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Aieman

Supervisors certification

We, certify that this thesis entitled "**Effect of Magnatic Field Energy on Aflatoxine Production by *Aspergillus flavus*"** was preperd by **"Aieman Muhammad Ahmad"** under our supervision at the College of Science / Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in **Biotechnology.**

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1.1 Introduction

Magnetobiology is the study of biological effects of mainly weak static and low-frequency magnetic fields, which do not cause heating of tissues. Magnetobiological effects have unique features that obviously distinguish them from thermal effects; often they are observed for alternating magnetic fields just in separate frequency and amplitude intervals.

Epidemiological studies that suggest links between exposure to magnetic fields and various harmful effects. The current focus on deleterious effects was a product of our poor current understanding of the mechanisms of biomagnetic interactions. After all, if one didn't had a good understanding on how a complex system works, any energetic perturbations (such as applied EMFs) were much more likely to adversely affect function than to be helpful.

Bioelectromagnetics is the study of the interaction between electro-magnetic fields and biological entities. Common areas of investigation include animal navigation utilizing the geomagnetic field, potential effects of man-made sources of electromagnetic fields. The term is similar to bioelectromagnetism, which deals with the ability of living cells, tissues, and organisms to produce electrical fields and the response of cells to electromagnetic fields.

The magnetic energy is the energy of fundamental nature and it control the spin of electrons around the nucleus of atoms and cells. Recent years have seen a growing interest in studying the impact of the magnetic field on living organisms, especially human and animal , but few studies have addressed the effect of magnetic field on plants and microorganisms, as well as the effect of magnetic field on the biological events may met with considerable interest, especially in the field of medicine, microbiology and biotechnology

Also, few studies have focused on the impact of magnetic field on growth and metabolism of microorganisms have been published, that the impact of the magnetic field energy lies in the stimulus to the events of significant changes in the characteristics of metabolic organisms, these are changes in the exchange of ions through the cell membrane in the movement of cells (Lin, *et al*., 2001).

Aspergillus flavus is the most famous species among over 185 known species within the genus *Aspergillus*. It is not the most abundant and widely distributed soil-born molds that can be found anywhere on earth but also produce aflatoxins among the carcinogenic natural products ever discovered. *A. flavus* is a saprobe capable of surviving on many organic nutrients sources like plant debris, tree leaves, decaying wood, animal fodder, cottons, dead insects and even human and animal patients.

Cereals of different kinds, such as rice, corn, peanut are infected by many fungi in the field and during harvest and during activities of transport, at storage, before and after the manufacturing operations on them. These fungi differ both quantitatively and qualitatively different conditions of environmental temperature and humidity. The fungus *A. flavus* that cause significant damage to the grains and cereals is very serious, through the production of the secondary metabolites known as aflatoxins which produced by two species *A.parasiticus, A.flavus* and this metabolites with disadvantage of high toxicity to humans and animals (Raper and Fennell, 1965).

A.flavus produce aflatoxins B_1, B_2, G_1 and G_2 . These four major aflatoxins are named based on their blue(B) or green (G) fluorescence under Ultraviolet (UV) light. Aflatoxine M_1 is

Hydroxylated derivative metabolized from aflatoxine B_1 by cows and screated in milk.

Aflatoxin B_1 is the most frequent of these compounds present in contaminated food samples and aflatoxins B_2, G_1 and G_2 are generally not reported in the absence of aflatoxin B_1.

Thus, the aflatoxins form a family of highly oxygenated heterocyclic compounds with closely similar chemical structures that are formed naturally by certain species of moulds (Klich, 2007).

Aims of the study

The study was aimed to:

- Detect the effect of northern, southern and both northern and southern poles of magnetic field energy on the growth of *Aspergillus flavus* on solid media.
- Detect the effect of northern, southern and both northern and southern poles of magnetic field energy on the growth in liquid media and total aflatoxin production.
- Estimate the protein concentration, pH, Enzymes (Amylase and Protease) Activity and reduced sugars of *Aspergillus flavus* culture after affected by magnetic field energy.

1.2- Literature Review

1.2.1 Magnate and magnetic field energy

Magnetite is the most magnetic of all the naturally occurring minerals on Earth. It is a ferromagnetic mineral with chemical formula Fe_3O_4. The formula for magnetite may also be written as $FeO \cdot Fe_2O_3$, which is one part wüstite (FeO) and one part hematite (Fe_2O_3). Naturally magnetized pieces of magnetite, called lodestone, will attract small pieces of iron, and this was how ancient people first noticed the property of magnetism. Lodestones were used as an early form of magnetic compass (Hurlbut and Klein, 1985). Magnetite typically carries the dominant magnetic signature in rocks, and so it has been a critical tool in pale magnetism, a science important in discovering and understanding plate tectonics and as historic data for magneto hydrodynamics and other scientific fields (Harrison, *et al*., 2002).

Magnetic fields are everywhere. There are all types of wave forms, intensities, and frequencies. Magnetic fields bathe the whole earth and have significant effects on living organisms. Magnetic fields cannot be felt, sensed, or seen like patterns of light, or heard like sounds, so their biological effects have largely gone unnoticed (Kirschvink, *et al*., 1985).

1.2.2: Effect of magnetic field energy on the living organisms

The effects of magnets and magnetic fields have been known for a very long time. Galen, a Greek physician around 200 BC, in his

book, *De Simplicium Medicamentorum Facultatibus*, mentions the use of magnets. In 1000 A.D., a famous Persian physician described his findings using magnetism to relieve various disorders. In the early 1500s, the great medical doctor, Paracelsus, wrote several treatises on the use of magnets and magnetism. While Paracelsus gave many hints for the nature and use of magnetism, who taught a whole philosophy of universal magnetism (Presman 1970; Binhi, 2003).

