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Effect of toxigenic *Aspergillus flavus* and aflatoxins on seed quality parameters of *Sorghum bicolor* (L.) Moench.

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Abstract

The *Aspergillus flavus* and aflatoxins are known to be detrimental to plant and animals affecting their productivity and yield. This study evaluated effects of toxigenic *A. flavus* and aflatoxins on physical parameters like seed germination, seedling vigor, root length, shoot length and also biochemical parameters like chlorophyll content, protein, sugars and amylase activity in sorghum seeds. The sorghum seeds were treated with 100, 250 and 500 µg ml^{-1} concentrations of aflatoxins and likewise, *A. flavus* spore suspension adjusted to $1x10^8$ spores ml^{-1} were also treated to seeds in different treatments. The experimental results revealed maximum inhibition of seed germination, seedling vigor, chlorophyll, proteins, total sugars and α-amylase activity in the sorghum seedlings was observed at 500 µg ml^{-1} followed by 250 and 100 µg ml^{-1}. But seed treatment with toxigenic *A. flavus* spore suspension showed slight inhibition all the above parameters tested when compared to untreated control, but there was no significant decrease was observed. The study highlighted negative effects of the *A. flavus* and aflatoxins on the tested seed quality parameters tested there by necessitating need of monitoring of toxigenic fungi and their metabolites in sorghum seeds.

Key words – Amylase – Chlorophyll – Great millet – Mycotoxins – Seed germination and Seedling vigour.

Introduction

Aflatoxins are potent carcinogenic, mutagenic and teratogenic metabolites produced primarily by the fungal species *Aspergillus flavus* and *A. parasiticus*. Most of the species of *Aspergillus* are dominant and play vital role in the seed biodeterioration (Amaike and Keller 2011; Aiyaz *et al.* 2015). Association of variety of fungi with seeds causes significant loss in seed quality and nutritional quality (Koirala *et al.* 2005). Fungal organisms play significant role in causing pre- and post-infections and considerable quality losses *viz.*, seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, seedling damage and their nutritive value during production, harvest and storage (Mohana *et al.* 2011; Aiyaz *et al.* 2015). It is well documented that, mycotoxins produced by specific filamentous fungi and also cause significant reductions in crop yield and economic losses (Bhatnagar and Garcia 2001; Divakara *et al.* 2015).

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The involvement of *Aspergillus* spp. as plant pathogens has not been seriously taken in to consideration, but there is accumulating evidence that the aflatoxins are phytotoxic compounds and secretion in to seeds/ grains interferes with seed germination, seedling growth, protein synthesis, carotenoid, and lipid content in various crops (Sinha and Kumari 1990; Agar and Alposy 2005). The levels of phytohormones such as GA-like substances (GAs), trans-Zeatin (*t*-Z) and indole acetic acid (IAA) have been decreased in the germinating maize seeds exposed to aflatoxin B_1 and G_1 (Agar *et al.* 2006).

These toxigenic fungi and aflatoxins were reported as prevalent contaminants of sorghum in India and other parts of the world (Silva *et al.* 2000; Yassin *et al.* 2010; Ratnavathi *et al.* 2012; Divakara *et al.* 2014). Presently a very few reports are available with respect to their effects on sorghum seed quality parameters. Hence in the present study an attempt has been made to study the effect of these toxigenic fungi and aflatoxins on seed quality parameters of sorghum.

Materials and methods

Source of sorghum seed samples

Seeds of sorghum cv. 8340 (Bayer Crop Science) was obtained from the commercial market of Mysore city, Karnataka, India and used throughout the experiments.

Aspergillus flavus

Toxigenic *A. flavus* (isolate S3 Accession No. in NCBI-KJ848670, KF309040) was isolated from sorghum seeds and assessed its toxigenicity as described in Divakara *et al.* (2014) and used for the treatment of sorghum seeds.

Chemicals

Aflatoxin B_1 was purchased from Sigma Aldrich (USA), and initially dissolved in one ml of Benzene: Acetonitrile (98:2) to make a stock solution of 1 mg ml^{-1}. Further dilutions of aflatoxin B_1 (100, 250, 500 µg ml^{-1}) were prepared to treat the sorghum seeds.

