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EFFECT OF STATIC MAGNETIC FIELD ON AFLATOXIN PRODUCTION UNDER SOLID STATE FERMENTATION

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ABSTRACT

This study was conducted to reduce aflatoxin B1 produced by *Aspergillus flavus* under the influence of magnetic field and under solid state fermentation. The fungus was exposed to the north pole and then compared its effect with the control treatment (without the magnetic field energy). The substrates used for the growth of *A. flavus* (corn, wheat, rice and wheat flour) were obtained from the local market and sterilized by autoclaving. A static magnetic field of 100 Gauss was subjected to the *A. flavus* for (7, 14 and 21) days of fermentation at 28°C and 25% moisture content. The concentration of aflatoxin B1 has been determined by Enzyme Linked Immunosorbent Assay (ELISA). At the end of each fermentation period and according to the extraction procedure provided with the ELISA kit. The extract was tested for the aflatoxin B1 concentration and the results were statistically analyzed. The results showed that the north pole significantly decreased the aflatoxin B1 concentration for the corn during (7, 14 and 21) days. The north pole also significantly decreased the aflatoxin B1 concentration for the wheat for the two periods (7and 14) days, but there were no significant differences in the last period (21days). While for the rice, the north pole significantly decreased the aflatoxin B1 concentration for the three periods (7, 14 and 21) days, but there are no significant differences in the last period (21days) between the control and the north pole treatment. There was no effect of the north pole for the wheat flour with some rise in the proportion of aflatoxin B1 as compared with control treatment and this would be due to the low moisture content in the three periods (7,14, 21) days. This study clearly demonstrated that there are significant effects of the static magnetic field in decreasing the aflatoxin B1 concentration produced by *Aspergillus flavus*.

KEYWORD: Static Magnetic Field, Aflatoxin, Solid State Fermentation.

INTRODUCTION

The magnetic field is the force that surrounds the magnet in which magnetic materials affected by it. Iron, cobalt and nickel are the only magnetic elements.[1] Plant cells affected by a magnetic field, according to many factors including species, intensity of magnetic field (MF) and the exposure period.[2][3] Recently, growing the interest in studying the effect of the magnetic field on living organisms, especially human and animal, but few studies has addressed to the effect of magnetic field on plants and microorganisms, also the effect of magnetic field energy on the field of medicine, microbiology and biotechnology.[4]

Aflatoxin is a toxic metabolite produced by different species of toxigenic fungi, called mycotoxins. There are 18 different types of aflatoxins identified; the major members are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2), M1 (AFM1) and M2 (AFM2) which are produced by *Aspergillus flavus* and/or *Aspergillus parasiticus*.[5] Aflatoxins biosynthesis as all secondary metabolites which strongly dependent on growth conditions such as substrate composition or physical factors such as pH, water activity, and temperature or modified atmospheres.[6][7]

Solid state fermentation is the process that occurs after a non-soluble material which acts as a support and nutrient source, with a small quantity of water, under the action of the fermenting agent.[8] SSF offers greatest possibilities for fungi. Unlike other microorganisms, fungi typically grow in nature on solid substrates such as pieces of wood, seeds, stems, roots and dried parts of animals such as skin, bones and fecal matter.[9]

MATERIALS and METHODS

Fungal species

The fungal species *Aspergillus flavus* were obtained from the College of Agriculture/ University of Baghdad, where it has been confirmed as aflatoxigenic species. *Aspergillus flavus* has been identified after growing on Potato Dextrose Agar (PDA) medium by observing the growth characteristics (color, texture, appearance and diameter of colonies) and microscopic (microstructure).[10]

Detection of Aflatoxin Production by *Aspergillus flavus* using Ammonium Vapor test

Aspergillus flavus and *Aspergillus niger* were grown as a single colony in the center of a plates contain yeast extract sucrose agar media and incubated in the dark at 28^{o}C. After 3 days, the plates were inverted and 2ml of ammonium hydroxide poured in the cover of the

plates.[11][12] This test was repeated with another set after 7 days. A color change was observed after 10 minutes.

Static Magnetic Field

Magnetic bar with thickness (2.9 cm) and single field strength of 100 Gauss which was measured by Gaussmeter. The magnetic bars North Pole was put on the bottom of the cultured flasks using adhesive tape.

