors, rancidity and discoloration, which have led to deteriorate the food commodities as provide loss of weight and toxicity because of enhancement of aflatoxin in food. Accumulation of toxic fungi is very common in wheat flour; more than 500 species of fungi are isolated from flour which belong to the genera, are Alternaria, *Aspergillus* and *Penicillium*. Flour manufacturing areas are on risk and danger of contamination with mycotoxins (Doolotkeldieva, 2010) [16].

Maize cultivars are primarily focused to develop maize crop with least incidence of various fungi. More than 50 diseases are isolated from field and stored maize grains. Damage becomes extreme due to abiotic and biotic factors in responsible of enhancing mycotoxins producing fungi in maize grains. Major species of *Aspergillus*, *Penicillium* and Fusarium are earlier reported in maize grains. Three different samples of maize grains were used for 20 weeks of storage period to check the level of fungi and seed germination. In all above samples, result showed the maximum occurrence of *Aspergillus* and fewer amounts of Fusarium and *Penicillium* spp were recorded. Reduction in germ portion of maize seed was also observed after storage period. This research identify the damage of maize grains primarily due to aflatoxins producing fungi especially *Aspergillus* spp (Tsedaley & Adugna, 2016) [43].

Infestation of aflatoxin B^1, fumonisin and some mycotoxin producing fungi had been studied in sorghum grains. Samples of sorghum grains were collected through some local storage areas and major districts of East Hararghe Ethiopia. Identification of mycotoxin level was done through immunosorbent assay (ELISA). Sorghum grain samples were highly infested with *Aspergillus* and Fusarium species. Aflatoxin B^1 was detected in sorghum grains samples but amount of aflatoxin B1 was high in stored samples of sorghum grains. There were total of 907-2041 µg kg^{-1} level of fumonisin identified in all samples of grains. This study indicates that maximum storage of sorghum grains increase the level of mycotoxins in food which lead towards chronic diseases (Taye, Ayalew, Chala, & Dejene, 2016) [39].

Contamination of cereals commodities such as maize, rice and milled are associated with various mycotoxic fungi in storage system. Technique of thin layer chromatography was used to isolate the mycoflora on grains. Fungi, *A. terreus*, *A. flavus*, *A. niger*, *A. oryzae*, *Penicillium italicum* W., *Penicillium spinulosum* T., *Rhizopus stolonifer* V. and *Fusarium* spp were isolated from grains. There were only three toxins such as aflatoxin B^1 fumonisin B^1 and zearalenone were identified from the samples. This study discussed the important stored commodities infested with various number of fungi (Amadi & Adeniyi, 2009) [6].

Wheat flour is contaminated with serious insect pests such as *T. castaneum* and toxic fungal species. Isolation and identification of mycotoxic fungi: *A. flavus*, *A. niveus*, *A. terreus* and *A. niger* from wheat flour and different stages of red flour beetle were conducted in this experiment. Both insect and fungus relationship reduced the quality of flour in terms of change in color and pungent smell. From wheat flour out of these four toxic fungal species, *A. flavus* was the most prevailed fungus present in the amount of 44.5% present in wheat flour. The other species were followed as 37.8% for *A. niveus*, 10.9% for *A. terreus* with and 6.7% for *A. niger* were present. Adult, larvae and casted skin of *T. castaneum* revealed the fact that *A. flavus* was the maximum in amount and *A. niger* the lowest one in all stages. This study explained the amount aflatoxins producing fungi were consistently associated with wheat flour and *T. castaneum* was responsible in dissemination of *Aspergillus* spp (Bosly & Kawanna, 2014) [12].