Treatment of sorghum seeds with toxigenic *A. flavus* and aflatoxins

For the treatment of sorghum seeds with toxigenic *A. flavus,* seven-day-old fresh culture of *A. flavus* was washed with sterile distilled water to obtain the spore suspension and the concentration was adjusted to $1x10^8$ spores ml^{-1} using haemocytometer. To the spore suspension, 0.5% CMC was added to facilitate the adherence of spores to seeds and incubated at 25±2°C on a rotary shaker incubator at 150 rpm for 6 h. For the treatment of aflatoxins to sorghum seeds, one hundred sorghum seeds for each treatment were initially soaked in sterile distilled water for one hour and subsequently soaked in different concentrations (100, 250 and 500 µg ml^{-1}) of aflatoxin solutions for 24 hours. Control seeds were also soaked in 10 ml sterile distilled water.

Effect of toxigenic *A. flavus* and aflatoxins on seed germination and seedling vigor

Effect of toxigenic *A. flavus* and aflatoxins on seed germination was determined by placing the seeds on moist filter papers in Perspex plates and kept for incubation at 25±2 ^oC. The germination percentage was determined on the 7^{th} day. Seedling vigor was calculated according to Abdul Baki and Anderson (1973).

Effect of toxigenic *A. flavus* and aflatoxins and on Chlorophyll content in sorghum seedlings

Chlorophyll (Chl) *a* and *b* contents of the newly emerged seedlings were estimated following the method of Arnon (1949). Briefly, the leaves (0.10 g) were ground with 80 % acetone in a pestle and mortar. The resultant homogenate was centrifuged and the resultant

supernatant was collected and the absorbance was read at 663 and 645 nm. The amount of chlorophyll a and b and total chlorophyll was calculated using the formula mentioned below and expressed as mg g^{-1} leaf tissue.

Chl_a= [(12.7 x A663) x (2.6 x A645)] x ml acetone/mg leaf tissue
Chl_b= [22.9 x A645) x (4.68 x A663) x ml acetone /mg leaf tissue

Effect of toxigenic *A. flavus* and aflatoxins on protein and total sugars

Effects of toxigenic *A. flavus* and aflatoxins on total proteins, total sugars were determined by following the procedures of Bradford (1976) and Dubois *et al.* (1956).

Effect of toxigenic *A. flavus* and aflatoxins on α-amylase activity

α-amylase activity in the germinating seeds was estimated by the modified method of Kang *et al.* (1997). Briefly, one g of the germinating seeds were withdrawn from each treatment and grind in 5 ml of pre-chilled 0.05M citrate buffer, pH 6.0; the resulting homogenate was centrifuged at 10000 r.p.m for 10 min. Enzyme activity was assayed in the supernatant as yield of crude enzyme. To the 0.1 ml of crude enzyme, 0.9 ml of 2% soluble starch was added and incubated in shaking water bath at 50 ^oC for 30 min. The reaction was stopped by adding DNS (Dinitro salicylic acid) reagent and boiled for at least 3 min for color development. Absorbance was read at 550 nm against blank. The blank contained all the assay reagents without the enzyme. Standard glucose curve was prepared from glucose concentrations of ranging from 0.0 to 1 mg ml^{-1} and expressed as release of reducing sugars g kg^{-1}.

Results

Effect of toxigenic *A. flavus* and aflatoxins on seed germination and seedling vigor

The results of the effect of toxigenic *A. flavus* and aflatoxins on seed germination and seedling vigor are shown in Fig. 1. Seed germination, seedling vigor, root length and shoot length were significantly decreased linearly with increased concentration of aflatoxins over untreated control. Maximum inhibition in seed germination, seedling vigor, root length and shoot length was caused by 500 µg ml^{-1} of aflatoxins treatment when compared to other two treatments (100 µg ml^{-1} and 250 µg ml^{-1}). But, treatment of toxigenic *A. flavus* spore suspension slightly inhibited the germination, seedling vigor, root length and shoot length when compared to untreated control, but there was no significant decrease was observed.

Effect of toxigenic *A. flavus* and aflatoxins on chlorophyll content in sorghum seedlings

Significant decrease in chlorophyll contents was observed when sorghum seeds were treated with different concentrations of aflatoxins (Fig. 2). Here also marked inhibition of chlorophyll content has been recorded over untreated control and significantly decreased linearly with increased concentration of aflatoxins. Maximum inhibition has been observed at 500 µg ml^{-1} of aflatoxins treatment when compared to other two treatments (100 µg ml^{-1} and 250 µg ml^{-1}). There was no significant decrease between control and toxigenic *A. flavus* spore suspension treatment on the chlorophyll contents of sorghum seedlings.