Spores' Suspension Preparation

According to[13] spore suspension was prepared with slight modifications as follows:

- Plates containing fungal isolates inoculated on PDA medium were incubated at 28°C for 7 days.
- 5 ml of DW was added to the plate for spores harvesting.
- A flask containing sterilized bread were inoculated with spore suspension and incubated at 28°C for 7days.
- One hundred ml of DW was added to the flask and mixed vigorously by hand.
- Through sterile cotton wool, the suspension of spores was filtered.
- The suspension was centrifuged at (3000 rpm for 5 min). Then, the supernatant was discarded and the spores then washed twice by DW and further re-centrifuged.
- Then, 1 ml of DW was added to the deposit and mixed vigorously.

Effect of Magnetic North Pole on Aflatoxin Production Under Solid State Fermentation of Corn, Wheat, Rice and Wheat Flour

To test the effect of the magnetic north pole on the fungi cultures under solid state fermentation the following steps were carried out for each of corn, wheat, rice and wheat flour:

- Twelve culture flasks were loaded with 25 g of the sample and autoclaved at 121°C for 15 min.
- Inoculate each flask with 10^6spores/ml of fungus.
- The twelve flasks were divided into three groups as follows: Each group consists of four flasks, two flasks used as control and two flasks were put under the effect of northern pole. Each group was extracted at regular times after 7, 14 and 21 days.

Extraction Procedure for Corn, Wheat and Wheat Flour

The samples were extracted according to the instruction provided with the kit, extraction solvent (70% methanol) was prepared. The ratio of the sample to extraction solvent is 1:5 (w/v). For corn and wheat, grind the sample to the particle size of fine instant coffee, while this step was not included for the wheat flour. The extraction solvent was added to the grind sample and shake then the extract was filtered through Whatman#1 filter paper and collect the filtrate and read the optical density (OD) at 450nm.

Extraction Procedure for Rice

The samples were extracted according to the instruction provided with the kit, extraction solvent (50% methanol) was prepared. The ratio of the sample to extraction solvent is 1:5 (w/v). Grind the rice to the particle size. The extraction solvent was added to the grind. Centrifuge the rice at 3,500rpm for 5 minutes to pellet the particulate. Collect the supernatant and and read the optical density (OD) at 450nm.

RESULTS

Detection of *Aspergillus Flavus* Aflatoxin Using Ammonium Hydroxide Vapor Test

A new and rapid method for detecting toxigenic strains of *A. flavus* are through vapor tests. By exposing the aflatoxigenic colonies to ammonium hydroxide vapors, will result in a quick color change of the reverse side.[14] After 3 days of incubation at 28°C on Yeast Extract Sucrose (YES) medium of each *A. flavus* and *A. niger*, the results revealed that *A. flavus* show moderate red pigmentation on the reverse side of the colony with the ammonium hydroxide vapor test, while *A. niger* show no colour change, as shown in figure (1 (a,b). Similarly, the test was done after 7 days of incubation at 28°C on YES medium. *A. flavus* produce strong red pigmentation on the reverse side of the colony, whereas *A. niger* produces no color change, as shown in figure (2 (a,b). The intensity of the color refers to the high toxicity of the fungal species.

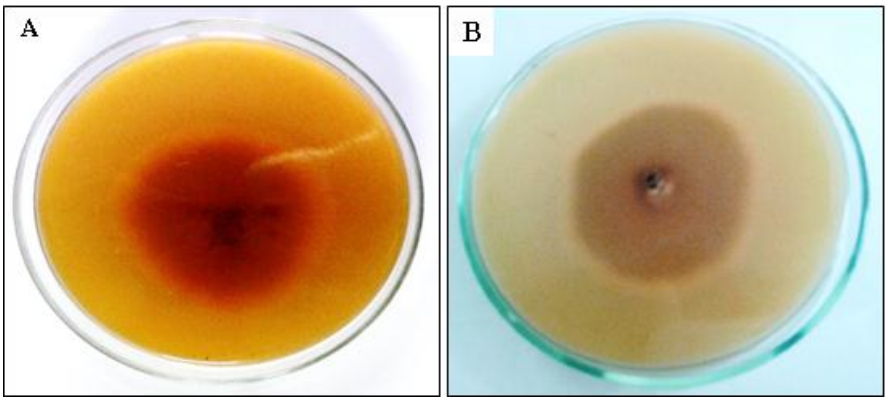

Figure 1: (a) *Aspergillus flavus* with moderate red color on the reverse side. (b) *Aspergillus niger* with no color change on the reverse side on YES medium after 3 days of incubation at 28°C.

