

Effect of total aflatoxin on the growth characteristics and chlorophyll level of sesame (*Sesamum indicum* L.)Anjorin Toba Samuel¹, Inje, Tersoo Valentine²¹Department of Crop Science, Faculty of Crop Science, University of Abuja, Abuja²Department of Biological Sciences, Faculty of Science, University of Abuja, Abujaoyindamola35@yahoo.com

Abstract: This study investigated the effect of total aflatoxin (1000µg/l, 500µg/l, 250µg/l, 125µg/l, and 0µg/l) on the chlorophyll level and some growth characteristics of sesame (*Sesame indicum*) using bogoro, E8, Ex-sudan varieties as the plant bioindicators. The experiment was conducted on the Department of Biological Sciences experimental field, Faculty of Science, University of Abuja, Abuja. The experimental design adopted was Randomized Complete Block Design (RCBD) involving three replicates. The seedling emergence, shoot length, root length, seedling vigour and chlorophyll level of the sesame cultivars at 10 Days After Sowing (DAS) under the control plot was significantly ($p<0.05$) higher than other treatments. All the parameters measured under the control plots of E8 and Ex-sudan cultivar were significantly ($p<0.05$) higher than other treatments. However, the number and area of leaves and number of flowers of *bogoro* cultivar at 56 DAS were significantly ($p<0.05$) higher at 125µg/l treatment than at other levels. The study showed an inhibitory effect on all the growth characteristics of sesame by total aflatoxin especially above 125µg/l. The biotest of mycotoxin on various crop seeds is imperative. Also the fate of total aflatoxin on the sown seeds in the rhizosphere requires investigation.

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1. Introduction

Sesame (*Sesamum indicum* L.) is a flowering plant in the genus *Sesamum* and the family Pedaliaceae. It is widely naturalized in tropical regions around the world and it is cultivated for its edible seeds, which grow in pods (Bedigian, 2010). Sesame is a common staple food and very drought-tolerant, in part due to its extensive root system, it requires adequate moisture for germination and early growth (FAO, 2012). For thousands of years, sesame seeds have been a source of food and oil. Sesame has one of the highest oil content of any seed, some varieties exceeding 50% oil content compared to soybean's 20% (Langham, 2008). Sesame oil is one of the most stable vegetable oils, with long shelf life, because of the high level of natural antioxidants (sesamin, sesamol, and sesamol). Oil from the seed is used in cooking, as salad oils and margarine, and contains about 47% oleic and 39% linoleic acid (Ivon *et al.*, 2005). Sesame seed is also rich in protein, at 25% by weight. The flour that remains after oil extraction is between 35 to 50 % protein, has good effective carbohydrates, and contains water-soluble antioxidants (sesaminolglucosides) that provide added shelf-life to many products. This flour, also called sesame meal, is an excellent high-protein feed for poultry and livestock. Sesame seeds may be baked into crackers, often in the form of sticks (Oplinger *et al.*, 2000). In Greece the seeds are also used in cakes. Fast-food restaurants use buns with tops sprinkled with

sesame seeds. Aflatoxin-producing *Aspergillus* could inhabit the soil, decaying vegetation, hay, and grains undergoing microbiological deterioration and it invades all types of organic substrate whenever conditions are favourable for its growth, favourable conditions include high moisture content (at least 70%) and high temperature (Machida, 2010; Leong *et al.*, 2011, McDaniel, 2011). Chlorophyll is an extremely important biomolecule, chlorin pigment found in the chloroplasts of plants. It is critical in photosynthesis as it allows plants to absorb energy from light (Anthony *et al.*, 2003). Chlorophyll molecules are specifically arranged in and around photosystems that are embedded in the thylakoid membranes of chloroplasts (Chen *et al.*, 2010). Chlorophyll content can be an indicator of the plant's condition.

Biotests of mycotoxin can facilitate an objective evaluation of the effect of a toxin level, due to the fact that all higher plants have certain sensitivity to different xenobiotics found in the soil environment. El-Taher *et al.*, 2012 recommended that the characteristics of plant bioindicators to be used in phytotests should have small and even seeds, uniform germination power and energy of seeds, a short emergence period (1-2 days), a short vegetation period, high biomass of stems, leaves or roots and have high sensitivity in relation to one chemical group such as aflatoxins, fumonisins. There is dearth of scientific reports on biotests of mycotoxin using crop seeds as the bioindicator in Nigeria, thus this research

investigated the effect of total aflatoxin on the seedling emergence, growth characteristics and chlorophyll level of three sesame varieties.