Effect of toxigenic *A. flavus* and aflatoxins on protein and total sugars

Studies on the effect of toxigenic *A. flavus*, aflatoxins on protein and total sugars have showed the significant difference among the control and treatments (Fig. 3). There was a marked decrease in the contents of total proteins and total sugars over untreated control seedlings. Maximum inhibition of has observed at 500 µg ml^{-1} of aflatoxins treatment when compared to other two treatments (100 µg ml^{-1} and 250 µg ml^{-1}). There was no significant decrease between

control and toxigenic *A. flavus* spore suspension treatment on total sugar contents of sorghum seedlings, but difference was observed in case of protein contents.

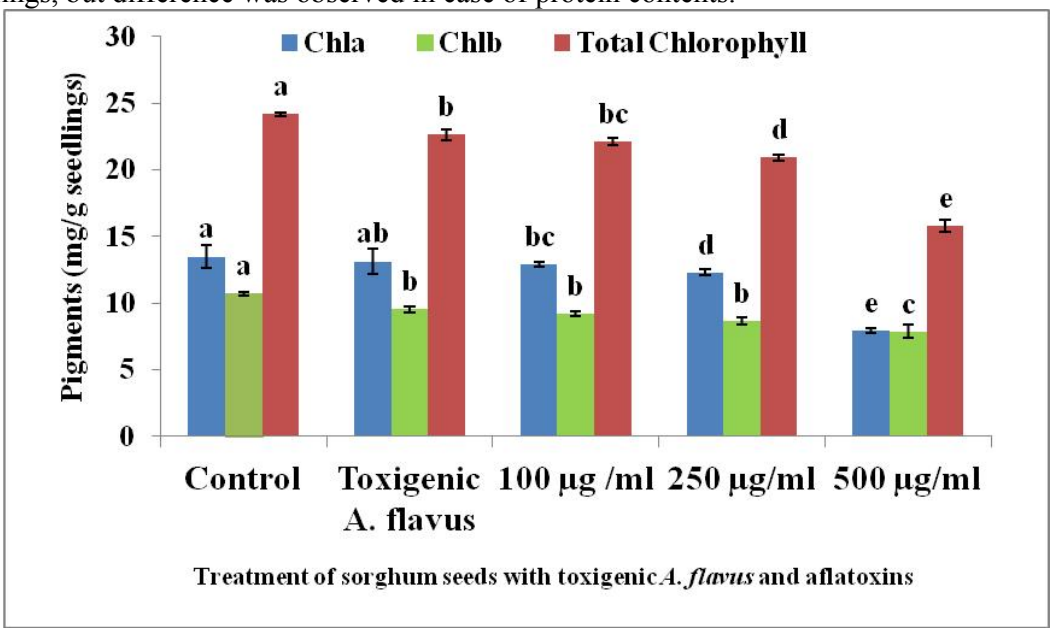

Fig. 1- Effect of toxigenic *A. flavus* and aflatoxins at different concentrations on seed germination and seedling vigor. Values are means of four independent replicates. Bars represent standard error.