Rice productions become deteriorate and unacceptable through the attack of *Paraeucosmetus pallicornis* D. in field level. This study mainly focused on the transmission of toxins producing fungi in rice through insects. Collection of *P. pallicornis* was done through four different regions of South Sulawesi Indonesia. Isolates of *Aspergillus* spp and *Gliocladium* spp were identified from collected samples of *P. pallicornis* from different regions. Variation in species of *Aspergillus* associated with insects showed different characteristics of rice growing areas. Inoculation of spores of fungi: *Aspergillus* and Gliocladium were sprayed on rice crop to check the level of toxicity of two different fungi. The rice seedlings were appeared to be more toxic with spores of *Aspergillus* rather than Gliocladium and *P. pallicornis* was more closely associated with *Aspergillus* spp. This study identified the positive relationship of *P. pallicornis* with *Aspergillus* spp in term of cause toxins in rice crop (Rosmana *et al*., 2014) [35].

Wheat flour was infested with *C. cephalonic*, *Ephestia kuehniella* Z. and *T. confusum* to evaluate the mycotoxins producing fungi. Losses in terms of quality and quantity, fungal infection and mycotoxins level were studied in two month of storage of wheat flour with presence of these insects. Changes in weight loss, population of insects, nutritional composition, insect-fungi association and fungal incidence were recorded in wheat flour. Almost 10 species of fungi isolated from control and infested wheat flour belonging to genus of *Aspergillus*, *Penicillium*, Cladosporium, Eurotium and Emercilla. The level of AB^1 and AB^2 were checked all in wheat flour samples. These findings documented the role of insects in infestation of wheat flour through aflatoxigenic fungi in storage system (Gabarty & El Nour, 2016) [19].

Insects, fungi and aflatoxin contamination are previously discussed in detailed in maize plant. Harvested maize samples were collected from Georgia to check the attack of insect pest. The levels of aflatoxin were asses through ELISA technique from pre and post-harvest maize samples. Result indicated the maximum level of aflatoxins range was identified in stored maize samples and lesser range of aflatoxins was observed in field contaminated maize samples. Numerous numbers of insects was identified with *A. flavus* in maize grain samples. Aflatoxin level was increased due to optimum temperature and humidity level. This study demonstrates the aflatoxin contamination due to insects in field and stored maize grains (Mc Millian, Wilson, & Widstrom, 1985) [26].

Factors responsible in damaging of grains

Storages areas are always on potential risk of mycotoxins, most likely by *Penicillium and Aspergillus fungus.* Several parameters have countered in increasing the chance if storage fungi such as relative humidity, temperature, insect damaged grains, and overall storage condition. These all factors simultaneously affect the germination ability of grain, exploit the carbohydrate level and enhance the microbial growth which ultimately decrease the nutrition of level of grain (Mohapatra, Kumar, Kotwaliwale, & Singh, 2017) [29].

Microbes carried within grains from field consider as initial level of damage and it can spread during improper handling in storage areas as well. However, this initial damage of microorganisms can enhanced very easily through insect pest attack. For example, a report on grain damage indicated that

grains affected from fungi increase the level of temperature (50-70 °C), initiate the insect pest attack that lead to deteriorate grain quality in storage places. Similarly, relative humidity was also recorded at higher level within damaged grain which enhances the mycotoxin contamination (Christensen & Kaufmann, 1965) [15].

There are numerous factors correlated in storages areas to enhance the damaged level such as abiotic and biotic factors. Study conducted to estimate the losses from stored grains during temporary infestation of *S. cerealella* and *S. oryzae*. Wheat grains were stored in aerated bottles in terms of four different treatments such as control wheat, *S. cerealella*+wheat, *S. oryzae*+wheat and both insects+wheat. Various elements: moisture and temperature contents, population of insects, CO_2 and seed germination level, fungal contamination, kernel and dust weight were evaluated from stored wheat for 2.5 months of storing period. Result were indicated the maximum number of *S. cerealella* in all treatments except control wheat which automatically enhanced the temperature, moisture, CO_2, dust weight and infection of bacteria and fungi. These results also deteriorate the seed germination and seed weight. The level of *A. glaucus* which produce aflatoxin in stored wheat was significantly higher due to *S. cerealella* involvement in treatments. The other insect: *S. oryzae* was not increased in number and also not provide any detrimental effects in storage time. These kind of study explained the damage level due to insect pests of wheat grains specially *S. cerealella* play maximum role in deterioration of stored wheat (Imura & Sinha, 1984) [22].

