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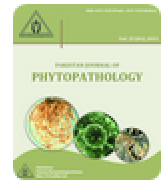

AFLATOXIN: CAN BE MANAGED BY SOIL AMENDMENTS IN GROUNDNUT?

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A B S T R A C T

Aflatoxins are metabolites mostly produced by *Aspergillus flavus* and *A parasiticus* habituating in soil and contaminating crop's produce throughout food chain from field, food factory to market and table. In groundnut Aflatoxins are considered to be a danger to human health, worldwide food safety and restricting the trade of produce of crop worldwide. Elimination of aflatoxin is not possible, however application of several pre-harvest and postharvest techniques can mitigate aflatoxin contamination. Present study has been conducted to investigate impact of application of different soil amendments viz; farm yard manure (FYM), cereal crop residue (CCR), and gypsum (GYP) in combination or alone at time of sowing and 50 days after sowing (DAS) to alleviate aflatoxin contamination in Groundnut under rainfed conditions. Experiment was conducted at two locations (Chakwal & Attock). Data of some chemical and physical soil properties i.e., electrical conductivity, potential of hydrogen, percentage of organic matter, available phosphorous (ppm), available potassium, saturation percentage and texture of the field before planting were recorded. Aflatoxin quantity was determined by using optical density value (OD) of enzymatic analysis of groundnut samples. Although all the amendments reduced aflatoxin significantly as compared with control but variation among various amendments was not significant. Maximum decrease of aflatoxin was observed in FYM (60.8%) followed by Gypsum (58.4%). It is worth to note amendments in soil not only reduced aflatoxin but enhanced the yield as well.

Keywords: Aflatoxin B1, Soil amendments, Cereal crop residue, Farm yard manure, Gypsum.

INTRODUCTION

Food security is the most important issue in the present situation of growing population to be ensured by abundant, safe and quality food rich in nutrition for a vigorous life (FAO, 1996). All these parameters contribute equally to guarantee food security and the tragedy of these features causes malnutrition, influence human health, as well as the socio-economic dynamics of civilized society. These are mycotoxins which damage twenty five percent of the world's food (Pankaj *et al.,* 2018) occupy the status of key factors responsible for creating insecurity of sustenance (Udomkun et al., 2017). *A. flavus* and *A parasiticus* habituating in soil produce aflatoxins in contaminated crop produce (Payne and Brown, 1998).

Most of farm produce is frequently contaminated by aflatoxins and consumption of this produce may pose a health hazardous situation in human-being as well as livestock (Kumar *et al.,* 2017; Ezikiel *et al.,* 2019). Aflatoxin's epidemic in tropical crops grown between 40^{o}degrees north and 40^{o} south along the equator (Williams *et al.,* 2004). Contamination by aflatoxin is more probable in warm, dry ecologies and is aggravated by dampness and poor drying for packing and marketing (Diener *et al.,* 1987). Although aflatoxins contaminate many grains, legumes, tree nuts, seeds and spices but peanut and corn are most commonly damaged by aflatoxin contamination. Growth of fungus on grain yield indicates bright chances of aflatoxins contamination. Aflatoxin being not visible so absence of mold growth is

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not guarantee of innocuous to munch (Brown, 2018). Aflatoxins disturb whole food chain by contaminating crops, causing food safety, food security and nourishment at verge and subsequently affect international trade of crops prone to contamination (Pleadin *et al.,* 2014). For faunae, consumption of contaminated feed may cause harmful effects limiting their potential to produce safe milk (Tajik *et al.,* 2007).

High levels of aflatoxin B1 (>20ppb; countries have different permissible levels) make food poisonous and can cause serious health issues, including h*epatocellular carcinoma* (HCC) with the enduring infection with HBV, HCV and aflatoxicosis (Wild and Montesano, 2009). AFB1 is not only the most commonly produced toxic strain but also the most vigorous carcinogenic agent (Marchese *et al.,* 2018). Consumption of Aflatoxin cause stunted growth of offspring (Jiang *et al.,* 2008; Jolly, 2014) that is why mitigating *aflatoxin* may ensure improvement in *development of youngsters* (Gong *et al.,* 2004). Aflatoxins are heat tolerant are not destroyed through normal cooking (Medina *et al.,* 2017), and the functions in body are incapable to eliminate them (Brown, 2018).