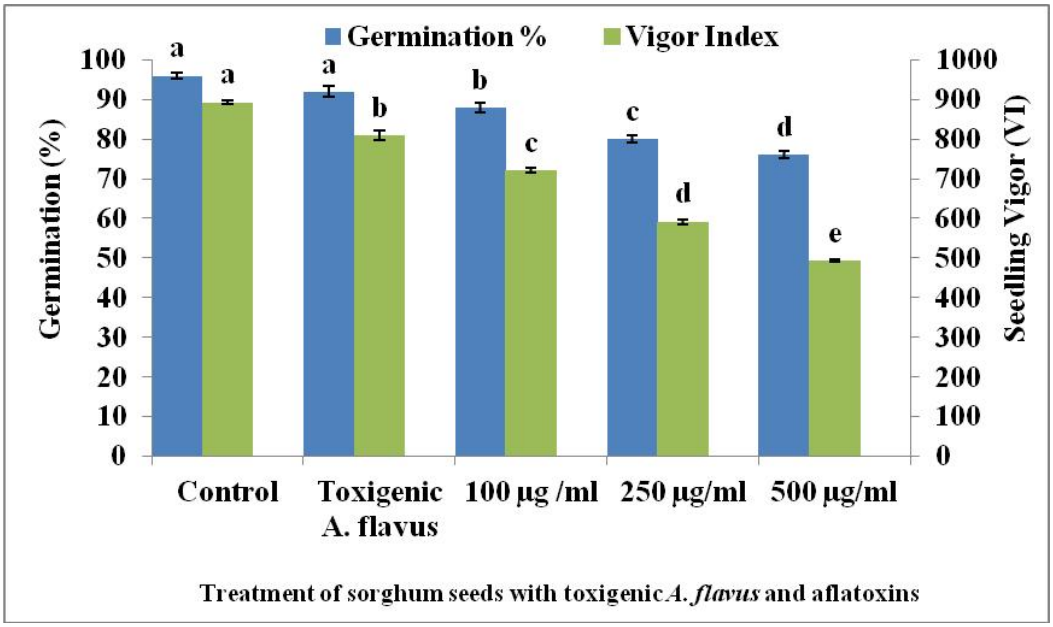

Fig. 2- Effect of toxigenic *A. flavus* on chlorophyll content in sorghum seedlings. Values are the means of four independent replicates. Bars represent standard error.

Effect of toxigenic *A. flavus* and aflatoxins on α-amylase activity

The results of the effect of toxigenic *A. flavus* and aflatoxins on α-amylase activity are shown in Fig. 4. α-amylase activity in sorghum seedlings has been decreased significantly with linear increase in the concentration of aflatoxin treatment when compared to untreated control. Maximum inhibition was observed at 500 µg ml^{-1} of aflatoxins treatment when compared to other two treatments (100 µg ml^{-1} and 250 µg ml^{-1}). Only slight difference was observed with toxigenic *A. flavus* treatment when compared to other treatments.

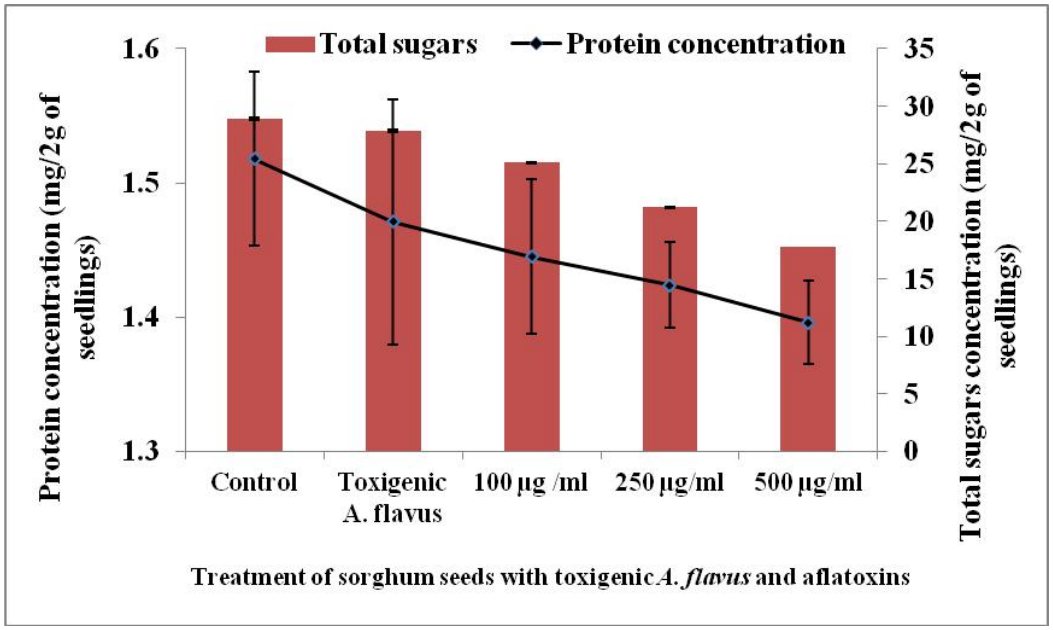

Fig. 3- Effect of toxigenic *A. flavus* on proteins content and total sugars in sorghum seedlings. Values are the means of four independent replicates. Bars represent standard error.