Different fungal pathogens were isolated from wheat crops in Pakistan. Wheat samples (185) were collected from different areas of Pakistan. Various fungi used to examine its effects on seed germination. Almost 50% wheat samples were contaminated with different fungi. The maximum rate of fungi isolated from seeds belongs to the genus *Aspergillus* spp and minimum from Alternaria spp. The level of AFB^1 isolated almost 48 samples of wheat and AFB^2 was isolated from 13 samples. Insects, *S. cerealella* and *S. oryzae* were used to infest the simulated bagged wheat for several weeks. Insects growth, temperature, CO_2 concentration, rate of moisture, fungal pathogen, seed germination, dust weight and toxicity of fat were used to evaluated from treatment of *S. cerealella*+wheat, *S. oryzae*+wheat and both insects+wheat. Single and mixed treatment of *S. cerealella* enhanced the temperature due to its metabolic heat. Number of *S. cerealella* increased more, total of eighteen species of fungi isolated but *A. glaucus* was the dominant one, some bacterial infection, dust rate, decrease in germination of wheat seeds and fat acidity were checked due *S. cerealella* associated wheat treatments (Bhardwaj, Srivastava, & Girish, 1977) [9].

Climate is a crucial factor in agriculture and food safety sector. The level of mycotoxin in terms of fungi is highly dependent on climatic condition such as maximum temperature involved in enhancing the level of mycotoxins in different regions. In contrast of minimum temperature, prevalence of mycotoxic fungi becomes less in particular region. Climatic effect was directly correlated with insect pest attack in stored commodities. Mycotoxins are the health hazard foodborne diseases which enhance with optimum growth condition i.e., relatively extreme temperature and moisture rate and stored grains insect pests. This study provide the susceptible climatic conditions essential for the growth of mycotoxins in food and also highlights the possible reasons to control over these toxins in stored grain food (Nesic, Milicevic, Nesic, & Ivanovic, 2015) [32].

Insects and storage fungi are two important biological factors counted to deteriorate the store commodity. Samples of wheat, oats and corn were used identify the number of maximum fungal colonies in storage period. *A. glaucus* was present in highest amount 84% in corn, 70% in oat and 37% of other fungi were isolated from wheat samples. These samples polluted with the major fungal specie were also having maximum number of insects. More amounts of *A. glaucus* were known to produce the number of *Cryptolestes pusillus* S., *O. surinamensis*, *R. dominica* and *T. castaneum* in wheat samples. Samples of corn, where *C. pusillus* was the only insect contacted with increasing number of *A. glaucus*. Sample of *A. advena* and *T. stercorea* were multiplicated more with seed borne fungi multiplication. Samples of highly contaminated with mycotoxins were used to enhance more insect's infestation. These results demonstrated that aflatoxins contamination enhanced due insects infestation in stored commodities (Sauer, Storey, & Walker, 1984) [36].

Life cycles of insects are sometimes dependent on external sources associated with in host. Insect, *Typhaea stercorea* was used to grow on isolates of *A. flavus*, *Eurotium rubrum* K., and *P. purpurogenum* fungi to determine its growth period. These findings indicated the difference in the life cycle pattern of *T. stercorea* on three isolates of mycotoxigenic fungi in which *A. flavus* was the only aflatoxin-producing fungus preferred by this insects for its growth (Tsai, Mason, & Woloshuk, 2007) [42].