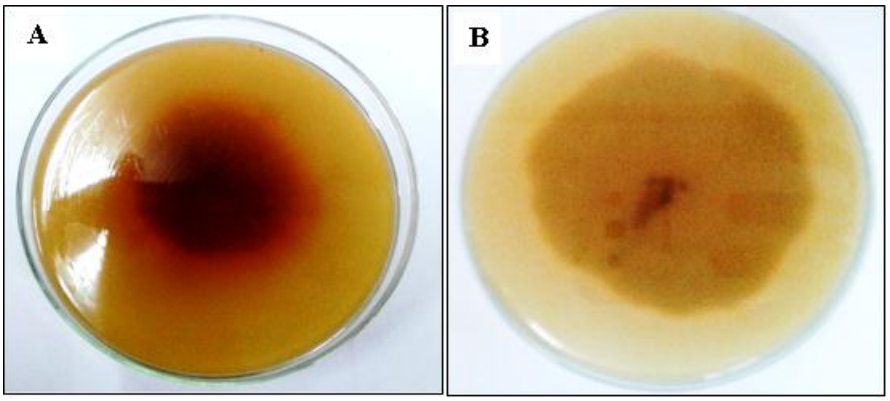

Figure 2: (a) *Aspergillus flavus* with moderate red color on the reverse side. (b)*Aspergillus niger* with no color change on the reverse side on YES medium after 7 days of incubation at 28°C.

The Effect of Magnetic North Pole on Aflatoxin B1 Concentration (Ppb) in Corn

The effect of magnetic northern pole on aflatoxin B1 production by *A. flavus* in corn were investigated. It was measured after (7, 14 and 21) days of fermentation on solid medium (corn) at 28°C in the dark with moisture content of 25%. The Control of all experiments was the solid medium without exposure to the effect of the magnetic north pole. Figure (3) shows the results of aflatoxin B1 concentrations in corn medium produced by the fungus, which were (0.54, 0.48 and 0.38) ppb when exposed to the northern pole compared to the control which were (1.84, 0.81 and 0.67) ppb after (7, 14 and 21) days respectively. The results also showed that the maximum production of aflatoxin B1 was on the seventh day and it was gradually depleted over time.

The statistical analysis of the results in figures (3) clearly showed that the north pole treatment significantly decreased aflatoxin B1 concentration as compared to the control for the three periods (7, 14 and 21) days.

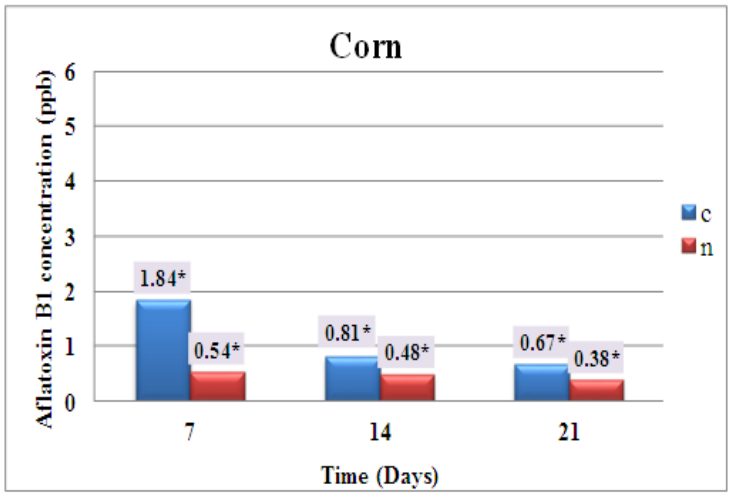

Figure 3: The effect of magnetic north pole on aflatoxin B1 concentration (ppb) produced by *A. flavus* after (7, 14 and 21) days in corn medium at 28°C and moisture content of 25%. C: Control, N: Exposed to the north pole. Symbol star (*): The mean difference is significant at the p< 0.05 level.