What these philosophers tried to elaborate upon was that there is an insubstantial invisible, magnetic power that draws things together. Individual elements might not really be considered magnetic but they all possess a magnetic aspect that attracts one thing to another (Moore, 1979;Ramchand, *et al*.,2001).

Magnetobiology is a subset of bioelectromagnetics. Bioelectro-magnetism and diamagnetism are the study of the production of electromagnetic and magnetic fields by biological organisms. The sensing of magnetic fields by organisms is known as magneto-reception (Hashish, *et al*., 2007).

Biological effects of weak low frequency magnetic fields, less than (1 Gauss) and 100 Hertz (Hz) correspondingly, constitutes a physics problem. The field intensity is not enough to cause any appreciable heating of biological tissues or irritate nerves by the induced electric currents (Andrw, 2007).

1.2.3 **Morphological description to *Aspergillus flavus***:

Aspergillus flavus is a fungus. It is a common mold in the environment, and can cause storage problems in stored grains. It can also be a human pathogen, associated with aspergillosis of the lungs

and sometimes causing corneal, otomycotic, and nasoorbital infections. Many strains produce significant quantities of aflatoxin, a carcinogenic and acutely toxic compound. *A .flavus* spores are allergenic and it is sometimes causes losses in silkworm (Klich MA. 2007).

A. flavus colonies grow rapidly and the growth is usually enhanced at 37 C^0. Colonies are yellow to dark yellowish-green, consisting of a dense felt of conidiophores or mature vesicles bearing phialides over their entire surface. Colony color may be influenced by culture medium additives, e.g., yeast extracts (Anon, 1989).

A. flavus is predominately a saprophyte and grows on dead plant and animal tissue in the soil. For this reason it is very important in nutrient recycling. However, *A. flavus* can also be pathogenic on several plant and animal species, including humans and domestic animals. It can infect seeds of corn, peanuts, cotton, and nut trees. Growth of the fungus on a food source often leads to contamination with Aflatoxin, four different aflatoxins, B_1, B_2, G_1 and G_2, have been identified with B_1 being the most toxic, carcinogenic, hepatotoxic and potentially mutagenic, while also being the most prevalent (Raper and Fennell, 1965)

Patients infected with *A. flavus* are often immunocompromised or neutropenic. The fungus also causes allergic diseases in asthmatics and patients suffering from cystic fibrosis. It is one of the main agents of human allergic bronchial aspergillosis (hypersensitivity pneumonitis) and par nasal sinusitis. It also occurs in external ears and may be involved in otitis; systemic infections have been reported in leukemic patients (Green, *et al*., 2002).

1.2.4 Aflatoxins

1.2.4.1 Historical OVERVIEW

In 1960, more than 100,000 turkeys died in England. The birds failed to thrive and had subcutaneous haemorrhages. Post-mortem examination revealed necrotic liver damage and cell proliferation in the bile ducts. The cause remained unknown and the disorder was called 'Turkey X disease' (Nesbit, *et al*., 1962).

The disorder was also subsequently detected in swine. During the same period, high incidences of hepatoma were found elsewhere in trout bred at fish farms. Epidemiological investigation eventually traced the problem to feed contamination, specifically a batch of Brazilian peanut meal which had been used as poultry feed for all these animals. This meal, which had been imported into the United Kingdom at the end of 1959, was subsequently termed Rosetti meal, after the name of the ship in which it was carried. It turned out to produce a new disease. Tests for known toxins proved negative (Blount, 1961).

After painstaking analysis, the Rosetti meal and the fish food were found to be contaminated with the relatively common fungus, *A. flavus*. Depending upon the growing conditions, certain strains of this fungus produce a variety of chemical compounds called aflatoxins. Aflatoxins are a group of chemically related mycotoxins which are produced by particular species of moulds. Their name derives from the fungus *A. flavus* on which much of the early work with these substances was performed (i.e. the genus *Aspergillus*, the species *flavus* and the suffix toxin).

Aflatoxins are found as a natural contaminant in several foods, such as peanuts, cotton seed, maize, rice, cocoa, soya, wheat, sorghum and barley (IARC, 1993). *Aspergillus flavus* has a cosmopolitan distribution. This mold and the related fungus, *A. parasiticus*, only produce aflatoxins under certain conditions. Peanuts are a good substrate for aflatoxin production. It was recognized that *Aspergillus flavus* did not appreciably affect the peanuts prior to harvesting (Galvano, *et al.,* 2001). The main determining factor for the growth and production of aflatoxin is the relative humidity. Few fungi grow on stored food at a humidity of less than 70%. At a relative humidity of 85% and a peanut water content of 30%, the fungus flourishes (Gong, *et al.,* 2002). Rapid post-harvest drying of the peanuts prevents attack by this fungus. When peanuts are left to dry in rainy or humid weather, this creates favourable conditions for aflatoxin production. Intact seeds are seldom infected. Damaged seeds, on the other hand (termites or mechanical damage) are more likely to be infected and to contain aflatoxin. When cows are fed infected feed, they secrete aflatoxins in their milk. Humans are exposed to aflatoxins through contaminated food. Toxins in particulates may cause aerogenic toxicity, e.g. in farmers working in a dusty environment with contaminated food products (Apelton, *et al*., 2000).