Stored grains are the prior to encountered various mycoflora associated with insects and mites. Quality and quantity of grains gets influenced with biotic and abiotic factors in storage system. Insects, mites and fungi respired in grains bulk due to their growth in entire period of storage was evaluated with water and temperature contents. These grains showed complex interaction of storage fungi with insects and mites. Insects and mites play important role in dissemination of mycotoxigenic fungi in storage system. Results showed the growth of Fusarium, *Aspergillus* and *Penicillium* spp were observed predominately in stored grain ecosystem. Due to mycotoxigenic fungi, several toxins such as aflatoxins, fumonisins and ochratoxin were also evaluated from grains. This study explained the interaction of insects and fungi in storage areas and abiotic factors were also changed according to the maximum growth of insect pests (Magan, Hope, Cairns, & Aldred, 2003) [25].

Sorghum and its products are affected with many different aflatoxigenic fungi and stored grain insects are closely associated with these fungi. Sorghum grain flour was inoculated with various groups of *Aspergillus* species and *P. citrinum* and three stored grain insects: *T. castaneum*, *Cynaeus angustus* L. and *C. pusillus*. Moisture level was also enhanced in all the treatments. Result showed that the maximum population growth of stored grain insects were detected in fungi inoculated treatment of sorghum grain flour rather than control. Two species of *Aspergillus*: *A. glaucus* and *A. candidus* were the appropriate medium in enhancement in the growth of stored grain insects. Mortality in *T. castaneum* and *C. pusillus* were also observed in sorghum grain flour inoculated with *P. citrinum* specie. Result indicated that *T. castaneum* was the only insect which preferred to feed and grow in flour infested with *Aspergillus* species. This study concluded that stored grains were highly dependent on fungi contaminated sorghum grain flour for their growth and survival (Wright & Burroughs, 1983) [44].

Insect and fungus are closely associated on tree growing areas in terms of causing severe infestation to several parts of the tree. Mycotoxigenic fungi are already studied in forest areas due to attack of insect pests. This study was conducted to assess the effects of inoculation of *F. solani* on *Pittosporum tobira* seedlings. Strains of *F. solani* were isolated from the bark beetle: *Scolytogenes birosimensis*. Result showed that seedlings were infected with severe disease symptoms of necrotic lesions and discoloration due to *F. solani* inoculation. Laboratory experiment revealed that the fact that seedlings of P. tobira was deteriorated after thirty days of inoculation of *F. solani*. Infestation through *F. solani* and *S. birosimensis* on seedlings of *P. tobira* were showed severe inoculum of diseases. This study was investigated to evaluate the major symptoms on seedlings of *P. tobira* due to mycotoxigenic fungi: *F. solani* and this fungus were closely related with *S. birosimensis* which enhanced the infection (Yamaoka *et al*., 2015) [45].

Cow pea and its products are infested with various fungal incidences which produce aflatoxins in human diet. Saturated and heat dried cow pea flour were checked to isolate mycoflora on their different level. Two kinds of agar were adopted to assess the strains of *A. flavus*. Artificially made cow pea flour and yeast extract sucrose agar were used to check the *A. flavus* colony through different temperature. Liquid chromatography technique was used to check the colonies of *A.flavus* on different artificial media. After incubation period, results provide the maximum *A. flavus* colony was identified on cow pea flour agar during maximum (60-120 ℃) range of temperature. The lesser amount of aflatoxins strains were identified on saturated cow pea flour under different media. This study showed the importance of heating method over saturated method which prevail the growth of *A. flavus* on cow pea flour (Houssou, Kpodo, Fandohan, Ahohuendo, & Hounhouigan, 2010) [21].

Most decaying fungus, *A.flavus* contaminants, incidence of aflatoxins and insects as an efficient carrier of mycotoxins producing fungi was isolated from peanut in its storage system. *Cryptolestes* spp was reported almost all collected contaminated sample of peanut in different months. Rate of *A.flavus* was maximum (79%) in peanut seeds and isolated 59% from insects. The level of aflatoxin B^1 was enhanced more (68.86kg and 69.12kg) in months of September and October. The highest amount of *A.flavus* (87.5%) was isolated in June due to association of insects with contaminated peanut samples. Both of insects-fungus relationship enhanced the risk of aflatoxin production in storage era of peanuts. This study mainly focused on isolation of aflatoxin producing from insects and peanut samples (Nesci, Montemarani, & Etcheverry, 2011) [31].