The Effect of Magnetic North Pole on Aflatoxin B1 Concentration (Ppb) in Wheat

Aflatoxin B1 production in wheat by *A. flavus* was determined under the effect of magnetic north pole. After (7, 14 and 21) days of fermentation on solid media (wheat) at 28°C in the dark with moisture content of 25%, the concentrations of aflatoxin B1 were (4.89, 0.62 and 0.53) ppb in controls while they were (0.27, 0.35 and 0.33) ppb when exposed to the northern pole respectively (Figure 4).

The statistical analysis of the results in figure (4) revealed that the north pole treatment significantly decreased the aflatoxin B1 concentration as compared with the control and for the two periods 7 and 14 days, however there are no significant differences between the northern treatment and control at the period of 21 days.

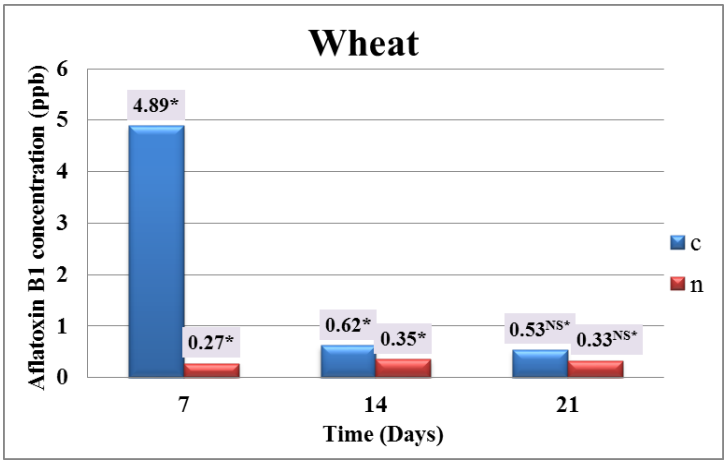

Figure 4: The effect of magnetic north pole on aflatoxin B1 concentration produced by *A. flavus* after (7, 14 and 21) days in wheat at 28°C and moisture content 25%. C: Control, N: Exposed to the north pole, the Symbol star (*): The mean difference is significant at the p< 0.05 level, *NS: nonsignificant at the p< 0.05 level.

The Effect of Magnetic North Pole on Aflatoxin B1 Concentration (Ppb) in Rice

The effect of the north magnetic pole on aflatoxin B1 production in rice was measured. It was estimated using ELISA technique after (7, 14 and 21) days of fermentation on solid medium (rice) at 28°C in the dark with moisture content of 25% without being exposed to the magnetic north pole or exposed to it**.** Figure (5) shows the concentration of aflatoxin B1 produced by the fungus in rice cereals medium. The concentration of the toxin of the exposed sample to the north pole was 0.16 ppb in comparison to the control sample which was 0.49 ppb at the end of 7 days of fermentation. The next 7 days of fermentation, the concentration of the toxin was 0.13 ppb of the control sample, whereas it was 0.03 ppb of the exposed sample to the north pole. After 21 days of fermentation, the concentration of the toxin was 0.05 ppb for the control while it was 0.02 for the treated samples with northern pole (Figure 5).

The statistical analysis of the results as shown in figure (5) clearly demonstrated that the north pole treatment significantly decreased the aflatoxin B1 concentration as compared with the control for the two periods 7 and 14 days, on the other hand there are no significant differences between the northern treatment and control at the period of 21 days.