2 Materials and methods

2.1 Collection of seed samples

The seeds of sesame such as Ex-sudan, Bogoro and E8 were collected from the National Cereal Research Institute (NCRI) Badeggi, Bida, Niger State in Nigeria. The E8 variety are characterized by early maturing, high yielding and yellowing of leaves, the Ex-sudan are characterized by high yielding and late maturing (120 days), while the Bogoro local are dwarf and short. The seeds were carefully cleaned and freed from foreign materials.

2.2 Experimental site and layout

The study was conducted in the field which were carried out in black polythene bags filled with rich sandy loam soil in texture, sand, silt, and clay content was 74, 16, and 10% respectively, slightly acidic in reaction ($p^H = 5.30$), low to medium in electrical conductivity (0.150dsm^{-1}), with low levels of organic carbon (0.087%) and nitrogen (0.175kg/ha). The bags were randomly arranged on the experimental field of Department of Biological Sciences, Faculty of Science, University of Abuja, Abuja. The experimental plots were arranged in Randomized Complete Block Design (RCBD) involving five concentration levels of total Aflatoxin (1000 $\mu\text{g/l}$, 500 $\mu\text{g/l}$, 250 $\mu\text{g/l}$, 125 $\mu\text{g/l}$ and 0 $\mu\text{g/L}$ of distilled water) to each of the three sesame varieties (Ex- sudan, Bogoro, E8) seeds before sowing.

2.3 Application of total aflatoxin to seed samples

The chemical, Total Aflatoxin; Aflastandard product code: p22/ p22A were procured from R. Biopharm Rhone Ltd, Indonesia through Chromogene Ltd and the bottle stored at 2°C - 8°C. The stock solutions were appropriately diluted at the time of sowing to obtain the required concentration. 1000 μg per litre of total Aflatoxin was serially diluted to obtain 500 μg , 250 μg , 125 μg , 0 μg per distilled water. All the sesame seeds were initially soaked in distilled water for an hour, before 10 gram of the seeds were subsequently transferred to the beaker (500ml) filled with each level of concentration. The setup was allowed to stay for three hours before sowing.

2.4 Sowing of treated Seeds

On the 28th August 2012, 10 seeds were sown per bags and covered lightly with top soils. Regular watering was carried out as required.

2.5 Data Collection

Data were collected on the germination %age, shoot length, root length, seedling vigour, chlorophyll level, number of leaves, area of leaves and number of flowers.

2.6 Seed emergence, shoot and root length The percentage of seedling emergence of the various

cultivars were taken by counting the plants that emerged after ten days, and was multiplied by hundred (100). The shoot and root length were measured in millimetre, which was taken by selective uprooting of 3 plants at a time.

2.7 Seedling vigour

The seedling vigour of various cultivars was taken by using the formula adopted by (Okelola, 2005):

$$(sl + rl) \times g\%$$

where sl= shoot length; rl = root length; g% = germination percentage

2.8 Chlorophyll level

The chlorophyll level in sesame was recorded by aid of a chlorophyll-metre (Atleaf®) assembled, tested and calibrated in the United States. It was used to determine the percentage of chlorophyll on 3 selected leaves per pot of sesame seedlings. Atleaf® is a powerful, handheld, easy to use device for noninvasively measuring the relative chlorophyll content of green leaf plants. Chlorophyll content can be an indicator of the plant's condition. The device is. The Plant relative chlorophyll concentration was measured by inserting a leaf into the device aperture, Green leaves of up to 3mm thickness can be measured. It only takes one press of the key to perform a basic measurement (Gitelson, 1999). The device has simple, one-key operation for measuring the optical density difference at 2 wavelengths (660nm and 940nm), displays 2 lines x 16 characters and it powered by 2 AA (1.5V) batteries.

2.8 Number of Leaves and flowers

The data on the number of leaves and flowers were taken by counting of the available leave on each cultivar under each treatment at 56DAS.

2.9 Area of leaves

The area of leaves of the sesame cultivars were measured on 56DAS using graph sheet with cm^2 grids.

2.10 Statistical Analysis of Data Data collected was subjected to ANOVA and the means were separated with Duncan Multiple Range (DMRT). Varietal x concentration effects were determined by using Statistical Analysis Package SPSS Statistical Version 17.0.