The other most significant research on Mite species, *Tyrophagus putrescentiae* S. explained about spreading mycotoxigenic fungi in storage places. The purpose of the study was use to examine the incidence of several fungi through *T. putrescentiae* which are environmentally and medically very important species. Huge amounts of fungal incident were counted by *T. putrescentiae* such as Alternaria sp., *Microsporum gypseum* E., and *A. chevalier*. Grains get affected by decreasing their nutritious level and cannot maintain the standard quality of the food according to markets. These pests also involved to cause serious health hazard diseases in terms of producing aflatoxins, mycotoxins fumonisins, and ochratoxin in stored commodities (Tripathi, 2018) [41].

Olfactory cues for host location

Volatile emissions play an important role in evoking the behavior of insects within stored grain bulk. Fungi act as an essential element to change the response of insects. As a secondary pest of stored grain, *Tribolium confusum* J. is attracted to the infested cereal commodities and organic volatiles play an important role in this attraction. A study has been done to recode the response of female, *T. castaneum* towards infested rice emitting volatiles and behavior was evaluated through different treatments of rice with in Y-tube olfactometer bioassay. Significant response was recorded through volatiles emitting of insect-infested rice and minimum or no response was evaluated towards volatiles emitting of whole rice (Giunti, Palmeri, Algeri, & Campolo, 2018) [20].

Wheat grains produced some volatile organic compound either grain was infested with fungi or not. The research was conducted to elucidate the response of the Indian meal moth, *Plodia interpunctella* H towards volatiles from damaged grains. The maximum response of the female moth was assessed through an olfactometer i.e., two compounds of 1-hexanol and nonanol were used to attract the female moth in the least concentration. The study clarified the positive response of female *P. interpunctella* towards volatiles emitting from fungi-contaminated wheat grains. (Būda *et al*., 2016) [13].

Consecutively, *Musca domestica* L. is a really important insect for the contamination of human diets through its nature of transmitting microbes in food. In the stored grain bulk, houseflies are attracted to volatiles coming from infested cereals and become efficient carriers of storage fungi. It produces mycotoxin in grains to cause carcinogenic diseases in humans and animals. Four important genera of fungi *Aspergillus*, Fusarium, Alternaria, and *Penicillium* spp were identified in maximum amount in houseflies which are the most harmful fungus (Abid *et al*., 2018) [1].

Stored grain insects are highly responsible for the contamination in terms of carrying mycotoxigenic spores while feeding and growing and they are also show immediate attraction towards volatiles emitting from fungi. Important stored grain pest: *T. castaneum* is the cosmopolitan insect and the efficient carrier of different fungi. In this study, *T. castaneum* was used to assess the response towards grains of sorghum and wheat, cotton seeds inoculated with fungi in laboratory, green house and field experiment. Result showed significant response of *T. castaneum* was mostly recorded in laboratory and greenhouse experiments. There was significant difference recorded in responses of *T. castaneum* to prefer inoculated cotton seeds over sorghum and wheat grains. Results showed cleared preference of *T. castaneum* and responded five times more in small scale experiments rather than in field experiments. These findings indicated that stored grain insect: *T. castaneum* was highly attracted towards food source inoculated with fungi in small scale experiments of laboratory and greenhouses (Ahmad, Daglish, Ridley, Burrill, & Walter, 2013) [2].