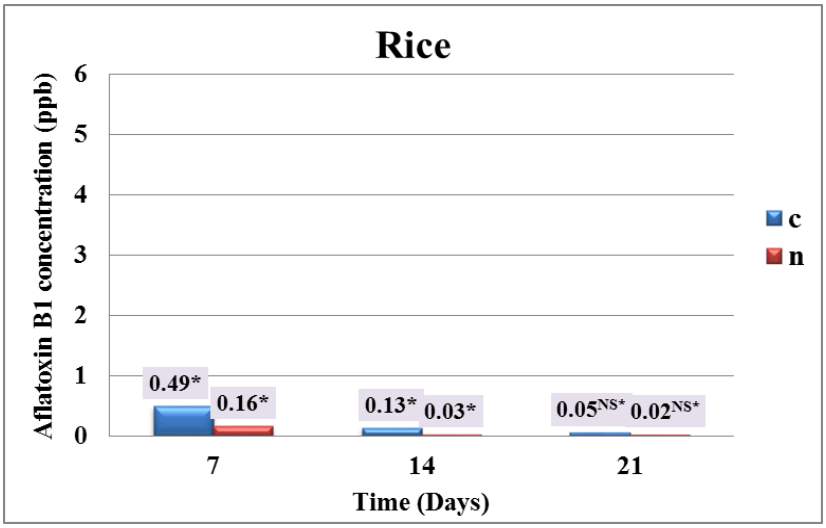

Figure 5: The effect of magnetic north pole on aflatoxin B1 concentration produced by *A. flavus* after (7, 14 and 21) days in rice at 28°C and moisture content 25%. C: Control, N: Exposed to the north pole. Symbol star (*): The mean difference is significant at the p< 0.05 level, *NS: nonsignificant at the p< 0.05 level.

The Effect of Magnetic North Pole on Aflatoxin B1 Concentration (Ppb) in Wheat Flour

The effect of magnetic north pole on aflatoxin B1 production in wheat flour was investigated. It was measured after 7, 14 and 21 days of fermentation on solid media (wheat flour) at 28°C in the dark with moisture content 25%. The Control of all experiments was the solid media without exposure to the effect of the magnetic north pole**.** Figure (6) revealed wheat flour results, which was 0.01 ppb when treated with northern pole and 0.32 ppb when treated with the control after 7 days, while it was 0.08 ppb when treated with northern pole and 0.21 ppb when treated with the control after 14 days, after 21 days it was 0.2 when treated with northern pole and 0.1 ppb when treated with the control.

The statistical analysis of the results in Figures (6) showed that the north pole treatment revealed no significant differences with some rise in the proportion of aflatoxin B1 as compared with control treatment for the three period (7, 14 and 21 days).

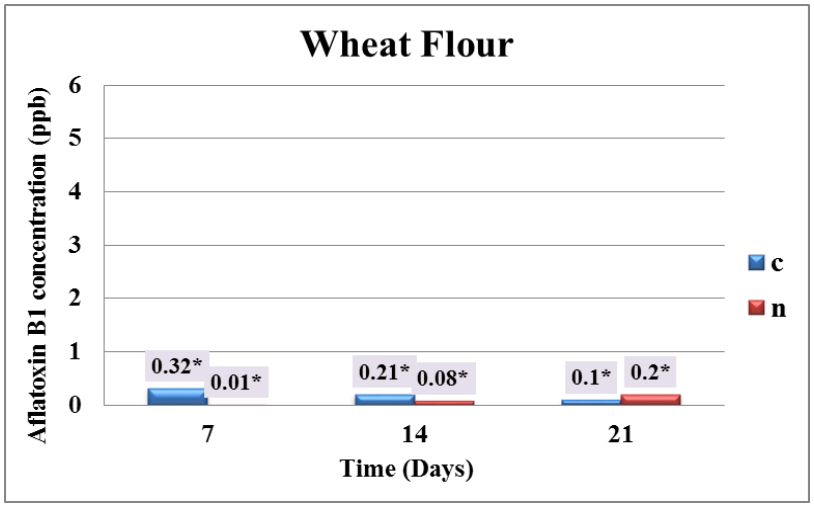

Figure 6: The effect of magnetic north pole on aflatoxin B1 concentration produced by *A. flavus* after (7, 14 and 21) days in wheat flour at 28°C and moisture content 25%. C: Control, N: Exposed to the north pole. Symbol star (*): The mean difference is significant at the p< 0.05 level.

