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Efficacy of Bitter Plant Extracts (*Azadirachta indica)* on Seed Germination, Chlorophyll and Carotenoid Synthesis in Mycotoxin Especially Aflatoxin B_1 Treated Maize Seeds (*Zea mays* L.)

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A B S T R A C T

Maize (*Zea mays* L.) is one of the most important cereal crop plants. It can be used in cooked, roasted, fried ground, crushed to prepare various food items like Kurkure, Popcorn, Corn syrup, Corn sauce, Cornmeal as well as it also useful as medicines and as raw materials for industries. Due to Climatic changes, it has been reported as a driver for emerging food and feed safety. This toxic infection result reduces grain quality due to contamination of maize seeds with the mycotoxin especially aflatoxin. One of the most effective ways to control the problems caused by aflatoxins due to prevent the growth of seed germination. Bitter plants like *Azadirachta indica* is one of the medicinal plants having antifungal or antimicrobial properties. Our findings reveals that the maximum inhibition in seed germination were 76.56 % on maize at 2ppm concentration of AFT- B_1. Also reports shows that, the minimum inhibitions in seed germination were recorded in chl a, chl b, Total chl a+b and carotenoids were 9.75, 22.22, 9.83 and 47.51 in maize seedlings due to treatment of AFT- B_1 with *A. indica* at 2 mg/ml concentration. Ultimately *Azadirachta indica* extracts reduce the inhibitory properties of AFT-B_1 produced by *A. flavus*. Furthermore, the bitter plant like *A. indica* investigated in the present study may provide potential leads for novel bioactive. This will ultimately lead to substantial, financial returns to the farmers as these Bitter plant like *Azadirachta indica.*

***Key words*:** *Azadirachta indica,* Maize seeds, *Aspergillus flavus,* Mycoflora, Aflatoxin B_1, Chlorophyll, Carotenoid

Maize (*Zea mays L.*) is one of the most important cereal crop plants. It has been a keystone model organism. Within the cereals, which include other plant model species like rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), maize is the most thoroughly researched genetic system [1]. It can be used in cooked, roasted, fried, ground, powder or crushed to prepare various food items like Kurkure, Popcorn, Corn syrup, Corn sauce, Cornmeal etc. Apart from food, maize is also useful as medicines [2] and as raw materials for industries. Climate changes has been reported as a driver for emerging food and feed safety issues in worldwide. It has been contaminated with various fungus like, *Aspergillus flavus*, *A. parasiticus*, *Fusarium* spp [3-6].

Aflatoxin B_1 produced by *A. flavus* has been already also reported [7-8]. Aflatoxins have the highest acute and chronic toxicity of all the mycotoxins, the maximum concentration in agricultural food products and their commodities is regulated [9]. This toxic infection results reduces grain quality due to contamination of mycotoxin especially aflatoxin in maize seeds [10-14]. Aflatoxin also adverse the growth of seed germination, synthesis of chlorophyll and carotenoids, resulting in virescence and albinism disease in the affected plant [15-16].

One of the most effective ways to control the problems caused by aflatoxins due to prevent the growth of seed germination as well as biochemical (Quality and Quantity) changes of maize seeds. Bitter plants like *Azadirachta indica* is one of the medicinal plants having triglycerides (esters formed from a molecule of glycerol and three molecules of fatty acids) and is very rich in azadirachtin- the key component acting as insect repellent, anti feedant, antifungal or antimicrobial and antiviral properties [17-18]. Only few reports are available on the action of bitter plant like *Azadirachta indica* on plant system. An attempt, has therefore been made to record the comprehensive data of AFT- B_1 and *Azadirachta indica* individually and in combination of seed germination, chlorophyll and carotenoid synthesis of maize seeds.

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MATERIALS AND METHODS

Collection of materials

Maize seeds (*Zea mays* L.) of Syngenta Sugar 75 corn obtained from Pooja seeds, Agricultural seed store, Bazar

Samiti Road, Shivdhara, Darbhanga, Bihar, 846004, were used throughout the experiments.

Toxins

Standards of Aflatoxin B_1 was obtained from Sigma Chemical Co. St. Louis, U.S.A.

Bitter plants

Bitter plants like *Azadirachta indica* were obtained from the Janta nursery, Karamganj, west of Naka No- 6, Darbhanga.

Preparation of plant extracts

Azadirachta indica (Neem seeds) were repeatedly washes to remove dust and other impurities material and subsequently dried in oven at 50 °C until it attained constant moisture content. Twenty grams of plant part were blended with 100 ml of sterile distil water to prepare a standard and various concentration such as 2 mg/ml, 4 mg/ml and 6 mg/ml of aqueous extract.