Life cycles of insects are sometimes dependent on external source associated with in host. Insect, *Typhaea stercorea* was used to grow on isolates of *A. flavus, Eurotium rubrum* K. and *P. purpurogenum* fungi to determine its growth period. Results provide the adequate data of *Typhaea stercorea* L. were complete its life cycle on this fungal isolates on proper culture medium. There were significant results were observed: *T. stercorea* was used to prefer isolates of *A. flavus* for its

potential growth rather than feed on the other isolates of *E. rubrum* and *P. purpurogenum*. Life cycle become completed at short period of time and female choose to laid maximum number of eggs on the isolates *A. flavus* fungi in a medium. Level of aflatoxin was also determined on the growth period of *T. stercorea*. These findings indicated the difference of life cycle pattern of *T. stercorea* on three isolates of mycotoxigenic fungi in which *A. flavus* was the only aflatoxin producing fungus preferred by this insects for its growth (Tsai *et al*., 2007) [42].

Mycotoxin contaminations are major threat to corn and its products due to efficient transmission of *F. verticillioides* through sap beetle. This study was used to investigate the response of sap beetle towards the volatiles emitted from *F. verticillioides*. Microextraction solid-phase method was implemented to evaluate the different olfactory volatiles. There were total 5 alcohol, acetaldehyde and ethyl acetate were observed in volatiles analysis. This mycotoxigenic fungus also emitted some phenolic compounds, hydrocarbon and 10-carbon ketone. Result showed that alcohol, aldehyde and ester were identified in maximum amount rather than least amount of phenolic compounds. During the wind-tunnel bioassay, sap beetle was attracted towards the entire volatiles compound of the *F. verticillioides* strain. Response and attraction of the sap beetle was enhanced due to maximum volatiles emission of alcohol, acetaldehyde and ethyl acetate. This study demonstrated that response of sap beetle was evaluated due to fungus emitted volatiles compounds and attraction and response of insect become enhanced due to maximum volatiles emissions (Bartelt & Wicklow, 1999) [8].

Maize grains were stored through different storage system to analysis the level of mycotoxigenic fungi, *Fusarium* and fumonisins contamination. Incidence through *Fusarium* was recorded in higher amount in non-ventilated domestic place of cemented flour rather than the other storage systems. The minimum contamination caused through Fusarium was recorded in ventilated bamboo granary place. The overall infection through Fusarium was recorded in all maize samples. The level of contamination through Fusarium and fumonisins concentration was enhanced through lepidopterous pests in stored maize. The use of ventilated storage areas to avoid the insect pests infestation become very important aspect to stored grains (Fandohan, Gnonlonfin, Hell, Marasas, & Wingfield, 2006) [17].

Wheat grains are severely infested with various mycotoxins during post-harvest stage. Various sample of wheat grains were used to analyze the mycotoxigenic fungi during several month of storage period. Total of 5 samples of wheat grains was tested in thirty days of intervals to isolate different species of fungi belongs to the genus of *Aspergillus*, *Penicillium* and Fusarium. Some aflatoxins: AFB^1, AFB^2 and AFG^1, $AFG^{2,}$ ochratoxin A, zearalenone, deoxinivalenol and some fumonisins were isolated in grains. The results demonstrated that all mycotoxins were identified in maximum samples of grains except fumonisins level was less isolated in all samples. The level of *Aspergillus* spp was found in maximum amount in all samples of wheat grains and *Penicillium* and *Fusarium* spp was less identified in all samples. This study proved that rate of mycotoxins belonging to *Aspergillus* spp was identified in higher amount in all samples of grains (Birck, Lorini, & Scussel, 2006) [11].

Transmission of toxigenic fungi through insects pest play important role in storage system. Stored corns are susceptible to various mycotoxigenic fungi through weevils. Corn grains samples were collected for the further inoculation of different treatments. Two toxigenic strains of *A. flavus* and *Fusarium verticillioides* were used for inoculation in different samples. Stored corn grains were inoculated with weevils and two mycotoxigenic fungi singly and mixed placed in different treatments. There were also sterilized corn samples assessed the mycotoxins through electron microscopy without intervention of weevils. These samples of treatments were stored and checked after 10 days of intervals. Results indicated the level of moisture content, mycotoxins, various fungi, weight, aflatoxins and fumonisins become increased where the weevil are associated with stored corn grains. This study provides the fact that the weevil are the efficient transmitter of mycotoxigenic fungi in stored corn (Ferreira-Castro, Potenza, Rocha, & Corrêa, 2012) [18].