3. Results

Table 1 shows that the seedling emergence of bogoro at 10DAS under the control plot was significantly ($p < 0.05$) higher than other treatments. There was no significant ($p > 0.05$) difference in the emergence of bogoro cultivar applied with 500 $\mu\text{g/l}$ and 100 $\mu\text{g/l}$. Also, the shoot and root length, seedlings vigour and chlorophyll level of the bogoro cultivar under the control plot was significantly ($p < 0.05$) higher than those treated with aflatoxin. Application of total aflatoxin at 500 or 1000 $\mu\text{g/l}$ significantly ($p < 0.05$)

reduced all the parameters measured than under other treatments.

The seedling emergence, shoot length, root length, seedling vigour and chlorophyll level of E8 sesame cultivar in the control plots were significantly ($p < 0.05$) higher than other treatments. There was no significant ($p > 0.05$) difference between the seedling emergence of the E8 seeds applied with 125 $\mu\text{g/l}$ and the control.

The germination emergence, shoot and root length, seedling vigour and chlorophyll level of Ex-

sudan cultivar in the control plot was significantly ($p < 0.05$) higher than other treatments. Total aflatoxin application at 1000 $\mu\text{g/l}$ resulted in the lowest significant reduction in the germination percentage, root length and the chlorophyll level. The shoot length of 125 $\mu\text{g/l}$ treatment of the Ex-sudan cultivar was the lowest. The interaction between the varieties and treatments led to highly significance ($p < 0.05$) difference on the germination percentage, shoot length, root length, seedling vigour and chlorophyll level as shown in the Table 1.

Table 1. Effect of total aflatoxin on the germination percentage, shoot length, root length, seedling vigour and chlorophyll level of three sesame cultivars at 10DAS

Variety	Concentration ($\mu\text{g/L}$)	Seedling emergence	Shoot length (mm)	Root length (mm)	Seedling vigour (x100)	Chlorophyll level (index)
Bogoro	0	96.00a	19.75a	16.50a	1895.96a	35.85a
	125	90.00b	17.50b	15.25b	1575.06b	31.77b
	250	70.00c	17.00b	12.25c	1190.66c	21.50c
	500	40.00d	12.75c	10.75d	510.00d	20.10d
	1000	40.00d	13.00c	10.80d	520.66d	20.20d
E8	0	99.00a	31.00a	36.75a	3068.66a	32.30a
	125	99.00a	16.50c	20.25d	1633.53c	30.22c
	250	75.00b	19.50b	32.00b	1852.56b	31.05b
	500	40.00c	12.75d	10.75e	510.00 e	20.10e
	1000	50.00c	16.00c	24.70c	1440.66d	28.60d
Ex-sudan	0	70.00a	28.25a	22.50a	1977.55a	34.24a
	125	55.00c	20.00d	20.00b	1100.66d	30.00b
	250	65.00b	23.40c	22.50a	1521.06b	29.93b
	500	55.00c	24.50c	19.80b	1343.83c	29.23b
	1000	45.00d	25.00b	18.75c	1125.66d	27.60c
Interaction (variety x level)		**	**	**	**	**