1.2.4.2 Natural occurring

Human exposure to aflatoxin occurs mainly through growth of the *Aspergillus* species *A. flavus* and *A. parasiticus*. Whether exposure is predominantly to Aflatoxine B_1, or to mixtures of various aflatoxins, depends upon the geographical distribution of the strains. *A. flavus*, which produces aflatoxins B_1 and B_2, occurs worldwide,

while *A. parasiticus*, which produces aflatoxins B_1, B_2, G_1 and G_2, occurs principally in the Americas and in Africa (Midio, *et al*., 2001).

Aflatoxins occur both in food crops in the field prior to harvest, and in improperly stored food where mould species have found an opportunity to grow. Fungal growth and aflatoxin contamination are a consequence of an interaction between the mould, the host organic material (i.e. crop, foodstuff) and the environment. The appropriate combination of these factors determines the degree of the colonization of the substrate, and the type and amount of aflatoxin produced.

Humidity, temperature and insect damage of the host substrate are major determining environmental factors in mould infestation and toxin production (Robens, *et al*., 1992).

In addition, specific crop growth stages, poor fertility, high crop densities and weed competition have all been associated with increased mould growth and toxin production. For example,
pre- harvest aflatoxin contamination of peanuts and corn is favored by high temperatures, prolonged drought conditions and high insect activity; while postharvest production of aflatoxins on corn and peanuts is favored by warm temperatures and high humidity.
Aflatoxins have been detected in milk, cheese, corn and other cereals, peanuts, cottonseed, nuts, figs and other foodstuffs. Milk and milk products and eggs are sometimes contaminated (generally with aflatoxine M_1 and M_2) because of the animals consumption of aflatoxine-contaminated feed (Rustom, 1997).

Worldwide, corn contamination is probably of the greatest concern because of its widespread cultivation and its frequent use as the staple diet in many countries. However, due to local practices, customs or conditions, other foodstuffs may represent the greatest problem in certain localities (Wyllei and Marchouse, 1978).

1.2.4.3 Chemical Features:

Aflatoxins can be produced by the four toxic species of *Asperagillus: A. flavus, A.parasiticus, A. nomius* and *A. pseudotamarii* as secondary metabolites (CAST, 2003). Occurrence of these molds and therefore aflatoxin contamination is limited to warm and humid climates. Chemically Aflatoxins are normally refers to the group of difuranocoumarins and classified in two broad groups according to their chemical structure; the difurocoumarocyclopentenone series (AFB_1, AFB_2, AFB_2A, AFM_1, AFM_2, AFM_2A and aflatoxicol) and the difurocoumarolactone series (AFG_1, AFG_2, AFG_2A, $AFGM_1$, $AFGM_2$, $AFGM_2A$ and AFB_3) (IARC, 1993). The aflatoxins display potency of toxicity, carcinogenicity, mutagenicity in the order of $AFB_1 > AFG_1 > AFB_2 > AFG_2$ as illustrated by their LD50 values for day-old ducklings (Azziz, *et al*., 2005). Structurally the dihydrofuran moiety, containing double bond, and the constituents liked to the coumarin moiety are of importance in producing biological effects. The aflatoxins fluorescence strongly in ultraviolet light (ca. 365 nm According to the color of the fluorescence the aflatoxins are grouped into aflatoxin B_1 and B_2 (AFB_1, AFB_2) for blue, and G_1 and G_2 (AFG_1, AFG_2) for green, where subscripts refer to the chromatographic mobility. Aflatoxin M_1 and M_2 (AFM_1, AFM_2), known as milk aflatoxins, are metabolites of AFB_1 and AFB_2 (Pitt, 2000).