Stored wheat grains are contaminated by different fungi which is an essential factor in the transmission of volatile organic compounds. Gas chromatography and mass spectrometry are the techniques to identify the fungi in stored wheat grains such *Alternaria alternata* Fr. and *A. infectoria* N. There were a total of 57 volatile compounds identified from stored wheat grains. These volatiles were also connected with the growth period of fungi. The study analyzes the knowledge about volatility in its initial stage for the prevention of losses in stored wheat. (Barkat, Du, Ren, Hardy, & Bayliss, 2017) [7].

The olfactory response is previously studied in detail to analyze the biological factors of insect pests within host. Role of toxigenic fungus: *F. verticillioides* were checked in different coleopteran and lepidopteran pests through olfactometer in maize crop. Results showed the maximum fecundity rate of *Eldana saccharina* W. and *Mussidia nigrivinella* R. were observed in spores-treated stems, leaves, and seeds of maize plants (Ako, Schulthess, Gumedzoe, & Cardwell, 2003) [3].

Likewise, wheat grains are contaminated by different fungi which is essential factor in transmission of volatile organic compound. Gas chromatography and mass spectrometry were the technique to identify the fungi in stored wheat grains such *Alternaria alternata* Fr. and *A. infectoria* N. There were total 57 volatiles compound identified from stored wheat grains. These volatiles were also connected with growth period of fungi. Initial colonies of *A. infectoria* produced volatiles more to enhance the growth of *A. alternata* which automatically produced volatiles. Frequently identified volatiles such as Cyclooctasiloxane and hexadecamethyl emitted by *A. alternata* and Pentadecane emitted by *A. infectoria*. This study analyzes the knowledge about volatile on its initial stage for prevention of losses in stored wheat. (Barkat *et al*., 2017) [7].

Similarly, Mycotoxin contaminations are a major threat to corn and its products due to the efficient transmission of *F. verticillioides* through sap beetle. The study was implemented through wind-tunnel bioassay; the sap beetle was attracted towards the entire volatiles compound of the *F. verticillioides* strain. Response and attraction of the sap beetle were enhanced due to the maximum volatile emission of alcohol, acetaldehyde, and ethyl acetate emitted from *F. verticillioides* (Bartelt & Wicklow, 1999) [8].

Conclusion

Mycotoxins observed from the start of the organizing crop cultivation and their effect has always been ignored for the past 40 years. The toxin first time detected from the

groundnut was named aflatoxin, produced by *A. flavus*. Insects and fungi are closely associated and act as detrimental factors in stored grains commodities. The primary focus of this study was to generate factual data on the amalgamation of mycotoxins in our food. Storage fungi are dependent on stored grain insects for their growth and proliferation on stored grains. Insects are highly responsible for increasing water and heat contents in grain bulk that provide a suitable medium for fungi to grow. Insects are previously involved in the efficient transmission of fungal spores especially disseminators of mycotoxins that cause carcinogenic diseases in humans in terms of contaminated diet. The significant and easiest route preferred by the insect to spread fungal spores is taking signals from volatile emitted from food. Also, it helps to maintain their life cycle for a longer period.

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Conflict of interest, the author has no conflicts of interest to declare that are relevant to consent of this article.

Ethical approval and Consent for publication
Not applicable- this study is exempted from ethical approval by departmental ethical review committee (ERC, Department of Biosciences, CUI, Islamabad) as no human or endangered animals were used as test organism.

Consent for Publication
This article has written solely, there is no need to require consent of anyone to publish only corresponding author agree to publish.
Consent to participate
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