Soil amendments play pivotal role in mitigation of aflatoxin contamination (Waliyar *et al.,* 2015). Enzyme-Linked Immunosorbent Assay (ELISA) is mostly used for aflatoxin detection in groundnut. Keeping in view the said situation, the present study, was conducted, focusing pre-harvest management using FYM, GYP, and CCR in single or in combination to mitigate aflatoxin B1 below permissible limit in groundnut. The current investigation highlights the role of soil amendments in management aflatoxin contamination in Groundnut during pre-harvest.

MATERIALS AND METHODS

Description of Study Area: We used a groundnut plot reported with aflatoxin contamination since last 5 years regularly at Barani Agricultural Research Institute, Chakwal and at least 2 years at farmers field at Attock enriched the plot by incorporating infected plant material into the soil. The experiments were sown in last week of march and harvested in second week of October at Barani Agricultural Research Institute, Chakwal and Farmer Field, Attock during 2018 and 2019. Soil amendments viz. farm yard manure (FYM), gypsum (GYP), and cereal crop residue (CCR) were applied in single or in combination at sowing time and 50 days after sowing @ 2.5T/ha for each amendment following Waliyar *et al.* (2008).

Table 1. Some chemical and physical soil properties of the field before planting.

Location	EC cm^{-1}	pH	Organic matter (%)	Available Phosphorus (ppm)	Available Potassium (ppm)	Saturation (%)	Texture
Attock	0.635	7.675	0.81	18.9	27.5	27.5	Sandy Loam
Chakwal	0.945	7.85	1.52	17.8	240	34.5	Sandy Loam

Enzyme Linked Immunosorbent Assay: Preparation of peanut sample: Samples of groundnut were furnished for detection of aflatoxin B1 from groundnut using ELISA AFBI Kit by MYBIOSOURCE. This analysis was built on the competitive enzyme immunoassay (Mona Eslami 2015) for the recognition of aflatoxin B1 in the specimen. The coupling antigens were already layered on the micro-wells. The aflatoxin B1 in the specimen and the coupling antigens pre-coated on the micro-well stripes compete for the anti- aflatoxin B1 antibodies. After adding the enzyme conjugate, the 3,3′,5,5′-Tetramethylbenzidine (TMB substrate) a chromogenic substance was added for staining. The optical density (OD) was measured by Bio Tek ELISA reader ELx800. OD value of the specimen had a negative correlation with the aflatoxin B1 in it. This numeral was compared to the standard curve and resultantly the concentration aflatoxin B1 was computed.

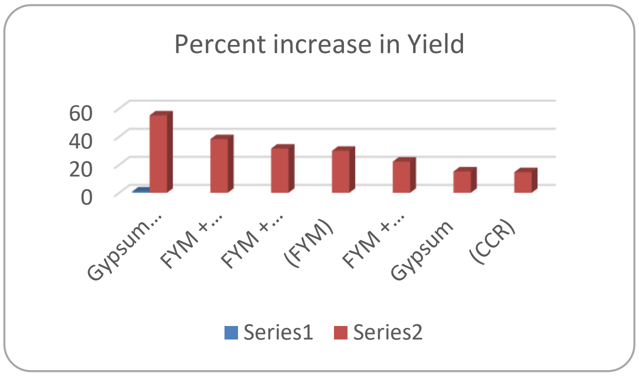
STATISTICAL ANALYSIS

The collected data were further subjected to statistical analysis by using Linear Model Statistics package (Version 8.1).