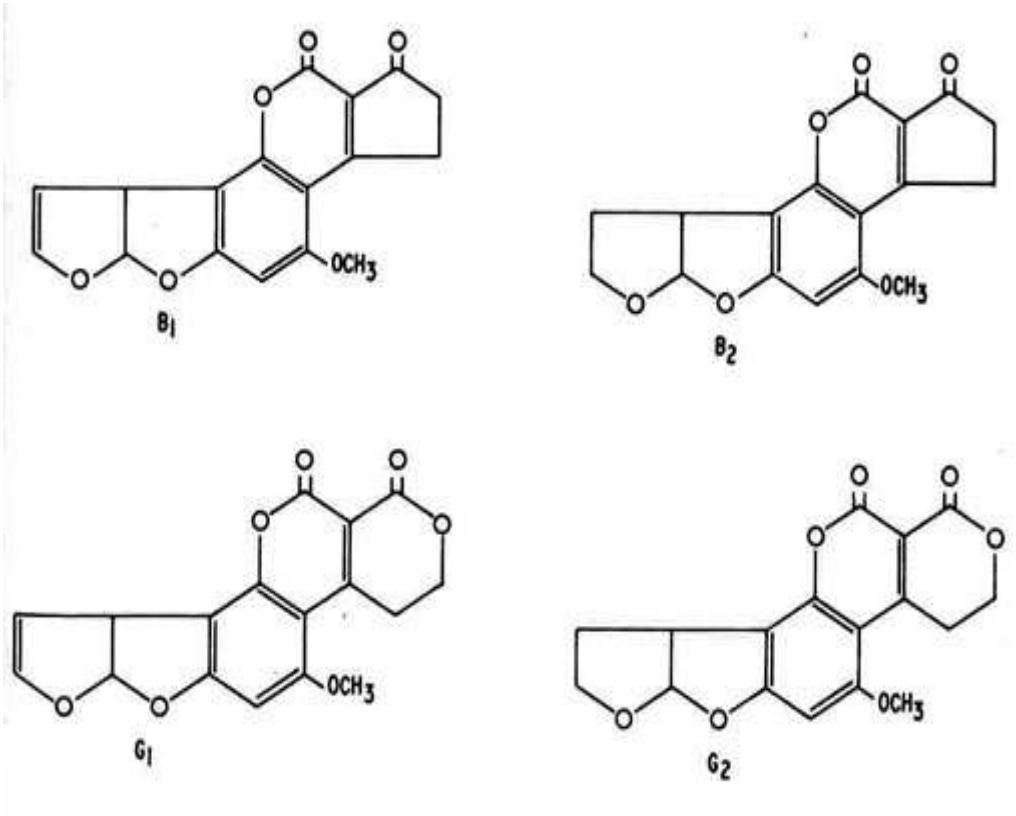


Figure 1. Structures of Aflatoxin B_1, B_2, G1 and G_2 (Midio, *et al*., 2001).

1.2.5 Metabolism of Aflatoxin B1:

Metabolism of AFB_1 can be divided into three phases, bioactivation (phase I), conjugation (phase II) and deconjugation (phase III), all of which can occur directly at the site of absorption, in the blood, after entering the liver as the main metabolizing organ, or in several extrahepatic tissues (Vermeulen, 1996). Aflatoxin B_1 itself is not a potent toxin, and phase I bioactivation is needed to exert toxic effects (Massey, *et al*., 1995). Phase I reactions are mainly oxidation of AFB_1 to hydroxylated metabolites such as aflatoxin M_1, aflatoxin Q_1 and aflatoxin P_1 and to the highly reactive AFB_1 8, 9 epoxide (Eaton, *et al*., 1994). This epoxide can occur in two isomers, the endo and the exoform, but only the exoisomer is of relevance in terms of

toxicity and carcinogenicity (Massey *etal*., 1995). The formed epoxide is highly unstable, and will readily bind to biological nucleophils such as nucleic acids (alkylation) to form stable adducts with RNA and DNA . Covalent binding of AFB_1 8, 9 epoxide to DNA is known to induce point mutations and DNA strand breaks, and is linked to the carcinogenic effects of AFB_1 exposure. In the presence of water, the epoxide will be rapidly and none enzymatically hydrolyzed to AFB_1 8, 9 dihydrodiole, which is able to form Schiff bases with primary amino groups in lysine residues (Sabbioni, *et al*., 1987). One of the proteins, readily available for AFB_1 adduct formation is serum albumin, forming a stable adduct persisting in the blood circulation of rats for several days and humans for several weeks. Therefore, levels of AFB_1 albumin or AFB_1 lysine after proteolytic digestion are widely used as biomarkers of AFB_1 exposure. The mechanism of diol formation and protein adduction is most likely involved in the acute toxic effects of aflatoxin (Eaton *etal*., 1994). Thus it is possible that aflatoxin could cause gross damage to cells at the intestinal interface, reducing nutrient uptake, or may specifically target important functional sites such as nutrient transporters or tight junctions (Turner, *et al*., 2005).

Cytochrome P450 enzymes (CYPs) are known to play the major role in oxidation of AFB_1 to the reactive epoxide in many tissues, although lipoxygenases and prostaglandin H synthase, in the presence of arachidonic acid (Goldbatt, 1969), have been shown to have the capacity, in humans, to catalyse this oxidation in extra hepatic organs.

The CYP enzymes are a family of haemoproteins with the capacity for monooxygenase enzymic metabolism of toxic hydrocarbons. The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) is required as a cofactor and oxygen is used as a substrate (Vermeulen, 1996), CYPs are additionally expressed extra

hepatically within most tissues and especially in the respiratory and intestinal tract, thereby providing intestinal cells *in vivo* with the capacity to bioactivate aflatoxin (Heathcot and Hibbert, 1978).

Hydroxylated AFB_1 metabolites (AFQ1, AFM1, AFP1) are less toxic because they are much poorer substrates for epoxidation (Theumer, *et al*., 2003), AFM1 has also been shown to exert direct toxic properties without metabolic activation, in contrast to AFB_1 (Long *etal*., 2009) Reduction of the 1keto group of AFB_1 produces the metabolite aflatoxicol (AFL) . This reduction is catalyzed by a cytosolic reductase and AFL can be readily oxidized back to AFB_1. Aflatoxicol is not considered a significant AFB_1 detoxification product since it has been shown to have comparable carcinogenicity and 70% the mutagenicity of AFB_1 (Eaton, *et al*., 1994) and can readily be oxidized back to AFB_1.

Phase II metabolism includes conjugation of phase I metabolites with glutathione or glucuronic acid and is considered detoxification to enhance water solubility and excretion (Massey, *et al*., 1995). Epoxide can be conjugated with glutathione with the help of glutathione-S-transferase (Wang, *et al*., 2001); an enzyme essential in the reduction and prevention of AFB_1 induced carcinogenicity. Conjugates of epoxide and hydroxylated AFB_1 metabolites are readily excreted via the bile into the intestinal tract, where they might be subject to bacterial deconjugation as phase III reaction, (Korde, *et al*., 2001).

1.2.6 Toxicity of aflatoxins

1.2.6.1 Effects on humans

The outbreak of aflatoxicosis in Kenya has resulted in 125 deaths among 317 cases of poisoning (Shane, 1993); the case fatality rate was 39%, caused by levels of AFB_1 in home grown maize reaching up to 8 mg/kg maize. Several previous outbreaks of

aflatoxicosis have occurred in Africa and India, mostly in adults with poor nutritional status and maize as staple food (IARC, 1993). The clinical picture indicated acute toxic liver injury manifested as jaundice with a mortality rate of 10-60% (Peraica, *et al*., 2003).