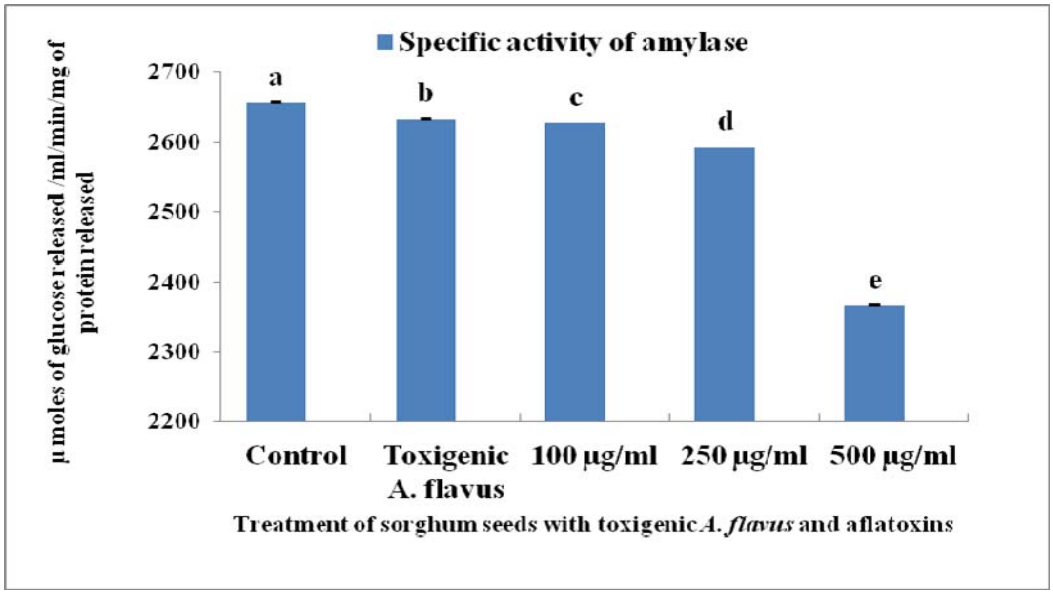

Fig. 4- Effect of toxigenic *A. flavus* on α-amylase activity in sorghum seedlings. Values are the means of four independent replicates. Bars represent standard error.

Discussion

In the present study, an attempt has been made to study the effect of toxigenic *A. flavus* and different concentrations of aflatoxins on seed quality parameters of sorghum have been evaluated. It is well known that, contamination of any seed/ grain with toxigenic *A. flavus* and all four types of aflatoxins have toxic effect on living organisms (Shi et al. 1994). Inhibition of seed germination and seedling growth by aflatoxin B_1 has been studied earlier in other crop plants (Janardhan *et al.* 2011; Navya *et al.* 2013; Divakara *et al.* 2015). Seed germination and seedling vigor of sorghum was greatly reduced with the treatment of different concentrations of aflatoxins. Maximum inhibition was recorded at 500 µg ml^{-1}. Seed germination and seedling growth of maize and broad bean has also been reduced with higher concentrations of aflatoxins (El-Naghy *et al.* 1999). Similarly, Yaqub Bhat & Fazal (2011) have studied the effect of *A. flavus* metabolites on wheat seed germination and seedling development and reported that the higher concentration of culture filtrate obtained from *A. flavus* reduced the seed germination, root and shoot lengths.

Aflatoxins have been reported to inhibit chlorophyll synthesis in maize plants (Prasad *et al.* 1996). Marked decrease in the chlorophyll content was observed in the present study are in agreement with the previous results. Inhibition of chlorophyll-a, chlorophyll-b and total chlorophyll content in maize seedlings has been reported (Shirurkar and Nilima 2012). Protein and total sugar contents in the treatments were greatly reduced with increased concentrations of aflatoxins. Decrease in protein contents by aflatoxin has been observed in the germinating maize seeds has been reported (El-Naghy *et al.* 1999). Changes in starch and sugar contents might be connected with the inhibition of α-amylase activity. Harris (1976) correlated the degradation of starch with the activation of starch hydrolyzing enzymes like α-amylase during seed germination, which converts the starch molecules in to simple sugars. Because of the reduction in α-amylase activity, lower levels of sugars have been observed in the treated seeds. In the present study, α-amylase activity has also been reduced greatly with the increased concentration of aflatoxins and maximum inhibition was observed at 500 µg ml^{-1}. In pea, transient changes in α-amylase activity were correlated with the changes in the rate of starch hydrolysis (Morohashi *et al.* 1989). Lower contents of sugars in high toxin treated seeds also suggest the least participation of this enzyme and have been reported earlier in maize seedlings (Prasad 1998).

Treatment of sorghum seeds with toxigenic *A. flavus* on seed quality parameters did not yield significant differences when compared to untreated control. But only slight differences have been observed. This is because of the usage of fresh seeds and *A. flavus* needs a storage period for establishment in seeds and to produce detectable differences in the seed quality parameters (McDonald and Harkness 1964, 1967). In conclusion, seeds selected for sowing should be devoid of any aflatoxin contamination to reduce the toxic effects on plant growth and development.

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Conflict of Interest

The authors do not have any conflicts of interest.

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