RESULTS

Aflatoxin B1 detection by Enzyme-Linked Immunosorbent Assay (ELISA): The work conducted revealed that application of farm yard manure followed by gypsum were most effective in mitigating aflatoxin although the various soil amendments decreased preharvest contamination likewise when compared with control (Table 2). Application of soil amendments at 50 days after sowing was more effective than applied at earlier growth stage (Table 3). Trial was conducted at two sites and significant difference in the mitigation of aflatoxin was recorded at both locations as impact of interaction between environment and with of soil amendments (Table 3).

Generally, contamination with aflatoxin is an issue that makes the food poisonous but does not diminish harvest. However, harvest data revealed that soil amendments increased the yield significantly as compared to control (Table 2). This situation is fitting because farming community is generally not well aware with the threats caused by aflatoxin but aims only at higher yield.



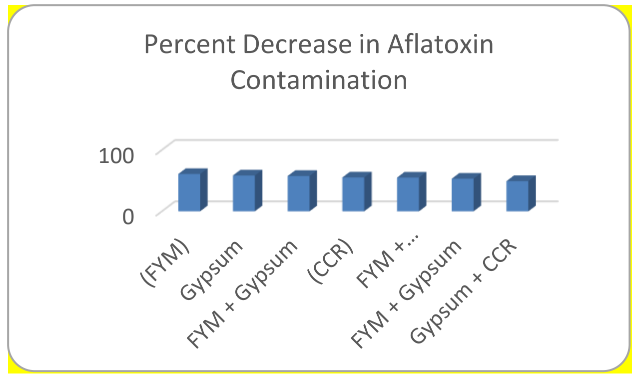
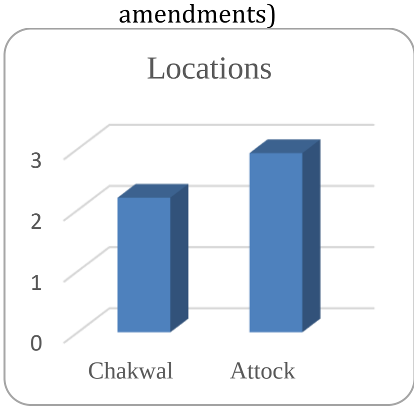
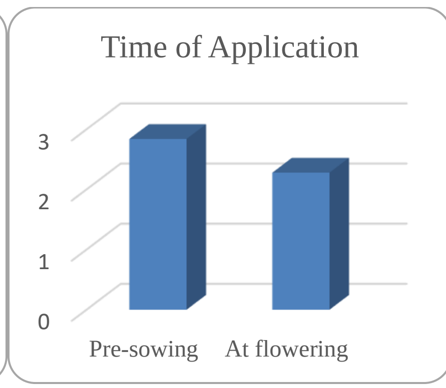


Figure 1 Percent increase in yield (Kg/ H) of groundnut and decrease in aflatoxin contamination (µg/kg) influenced b amendments)





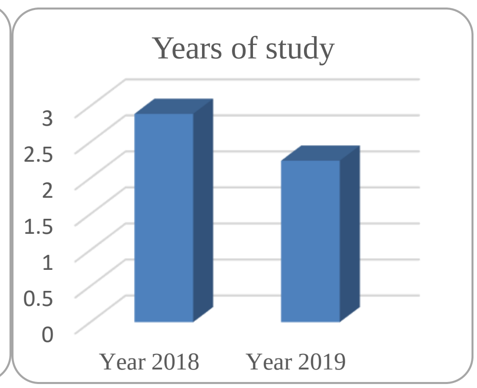


Figure 2. Variation in aflatoxin contamination in groundnut influenced by soil amendments at locations, time of application and years of experiment

DISCUSSION

Table 2 shows soil amendments either organic (FYM or CCR) or inorganic (Gypsum) decreased aflatoxin contamination significantly than control in our studies. Maximum reduction of aflatoxin was by FYM followed by Gypsum although difference is insignificant. This finding corroborates previous work.