In humans, numerous studies have linked the incidence of primary hepatocellular carcinoma with the intake of aflatoxins, leading to the classification of AFB_1 as class 1 human carcinogen by the IARC (IARC, 1993). Areas of high incidence of liver cancer such as China, Taiwan and subSaharan Africa, also have the highest prevalence for aflatoxin exposure and hepatitis B virus, leading to the theory that these two hepato-carcinogens act synergistically (Kew, 2003).

Fetal and childhood environment, including the nutritional status of the pregnant mother and the infant, are considered critical for growth and risk of disease in earlier life. Mal-nourishment is one of the common problems in developing countries. Apart from these, they are also exposed to high levels of mycotoxins, aflatoxins are the major among these. It has been proved that these aflatoxins are immunogenic, teratogenic, and they retard the growth among experimental animals (Kaaya, *et al*., 2002). High exposure of these aflatoxins occurs through out these regions. A study in West Africa showed a significant correlation among the aflatoxin exposure and stunted growth in children who are exposed to aflatoxin right for neonatal stages. Apart from that due to the capacity of aflatoxins to cross the placental barrier, can cause genetic defects at foetal stages itself (Maxwell, *et al*., 2006; Gong, *et al*., 2002).

Aflatoxins have been suggested as an etiological factor in encephalopathy and fatty degeneration of viscera, similar to Reye syndrome, which is common in countries with a hot and humid

climate. The clinical picture includes enlarged, pale, fatty liver and kidneys and severe cerebral oedema. Aflatoxins have been found in blood during the acute phase of the disease, and in the liver of affected children. However, use of aspirin or phenothiazines is also suspected to be involved in the etiology (Martins and Martins., 2005; Olaniran, *et al*., 2006).

In previous studies, aflatoxins were found in the brain and lungs of children who had died from kwashiorkor and in control children who had died from various other diseases. It was suggested that the presence of aflatoxins in the brains of control children might be due to metabolic imbalance or to a failure in the excretory mechanisms of children with conditions such as measles (which in 25% of cases precedes kwashiorkor), renal failure, pyloric stenosis, gastroenteritis (Turner, *et al*.,2002). Aflatoxins in the lungs were found in all children diagnosed to have pneumonia, irrespective of the presence of kwashiorkor. This could be due a reduced clearing ability of the lungs in pulmonary diseases or to exposure via the respiratory route (Linear, 1969).

Damage to mitochondria can lead to mitochondrial diseases and may be responsible for aging mechanisms. The damage can be to mitochondrial DNA (adducts and mutations), mitochondrial membranes, increased cell death (apoptosis) as well as to disruption of energy production .Aflatoxin B_1 preferentially attacks mitochondrial DNA (mtDNA) during heptocarcinogenesis vs nuclear DNA (Sakuno, *et al*., 2003), mtDNA is protected in aflatoxicosis resistant rodents from DNA adducts that effect mitochondrial transcription and translation (Seitz and Stickel, 2006). The mycotoxin alters energy-linked functions of ADP phosphorylation and FAD- and NAD-linked oxidizing substrates (Korde *et al*., 2003) and a-ketoglutarate-succinate

cytochrome reductases it causes ultrastuctural changes in mitochondria, and also induces mitchondrial directed apoptosis (Wang, *et al*., 2001), the disruption of mitochondria may well lead to dysfunction of various organs and concomitant symptoms that escape standard medical diagnostics. For example, it is believed that certain mitochondrial diseases result from the ability of the nucleus to detect energetic deficits in its area. The nucleus attempts to compensate for the power shortages (e.g. lack of ATP) by triggering the replication of any nearby mitochondria. Unfortunately, this response promotes replication of the very mitochondria that are casuing the local energy shortage, further aggravating the problem (Pasupath , 1999; Marryann, *et al*., 2001).

1.2.6.2 Effect on animals:

No animal species is resistant to the acute toxic effects of aflatoxins. A wide variation in LD50 values has been obtained in animal species tested with single doses of aflatoxins. For most species, the LD50 value ranges from 0.5 to 10-mg/kg body weight. Animal species respond differently in their susceptibility to the chronic and acute toxicity of aflatoxins. Environmental factors, exposure level, and duration of exposure beside age, health, and nutritional status of diet can influence the toxicity (Kpodo, 2007).The general effect of aflatoxicosis on animal can be categorized into two general forms.

Acute toxicity is caused when large doses of aflatoxin are ingested. This is common in livestock. The principal target organ for aflatoxins is the liver. After the invasion of aflatoxins into the liver, lipids infiltrate hepatocytes and leads to necrosis or liver cell death (Hawang and Draugon., 2005). This is mainly because aflatoxin metabolites react negatively with different cell proteins, which leads

to inhibition of carbohydrate and lipid metabolism and protein synthesis (Yabe, *et al*., 2004). In correlation with the decrease in liver function, there is a dearngement of the blood clotting mechanism, icterus (jaundice), and a decrease in essential serum proteins synthesized by the liver (Price ,*et al*., 2006). Other general signs of Aflatoxicosis are edema of the lower extremities, abdominal pain, and vomiting. The most sever case of acute poisoning of aflatoxin was reported in north-west India in 1974 where 25% of the exposed population died after ingestion of the molded maize with aflatoxin levels ranging from 6.25 to 156 mg/kg (Ali, *et al*.,1994) .