Seed germination

After soaking of seeds in *A. indica* plant extracts (2 mg/ml, 4 mg/ml and 6 mg/ml) with AFT- B_1 (2 ppm) concentration for 1 hr and then were placed in sterilized Petri dishes on three layers of blotting papers soaked in distilled water, each dish containing 8-10 seeds and 3 dishes was selected as replicates. For each *A. indica* extracts with different concentration. Then, the petri dishes will be kept in the incubation chamber at 28 ± 2 °C at light interval 12/12 hr and data will be recorded after 5-7 days. The percentage of seed germination were calculated by the following formula:

$$GI = \frac{\text{Total number of seed germinated}}{\text{Total number of seed observed}} \times 100$$

This data was analyzed statistically i.e., 't' – test for seed germination and 'F'- test for seedling growth. Statical calculations were carried out using ANOVA test [19].

Chlorophyll and carotenoid synthesis

Chlorophyll and carotenoid contents of the newly emerged leaves were estimated by the method of Arnon [20] and Davis [21], respectively. 250 mg leaf tissues were extracted in 5 ml 80% of acetone. Resulting green liquid/ extract was transferred to Buchner Funnel containing Whatman No. 1 filter paper. Extraction of tissue was repeated 2-3 times with 5 ml of 80% acetone which was subsequently filtered into the flask containing initial extract. With another 5 ml of acetone (80%), the mortar, pestle and sides of the funnel were rinsed. Finally, the volume of filtrate was made to 25 ml by adding extra amount of 80% acetone. The optical density of the extract was recorded with Beckman DU-64 spectrophotometer set at 480, 645 and 663 nm against blank (80% acetone). The amount of chlorophyll present in the extract was calculated by the following formulae in terms of mg/g dry weight.

$$\text{Mg chl a/g tissue} = 12.7\,(D\,663) - 2.69\,(D\,645) \times \frac{V}{1000 \times W}$$

$$\text{Mg chl b/g tissue} = 22.9\,(D\,645) - 4.68\,(D\,663) \times \frac{V}{1000 \times W}$$

Total chlorophyll = chl a + chl b

Where,
D = Optical density
V = Final volume of the 80% acetone chlorophyll extract.
W = Fresh weight in gm of the tissue extracted.

Determination of the total carotenoid contents of the tissue in presence of chlorophyll was made by the method of Davis [21]. Contribution by chl a and chl b to the extinction at 480 nm were determined using the extinction co-efficient of the chlorophyll at that wave length. The increase in absorbency at 480 nm which is due to carotenoid formation ($\Delta E\ Car_{480}$) is given by mg/g leaf tissue.

$$\Delta E\ Car_{480} = \Delta E\ 480 + 0.114\ \Delta E\ 663 - 0.638\ \Delta E\ 645 \times \frac{V}{1000 \times W}$$

Where,
$\Delta E\ Car_{480}$ = Total Carotenoids
ΔE = Extinction Coefficient
V and W = As in Chlorophyll

RESULTS AND DISCUSSION

Effect of Azadirachta indica extracts on seed germination

Result obtained in (Table 1) and Histogram- A, showed the effect of *Azadirachta indica* extracts on maize seed germination. In this experiment, *Azadirachta indica* plant extracts were used for controlling fungi on maize seeds with different concentration like 2 mg/ml, 4 mg/ml and 6 mg/ml with 2 ppm concentration of AFT -B_1, respectively. *A. indica* with different concentration and AFT- B_1 showed an increase of germination with 22.98, 52.56, 66.51 and 78.58% respectively when compared with control (98.06%), respectively.

Table 1 Effect of *Azadirachta indica* (*A. indica*) / (A) extracts with AFT B_1 on maize seeds germination

Seed	Treatment with diff. con^n	Germination X ± S. E	Difference with control	Percent inhibition
Maize seeds	Control	98.06 ± 0.23	-	-
	AFT- B_1 (2 ppm)	22.98 ± 0.19	75.08	76.56
	AFT- B_1 + 2 mg/ml (A)	52.56 ± 0.17	45.5	46.40
	AFT- B_1 + 4 mg/ml (A)	66.51 ± 0.16	31.55	32.17
	AFT- B_1 + 6 mg/ml (A)	78.58 ± 0.13	19.48	19.86

This data was analyzed statistically i.e., 't' – test for seed germination
A = *A. indica*

Effect of Azadirachta indica on AFT B_1 treated chlorophyll and carotenoid content of maize seeds

The effect of AFT -B_1 and *Azadirachta indica* plant extracts alone and in combination on chlorophyll and carotenoid synthesis in the cotyledonary leaves of maize is depicted in table -02 and (Fig 1). The minimum inhibitions record in chl a, chl b, Total chlorophyll and carotenoid were, 9.75, 22.22, 9.83 and 47.51% in maize seeds due to treatment of AFT- B_1 with *A. indica* at 2 mg/ml concentration, respectively. Toxin with *A. indica* (4 mg/ml) and *A. indica* (6 mg/ml) showed more or less inhibition in chl a, chl b, total chlorophyll a + b and carotenoids i.e., 19.51, 33.33, 2.45 and 56.73 % followed by 29.26, 50, 24.59 and 78.72%, respectively.