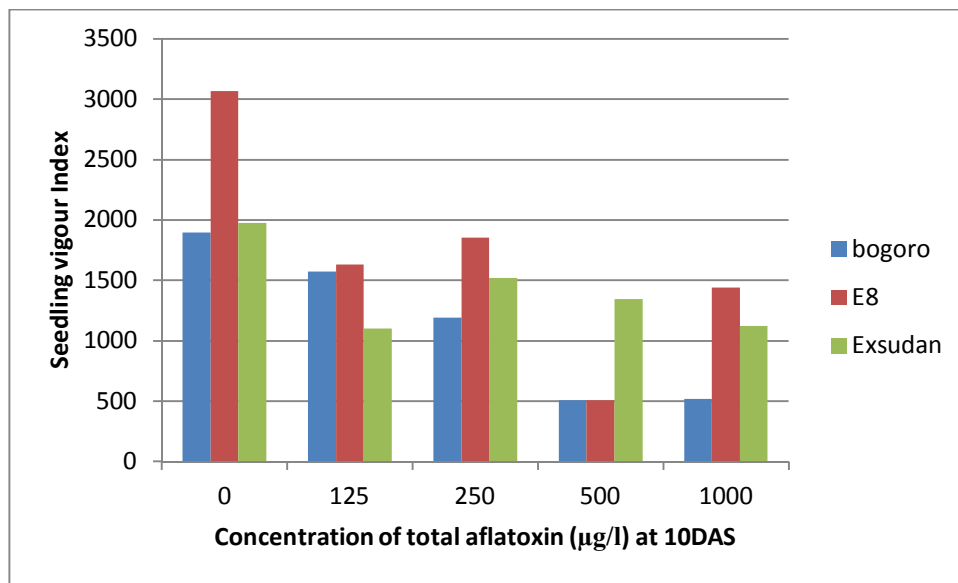


Figure 1. Effect of total aflatoxin on the seedling vigour on sesame cultivars

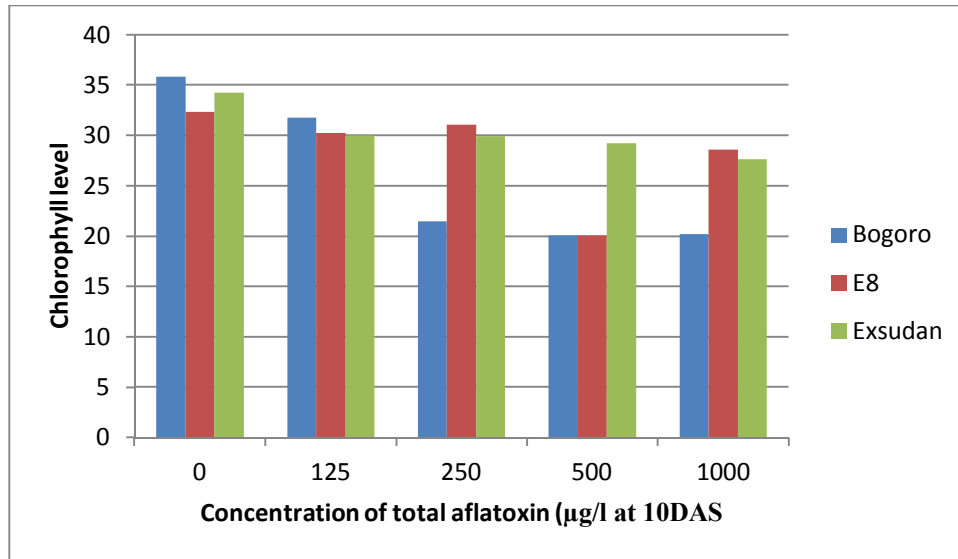


Figure 2. Effect of total aflatoxin on the chlorophyll level on sesame cultivars

The effects of total aflatoxin application on the number of leaves, area of leaves and number of flowers of three cultivars of sesame at 56DAS on the field are as shown in Table 2. The number of leaves and number of flowers of the 3 sesame cultivars applied with total aflatoxin were adversely affected.

It was indicated that the number of leaves (56DAS) in bogoro under the 1000µg/l treatment was significantly ($p < 0.05$) higher than other levels of treatments. There was no significant ($p > 0.05$) difference in the number of leaves of bogoro applied

with 500ug/l and the control. The area of leaves of bogoro treatment in the 1000µg/l treatment was significant ($p < 0.05$) higher than other treatments. There was no significant ($p > 0.05$) difference between the area of leaves of the bogoro seeds applied with 500µg/l, 125µg/l and the control. The number of flowers under the 1000µg/l treatment in the bogoro cultivar was significantly ($p < 0.05$) higher than other treatments. Total aflatoxin application at 250µg/l resulted in the lowest significant reduction in the number of flowers of the bogoro cultivar.

Table 2. Effect of total aflatoxin on the no of leaves, area of leaves and no f flowers on three sesame cultivars at 56 DAS

Variety	Concentration (µg /L)	No of leaves	Area of leaves (mm ²)	Number of flowers
Bogoro	0	11.16b	11.22c	3.16b
	125	19.00a	18.64a	6.66a
	250	10.33c	16.56b	2.50d
	500	9.66d	11.05c	1.80e
	1000	8.85d	15.49b	2.66c
E8	0	13.83a	29.87a	7.66a
	125	12.83b	16.86c	7.00b
	250	12.33c	22.15b	6.00b
	500	9.66d	11.05d	1.80c
	1000	11.66e	13.00d	2.16c
Ex-sudan	0	63.00a	28.35a	8.00a
	125	51.50b	22.40b	8.00a
	250	49.00c	20.25c	5.00b
	500	46.00d	16.10d	3.50b
	1000	26.50e	10.85 e	3.00c
	Interaction (varietal x level)	**	**	**

The number of leaves of the E8 cultivar in the control plot was significantly ($p < 0.05$) higher than other treatments. Application of aflatoxin at 500µg/l

treatment significantly ($p < 0.05$) reduced all the parameters measured than under other treatments. Area of leaves of the E8 cultivar in the control plot was

significantly ($p < 0.05$) higher than other treatment. There was no significant ($p > 0.05$) difference between the area of leaves of the E8 seeds applied with 500 $\mu\text{g/l}$ and 1000 $\mu\text{g/l}$. The number of flowers of E8 cultivars under the control plot was significantly ($p < 0.05$) higher than other treatment. There was no significant ($p > 0.05$) difference between the number of flower of E8 cultivar of treatments applied with 500 $\mu\text{g/l}$ and 1000 $\mu\text{g/l}$.