Chronic toxicity is due to long term exposure of moderate to low aflatoxin concentration. The symptoms include decrease in growth rate, poor feed utilization and decrease body weight, lowered milk or egg production, and immune suppression. reduce complement activity, suppression of the cell mediated immune responses , helper T-cells numbers and interleukin 2(IL-2) production decrease significantly when mice were treated with AFB1 (Hatori, *et al*.,1991) .

Aflatoxin was also shown to reduce the antibody response to vaccines, study conducted that in poultry the daily diet exposure to aflatoxins concentration 200ppb reduce antibody titers to vaccines for newcastle disease, infectious bronchitis and infectious bursal disease (Verma, *et al*., 2008).

Liver damage is apparent due to the yellow color that is characteristic of jaundice, and the gall bladder becomes swollen (Robens, *et al*., 1992; Mclean and Dutton 1995). Aflatoxins in poultry also decrease the production of Vitamin A in the liver, and it has secondary effects such as decreased blood calcium levels, decreased bone strength, and a decreased tissue and serum tocopherol level. This

decrease in tocopherol levels can lead to Vitamin A and E deficiencies.

Chronic aflatoxicosis in adult ruminants can cause anorexia, drying and peeling of the skin on the muzzle, rectal prolapse, and abdominal edema. Aflatoxicosis has also been shown to cause decreased fertility, abortion, and lowered birth weights in sheep. Some evidence on aflatoxicosis shows an effect on rumen microflora. This is characterized by a decrease in cellulolysis, and ammonium formation (Green, *et al*., 2002).

1.2.7 Methods of Aflatoxins decontamination:

Foodstuffs distributed to the population should not represent a potential health hazard; therefore detoxification of mycotoxin-contaminated product has been a continuing challenge for the food industry. A great deal of concern has been directed towards aflatoxins because of their potency and ubiquity. Most of the factors obtained from studies on aflatoxins can be applied to other mycotoxins.

The sensitivity of mycotoxins to physical or chemical treatment is affected by many factors, including moisture content, location of the toxin in the food, forms of the food, storage conditions, and interactions of the toxins with food components. It is important to understand these factors before a specific method can be recommended (Mishra and Das, 2003).

2-7-1 Physical Methods:

2-7-1-1 Thermal inactivation

Aflatoxins are resistant to thermal inactivation and are not destroyed completely by boiling water, autoclaving, or a variety of food and feed processing procedure (Kabak and Var, 2008). Aflatoxins may be destroyed partially by conventional processing procedures such as oil and dry roasting of peanuts to be used as salted nuts, in confections, or in peanut butter. It had been reported a 45-83% reduction in aflatoxin that was dependent on roasting conditions and initial aflatoxin concentrations in raw peanuts. In other studies, roasting conditions resulted in a significant decrease in the aflatoxin content of nuts, oilseed meals and corn. The degradation of aflatoxins was a direct function of temperature, heating interval, and moisture content (Moss, 1996).

Only partial destruction of aflatoxins in contaminated wheat occurs during the various stages of bread making (Tunail, 2000). Baking temperatures do not significantly alter the levels of aflatoxin in dough (Pyu, *et al*., 2003).

A considerable reduction in aflatoxin levels has been associated with the lime water treatment of corn to produce tortillas. However, subsequent studies had shown that much of the original aflatoxin was reformed on acidification of the products (Pineda Valdes and Bullerman, 2003). Lime (regenerating calcium hydroxide in water) was postulated to react with aflatoxin, resulting in (1) a loss of indigenous fluorescence and (2) a major change in the extractability of aflatoxin in solvents such as chloroform. Evidence suggests that aflatoxin may be “masked” chemically in alkaline-processed corn (i.e., tortillas and corn chips) and, thus, may escape analytical detection. More importantly, reformation of parent aflatoxin may occur under

the acidic conditions that were found in the stomach (Turner, *et al*., 2005).

Specific criteria had been established for evaluating the acceptance of a given aflatoxin reduction or decontamination procedure. The process must: (1) inactivate, destroy, or remove the toxin; (2) not produce or leave toxic residues in food; (3) retain nutritive value and feed acceptability of the product; and, if possible, (4) destroy fungal spores (Park, *et al*., 2006). Although some destruction of aflatoxins in feed grade meals had been achieved by conventional processing procedures, heat and moisture alone do not provide a very effective method of detoxification.

2.7.1.2 **Irradiation**

Studies had demonstrated that exposures of contaminate peanut oil to short wave and long wave UV light causes a marked reduction in the concentration of aflatoxins (Scott, *et al*., 1998). Exposure of contaminated milk to UV light for 20 min at 25°C decreased the aflatoxin M1 content by 89.1% in the presence of 0.05% peroxide, compared with 60.7% in peroxide-free milk (Molins, *et al*., 2001).

Aziz and Smyk (2008) found comparative mutagenic activity for equal concentrations of aflatoxin B_1 and mixtures of aflatoxin B_1 and B_2 using the Salmonella assay. Aflatoxins produce singlet oxygen after exposure to UV light and singlet oxygen further activates these chemicals to mutagens.

2.7.1.3 **Solvent extraction and mechanical separation**

Several suitable solvent systems are capable of extracting aflatoxins from different commodities with minimal effects on protein content or nutritional quality. Theses systems include 95% ethanol,

90% aqueous acetone, 80% isopropanol, hexane-ethanol, hexane-methanol, hexane-acetone-water, and hexane-ethanol-water. However, current extraction technology for the detoxification of aflatoxin-contaminated oilseed meals appears to be impractical and cost prohibitive (Yilmaz and Ozay, 2001). Most of the aflatoxin associated with contaminated corn or peanuts can be found in a relatively small number of kernels or seeds, providing an excellent opportunity to reduce the level of aflatoxin contamination by a variety of separation approaches.