Azadirachta indica extracts were found to be inhibit to ergosterol biosynthesis of *A. parasiticus*, this effect might be attributed to inhibition of enzyme (S), which is involved in the

biosynthetic pathway of ergosterol [22]. It has also found to be an inhibitory effect on the growth of *A. flavus* and *A. parasiticus* also reported by Thanaboripat *et al.* [23]. By adding the appropriate amount of *A. indica* extracts (2 mg/ml, 4 mg/ml and 6 mg/ml) with AFT- B_1 (2 ppm con^n) showed an increase the seed germination.

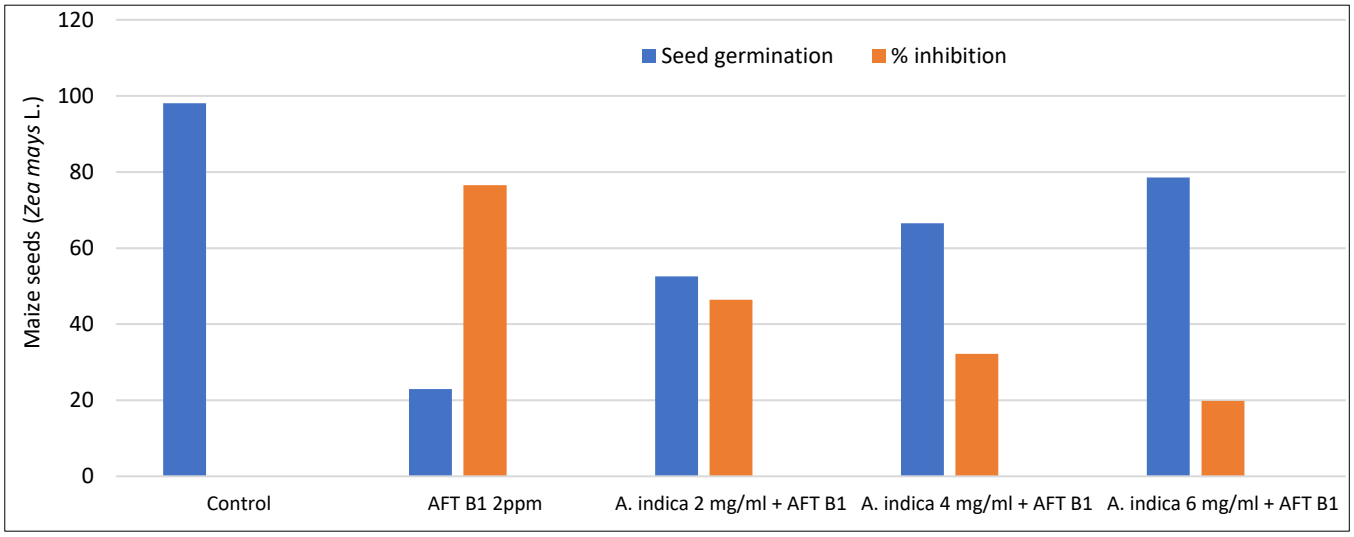

Fig 1 Effect of *A. indica* with AFT-B_1 on maize seeds germination

Table 2 *Azadirachta indica* on AFT B_1 treated chlorophyll and carotenoid content of maize seeds

Observation	% chl a (X ± S. E)	% Inhibition	% chl b (X ± S. E)	% Inhibition	Total chl (a + b)	% Inhibition	% Carotenoid	% Inhibition
Control	0.82 ± 0.017	-	0.36 ± 0.017	-	1.22 ± 0.044	-	1.41 ± 0.001	-
AFT- B_1 (2 ppm)	0.24 ± 0.017	70.73	0.26 ± 0.016	27.7	1.55 ± 0.019	27.04	1.21 ± 0.001	14.18
AFT- B_1 + 2 mg/ml	0.74 ± 0.016	9.75	0.28 ± 0.005	22.22	1.34 ± 0.025	9.83	0.74 ± 0.03	47.51
AFT- B_1 + 4 mg/ml	0.66 ± 0.021	19.51	0.24 ± 0.008	33.33	1.19 ± 0.034	2.45	0.61 ± 0.04	56.73
AFT- B_1 + 6 mg/ml	0.58 ± 0.021	29.26	0.18 ± 0.011	50.00	0.92 ± 0.025	24.59	0.30 ± 0.001	78.72