The number of leaves, area of leaves and number of flowers of Ex-sudan cultivar in the control plot was significantly ($p < 0.05$) higher than other treatments. Total aflatoxin application at 1000 $\mu\text{g/l}$ resulted in the lowest significant reduction in the number and area of leaves and number of flowers. The interaction between the varieties and treatment produced a highly significance difference on the number and area of leaves and number of flowers (Table 2).

4. Discussion

The phytotoxic effect of toxins may be observed on the basis of the reduction of dry or fresh weight of roots or above ground parts (stems, leaves) of test plants (Demczuk et al., 2004); On the basis of selected parameters, such as the reduction of root length, the toxic effect of a toxin may be determined already after approx. 24h, while the dynamics of root growth - after 3-5 days from the onset of the test. In turn, the reduction in fresh or dry weight of aboveground parts of plants may be established after approximately 10-14 days in a conventional biotest (Sekutowski and Sadowski, 2006).

In this study, there was an inhibitory response on the chlorophyll and growth characteristics of the biotest sesame cultivars, at high concentration of aflatoxin application. Sinha and Kumari (1990) and Adekunle and Basir (1997) reported that cowpea (*Vigna unguiculata* L. Walp) seed germination and chlorophyll formation were inhibited with increased aflatoxin concentration. Fratamico, et al., (2008) observed that preharvest aflatoxin contamination of peanuts and corn is favoured by high temperatures, prolonged drought conditions, and high insect activity; while postharvest production of aflatoxins on corn and peanuts is favoured by warm temperatures and high humidity. Crison (1997) observed that seedling growth was not inhibited at lower concentration of aflatoxin per ml of agar substrate but there was an inhibitory effect on elongation of the hypocotyls and the root in the species studied at higher tage age of aflatoxin.

Application of 125 $\mu\text{g/l}$ aflatoxin showed a remarkably different effect on bogoro cultivar by stimulating increased number and area of leaves. Many toxic substances like pesticides have been reported to have shown growth stimulating or hormone-like properties at sublethal concentrations (Wu et al., 1972).

This is by the stimulation of DNA-directed RNA and protein synthesis

To periodically quantify the concentration of a tested toxin in the rhizosphere, a set of PhytotoxkitTM biotests allowing for the evaluation of phytotoxicity of tested samples within a short time (1-3 days) could be used Sekutowski and Sadowski 2009, Mahoney, 2010). The determination of the level of degradation rate and translocation of toxic active ingredients in the soil is significant in crop environment. Instrumental methods, such as liquid high performance chromatography (HPLC), make it possible to determine the total content of toxins in the soil days or weeks after the application of the toxin (Ahmad and Crawford 1990, Sekutowski and Sadowski, 2006).

When selecting a bioindicator for a test it is necessary to take into consideration the age and the location of they plant and the sensitivity of individual tissues to the tested toxin. Sadowski and Crawford (1990) reported that the youngest roots and leaves of test plants turned out to be most sensitive. They further observed that an increase in the initial soil moisture content by 2% caused a marked shift of toxin deeper within the soil profile practically for all the tested active ingredients.

It is evident from this research that the most on seed emergence of sesame cultivars was achieved at It was evident that high concentration of aflatoxins at 500 and 1000 $\mu\text{g/l}$. had significant inhibitory effect on the shoot length, root length, seedling vigour, and chlorophyll level in sesame. At 125 $\mu\text{g/l}$ concentration aflatoxin, a significant stimulatory effect on the number of leaves, area of leaves and number of flowers of bogoro cultivar was observed. In all situations, the chlorophyll level of all sesame cultivars was higher at lower concentration and was more obvious on E8 and Ex-sudan cultivars.

Contaminated seeds with aflatoxigenic fungi should not be used for propagation except they are pretreated with fungitoxicant. There is a need for further study on the effects of other mycotoxins on crop seeds and the rate of degradation and translocation of toxin in the crop rhizosphere.

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