Conventional methods used to clean corn (e.g. dry cleaning, wet cleaning, density separation, and preferential fragmentation) are somewhat effective in reducing the aflatoxin content. Milling of corn resulted in better results (Baily, *et al*., 2004).

The distribution of aflatoxins was apparently low in grits and high in the germ, hull, or degermer fines of dry milled corn (Scott, *et al*., 1998). Aflatoxin occurred mainly in the steep water and fiber of wet milled corn, with smaller amounts present in the gluten and germ (Ankara, *et al*., 2003).

2.7.1.4 **Adsorption from solution**

Adsorbent materials including activated carbon and clay and zeolitic minerals have been shown to bind and remove aflatoxins from aqueous solutions such as water, Sorensen buffer, Czapek's medium, Pilsner beer, sorghum beer, whole milk, and skimmed milk (Bueno, *et al*.,2007). The adsorption of aflatoxins by clay (which is incorporated to remove pigments from crude oils) is a major factor resulting in the significant reduction of aflatoxins in refined peanut and corn oils (Philips, *et al*. 2009). Reports indicate that hyllosilicate clay

effectively removes aflatoxins from contaminated peanut oil and prevents its mutagenicity and toxicity *in vitro*

1.2.7.2 **Chemical methods**

A diverse group of chemicals had been tested for the ability to degrade and inactivate aflatoxins. These chemicals include numerous acids, bases, aldehydes, bisulfite, oxidizing agents, and various gases. A number of these chemicals can react to destroy (or degrade) aflatoxins effectively but most were impractical or potentially unsafe because of the formation of toxic residues or the perturbation of nutrient content, flavor, odor, color, texture, or functional properties of the product (Bailey, *et al*.,1995).

1.2.7.2.1 **Ammoniation**

Treatment of grain with ammonia appears to be available approach to the detoxification of aflatoxins. Ammoniation (under appropriate conditions) results in a significant reduction in the level of aflatoxins in contaminated peanut and cottonseed meals and corn (Maxwell, *et al*., 2006). The ammoniation process, using ammonium hydroxide or gaseous ammonia, has been shown to reduce aflatoxin levels in corn, peanut-meal cakes, and whole cottonseed which more than 99%. If the reaction is allowed to proceed sufficiently, the process is irreversible. Primarily, two procedures are used: a high pressure and temperature process (HP/HT) at feed mills or an atmospheric pressure and ambient temperature procedure (AP/AT) on the farm (Weng, *et al*., 1994; Millan, *et al*., 2003).

The HP/HT process involves the treatment of the contaminated product with anhydrous ammonia and water in a contained vessel. The amount of ammonia (0.5- 2%), moisture (12-16%), pressure (35-50

psi), time (20-60 min), and temperature (80- 120%) varies with respect to the initial levels of aflatoxin in the product (Brackett, 1998). The AP/AT process also uses a 13% ammonia solution, which is sprayed on the seed as it is packed into a plastic silage-type bag (approximately 10 feet diameter by 100 feet long). After this procedure, the bag is sealed and held at ambient temperature (25-45C^{o}) for 12-14 days. The holding time vary according to the ambient temperature, that was, a lower ambient temperature will require a longer holding time (Midio, *et al*., 2001).

The amount of ammonia (1-5%), moisture (12-16%), and time (14-42 days) will vary according to the initial levels of aflatoxin present. With this process, the bag is probed and tested periodically until test results show that aflatoxin levels are equal to or below 20 ppb. The safety of ammoniated corn has been evaluated in rainbow trout, chickens, and rats. The finding results provide strong evidence that chemical treatment via ammoniation can provide an effective strategy to detoxify aflatoxin-contaminated crops (Peracia, *et al*., 2003).

The mechanism for this action appears to involve hydrolysis of the lactone ring and chemical conversion of the parent compound aflatoxin B_1 to numerous products that exhibit greatly decreased toxicity (Midio, *et al*., 2001)..

1.2.7.2.2 **Treatment with Bisulfite**

Sodium bisulfite has been shown to react with aflatoxins (B_1, G_1, M_1, and aflatoxicol) under various conditions of temperature, concentration, and time to form water-soluble products. Several studies indicate that peroxide and heat enhance the destruction of

aflatoxin B1 by sodium bisulfite added to dried figs (Bailey, *et al*.,1995).

1.2.7.2.3 **Heterogeneous catalytic degradation**

The formation of aflatoxin B_1 adsorption complexes on the surfaces of certain inorganic materials (including various alumina, silica, alumino- silicate, and chemically modified aluminosilicate) may promote hetero-geneous catalytic degradation of the parent molecule after desorption. Hscheket, *et al*. (2002) observed that organic 56 extracts from reactions of aflatoxin B_1 with alumina, zeolite, and phyllosilicate contained varying levels of aflatoxin degradation products including aflatoxin B_2, which was identified as the major product in most casts. The parent aflatoxin that was reacted with activated charcoal was recovered from the complex unchanged. Perhaps chemical degradation of aflatoxins by reactive inorganic adsorbents represents another useful chemical approach to detoxification (Coke , 1998).

1.2.7.2.4 **Reduction in bioavailable aflatoxin by selective chemisorption**

Phyllosilicate clay (HSCAS or NovaSil™) currently available as anticaking agent for animal feeds has been reported to (1) tightly bind aflatoxins in aqueous suspension (2) markedly diminish aflatoxin uptake by the blood and distribution to target organs; (3) prevent aflatoxicosis in farm animals, including chickens, turkey, poults, goats, pigs, and mink; and (4) decrease the level of aflatoxin M_1 residues in milk from lactating dairy cattle (Galvano, *et al*., 2001 ; Wang, *et al*., 2005).