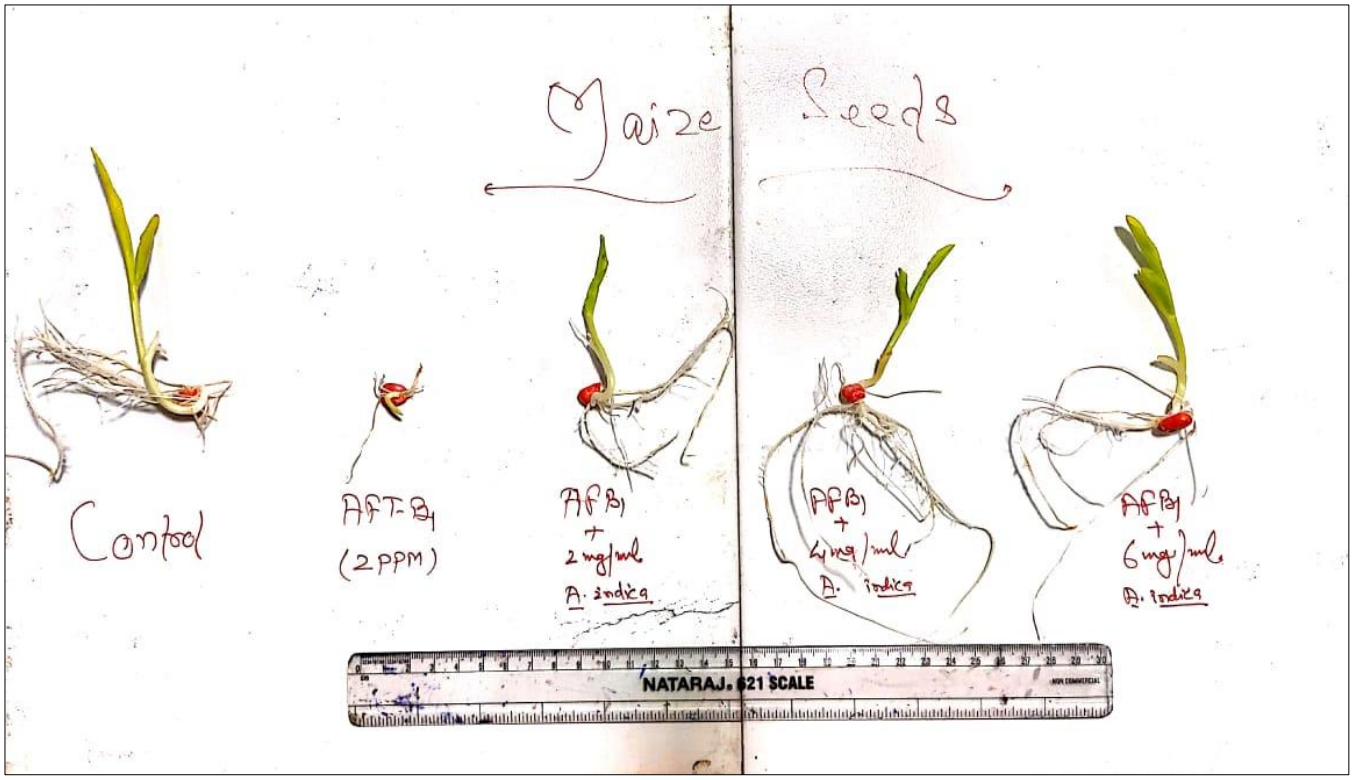

Fig 2 effect of *Azadirachta indica* with different concentration and AFT- B_1 treated Seed germination and chlorophyll content of maize seeds (*Zea mays* L.)

Our findings showed that the toxins in combination with *Azadirachta indica* extracts was also detrimental to the seed germination student t- test analysis showed significant effect on seed germination due to toxins with *Azadirachta indica* treatment. In addition to restricted seedling growth, chl a, chl b, total chlorophyll as well as carotenoid contents were also

inhibited due to AFT- B_1. *Azadirachta indica* with AFT- B_1 on the other hand increase the levels of chl a, chl b, total chlorophyll and carotenoids in maize seedling, respectively (Table 2).

CONCLUSION

In conclusion *Azadirachta indica* is a significant inhibitor of growth of *Aspergillus flavus* subsequently reverse the aflatoxin production in cereal crops. It has also one of the approaches to control the aflatoxin inhibitory properties to enhance the food production to accommodate the growing population demand in developing countries like India.

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