Calcium hydroxide, FYM and CCR when used as soil amendments or fertilizer reduced aflatoxin contamination in groundnut (Waliyar *et al.,* 2006; 2008) and similar results are recorded in present work (Table 2). Although agronomists generally treat earth as substrate for plant nourishment, it is in fact a multifaceted ecology hosting microbe, molds, protistans, and faunae (Muller *et al.,* 2016) soil offers a fitness benefit to *A. flavus* for aflatoxin production (Drott *et al.,* 2017). Scientific community has been taking interest in recognizing the association of environmental factors that encourage development of plants since ancient times. Mycorrhizal fungi and nitrogen fixing bacteria both documented as root symbionts in leguminous plants is well known example of such association (Morton, 1981). The adding of organic (carbon-based) constituents to soils such as animal manures, crop remains, green manures have a direct impact on soil organic matter. Organic amendments recover health of soil, amend metal toxicity, influence positively the texture, nutrient contents, pH level and increase microbial activities (Escobar and Hue, 2008). An excessive improvement has been made in the acquaintance on configuration of rhizosphere in recent past. Bacteria have evolved several adaptations to flourish in the rhizosphere niche. On farm composts improve the soil fertility by adding organic matter and nutrients, that are utilized by soil flora and faunae (Jacoby *et al.,* 2017). Study conducted by Dania & Eze (2020) *revealed* seed treatment of peanut with *Trichoderma harzianum* and cattle dung in combination reduced *A. flavus* population better than all other treatments and produced the highest pod yield, which was significantly higher than carbendazim fungicide.

Calcium strengthens the cell wall, while manure enhance microbial activity and decelerate soil infections (Hell and Mutegi, 2011). Gypsum, contains 23.3% calcium (Batte and Forster, 2015; Chen and Dick, 2011), and it was proved the most effective in reducing aflatoxin contamination among various fertilizers evaluated (Eche *et al.,* 2017). Addition of calcium as soil amendment in form of lime decrease aflatoxins contamination by 72% (Waliyar *et al.,* 2008). Calcium is one of the important elements for sound pod formation of peanut (Cox *et al.,* 1976; Jain *et al.,* 2011). Gypsum as source of calcium is also used to curtail pre-harvest aflatoxin contamination (Reding *et al.,* 1993; Gebreselassie *et al.,* 2014). Waliyar *et al.,* 2013 observed gypsum reduce pre-harvest aflatoxin contamination but did not influence agronomic traits however others (Kabir *et al.* 2013; Bairagi *et al.,* 2017) noted application of gypsum not only decreased aflatoxin contamination but increased the yield as well. In our study yield was increased from 15-55% when gypsum was used alone or in combination with other amendments (Table 2). These findings are in line with observations of Kabir et al. (2013); Bairagi et al. (2017).

A significant less aflatoxin contamination was recorded at Chakwal than at Attock (Table 3). Differential findings at both location is attributed to difference in chemistry of soil. This situation may because of lower levels of Potassium ions at Attock than at Chakwal (Table 1). In previous studies various Potassium salts had proved to mitigate aflatoxin production (Davis and Diener 1967) and inhibition of Aspergillus flavus growth (Bullerman 1983).

CONCLUSION

It can be concluded from the results that soil amendments evaluated mitigated aflatoxin contamination significantly as compared to control however, various amendments had no significant difference in their performance among themselves. Application of FYM and Gypsum in combination was found the most effective in decreasing the contamination of aflatoxin. It is pleasing that soil amendments not only resulted in decreased contamination of aflatoxin but improved the yield as compared to control as well. The data generated will be helpful in development of control measures of aflatoxins to ensure food and feed safety for health-conscious consumers. Moreover, providing acquaintance on aflatoxins toxicity will guarantee food safety and meet the demands of the rising population by reducing the occurrence of outbursts due to aflatoxins.

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Sayad R. Ali	:	Conduct trials.
Hafiz H. Nawaz	:	Reviewed manuscript to improve its language.
Rubab Altaf	:	Reviewed manuscript for its technical aspects.
Fakeha T. Jannat	:	Correct references for review of literature.
Sairah Syed	:	Provided references for review of literature.