DISCUSSION

A number of *Aspergillus* species have shown an ability to degrade aflatoxin, the degradation rates of AFB1 and AFG1 proportionally increased with the increase of the initial aflatoxin concentration or the size of mycelia inoculum.[15] *Aspergillus* sp. will normally have a period of active synthesis and accumulation of the aflatoxins in the medium, which is followed by reabsorption and metabolism by the producer mycelium, the metabolism or degradation of aflatoxins is enzymicly and occurs largely by the involvement of the cytochrome P-450 monooxygenase enzyme system in this activity.[16]

The fungal biodegradation revealed that the percentage of aflatoxin B1 degradation differed with the age of mycelium for both *A. flavus* and *A. parasiticus*. The highest rate of aflatoxin B1 degradation was associated with nine days old mycelia for both fungi.[17] Large yield of aflatoxins is associated with high carbohydrate concentrations, such as wheat and to a lesser extent in rice, oilseeds such as cottonseed, soybean and peanuts.[18]

It well known that there are many factors affects aflatoxin production in cereals included physical and chemical factors. The physical factors include temperature and moisture. The chemical factors include the composition and the nature of the substrate.[19] It was found that the MF decreases the water conductivity, which is inversely proportional to the flow rate and increases the amount of evaporated water, even after the water distillation. The effects are

due to the hydrogen bond network strengthening and the perturbation of gas/liquid interface from the air nano bubbles in the water.[20] Drought stress intensity increased aflatoxin concentration in plants, it was suggested that drought stress for less than ten days was enough to cause significant aflatoxin contamination in the field, the aflatoxin contamination is often related to the intensity of drought stress, contamination increased with drought stress severity this suggests the drought intensity increased aflatoxin accumulation in plants.[21]

The magnetic field has an important role in cation uptake capacity and it may consider as a substitution of chemical additives, as it can reduce toxins and thus enhance food safety.[22] The present data proved the cellular membrane of the microorganism had been affected by the external magnetic field, and then one expects a disturbance in their metabolic activity and, consequently, a change in their cell division.[23]

A theory that explains the effect of magnetic field on calcium ions bound in calcium-binding proteins, such as calmodulin. The calcium ions on and on vibrate about an equilibrium position in the binding site of calmodulin. A static magnetic field causes the calmodulin vibration to rotate, or proceed in the direction of the magnetic field at a frequency that exactly equals the frequency of the bound calcium, this will result in disturbing the bond between the calcium ion and the calmodulin.[24] This can affect transport into the cells and result in biological changes in the organism. The concentration of calcium ions in the cytosol (the main part of the cell) is normally kept about a thousand times lower than that outside by metabolically driven ion pumps in its membranes. Many metabolic processes are then regulated by letting small amounts of calcium into the cytosol when needed.[25] The inhibitory effect of magnetic field resulted from the interaction between electric charges of the magnetic field and that of the cytoplasm membrane resulting in the partial abolishment of electric potential of the cytoplasm membrane and this cause subsequent decrease in the macromolecular biosynthesis. The magnetic field may also cause damage of bacterial DNA and inhibition of its replication.[26]

Another theory assumed that biological free radicals are most commonly nitrogen or oxygen based with an unpaired electron, has the terms either RNS (reactive nitrogen species), such as nitric oxide (NO) or ROS (reactive oxygen species) such as superoxide anion ($O2^-$), hydroxyl radical ($OH\cdot$) and singlet oxygen ($^1O2$), the ROS and RNS play an important role in intracellular signaling and intercellular communication. It has been suggested that SMF affect cells through the radical pair mechanism, an SMF influences the spin of electrons in free

radicals, which may lead to changes in chemical reaction kinetics and possibly altering cellular function.[27]

The affected free radicals could effect on the enzymes which are involved in aflatoxin biosynthesis, such as a reductase and a cyclase because they attack sites of increased electron density like the nitrogen atom present in proteins and carbon-carbon double bonds present in polyunsaturated fatty acids and phospholipids.[28] The biosynthesis of Aflatoxin affected by one of the two stages from malonyl CoA, first with the formation of hexanoyl CoA, followed by formation of a decaketide anthraquinone.

CONCLUSION

- Magnetic field generally affects aflatoxin B1 concentration which produced by *Aspergillus flavus* in solid substrate.
- The north pole decreased aflatoxin B1 concentration in different solid substrates (corn, wheat, rice and wheat flour) as well as in three periods (7, 14 and 21) days as compared with the control under the same fermentation conditions.
- The maximum concentration of aflatoxin B1 was on the first week and it gradually lowered in the next two weeks.

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