1.2.8 Biochemical findings

The growing interest in what is commonly known as healthy and safe food, that is food with a high nutritive value, can be observed world-wide. The changing conditions of various food products production, transport, storage and treatment cause that they are exposed to various Environmental factors which could cause their damage. One of these threats is microorganisms, due to their metabolic activity. That is why new safe methods which could help reduce the number of Microorganisms, which are always present in the raw material and food products, are being looked for. One of the non-thermal methods of microorganism reduction is the magnetic field effect. In fact, there are only a few works on that very subject (Mehedintu and Berg: 1997).

The findings indicate that there is an association between the exposure to extremely low frequency electromagnetic fields and the oxidative stress through distressing redox balance leading to biological disturbances. Such disorders effect directly or indirectly on the biochemical reactions of enzymes such as Amylase and protease, protein concentration, reduced sugar concentration and potential of hydrogen ions (Binczycia, *et al*., 2003).

Amylase is an enzyme that catalyses the breakdown of starch into sugars. Where it begins the chemical process of digestion. Foods that contain much starch but little sugar.

Currently, two major classes of α-amylase are commercially produced through microbial fermentation. Unlike the bacterial α-amylase that attacks the alpha 1, 4 bonds, the fungal α-amylase attacks the second linkage from non-reducing terminals (i.e. C4 end) of the straight segment, resulting in two glucose units at a time (Beranfield, 1955).

While protease is any enzyme that conducts proteolysis, that is, begins protein catabolism by hydrolysis of the peptide bonds that link amino acids together in the polypeptide chain forming the protein.

1.2.9 Enzyme-Linked Immuno-Sorbent Assay (ELISA)

ELISA is a popular format of a "wet-lab" type analytic biochemistry assay that uses one sub-type of heterogeneous, solid-phase enzyme immunoassay (EIA) to detect the presence of a substance, usually an antigen, in a liquid sample or wet sample.

The principle of ELISA involves detection of an "analyte" (i.e. the specific substance whose presence is being quantitatively or qualitatively analyzed) in a liquid sample by a method that continues to use liquid reagents during the "analysis" (i.e. controlled sequence of biochemical reactions that will generate a signal which can be easily measured quantified and interpreted as a measure of the amount of analyte in the sample) that stays liquid and remains inside a reaction chamber or well that is needed to keep the reactants contained.

As a heterogenous assay, In ELISA a liquid sample is added onto a stationary solid phase with special binding properties and is followed by multiple liquid reagents that are sequentially added, incubated and washed followed by some optical change (e.g. color development by the product of an enzymatic reaction) in the final liquid in the well from which the quantity of the analyte is measured. The qualitative "reading" usually based on detection of intensity of transmitted light by spectrophotometric which involves quantization of transmission of some specific type of light through the liquid . The sensitivity of detection depends on amplification of the signal during the analytic reactions. Since enzyme reactions are very well known amplification

processes, the signal is generated by enzymes which are linked to the detection reagents in fixed proportions to allow accurate quantification - thus the name "Enzyme linked".

The analyte is also called the ligand because it will specifically bind to a detection reagent and thus ELISA falls under the bigger category of Ligand Binding Assays. The ligand-specific binding reagent is "immobilized" i.e. usually coated and dried onto the transparent bottom and sometimes also side wall of a well, which is usually constructed as a multi-well plate known as the "Elisa Plate". Conventionally, like other forms of immunoassays the specificity of Antigen-Antibody type reaction is used because it is easy to raise an antibody specifically against an antigen in bulk as a reagent. Alternatively if the analyte itself is an antibody its target antigen can be used as the binding reagent (Sadasivam, 1996).

2.1 Materials:

2.1.1 Apparatus and equipments:

Apparatus and equipments	Company	Origin
Electric oven	Gallen Kamp	England
ELISA reader	Bio Tek.	
Filter paper	Whatman	
Magnetic stirrer hotplate	Stuart	
Autoclave	Express	Germany
Incubator	Memmert	
Millipore filters	Sartorius Membarane	
Shaking incubator	GLF	
Water path		
pH- meter	Radiometer	Denmark
Electric sensitive balance	Delta Range	Switzerland
Micropipette	Crison	France
Cooled centrifuge	Crison	Spain
spectrophotometer	Mercury	China
Digital camera		
Microscope	Olympus	Japan

2.1.2 Chemicals

Materials	Company	Origin
Sodium acetate	Fluka	Switzerland
Sodium		
Ethanol	Local market	Iraq

Starch	Oxoid	England
Casein		
KNO_3	BDH	
KH_2PO_4		
NH_2PO_4		
$MgSO_4.7H_2O$		
$FeSO_4.7H_2O$		
Glacial acetic acid	Koch -Light	
Sodium potassium tartarate	Sigma	USA
sodium tungstate		
sodium molybdate		
phosphoric acid		
hydrochloric acid		
bromine		
Citric acid	Himedia	India
Dibasic sodium phosphate		
Tris(hydroxymethyl)aminomethane		
KCl		

2.1.3 Media

2.1.3.1 Ready- to- use media:

All bellowed listed media used in this study were prepared according to the instruction on the containers of their manufacturing companies:

Media	Company	origin
Potato Dextrose Ager	Biolife	Italy
Sabaroid Dextrose Ager		

2.1.3.2 Laboratory prepared media:

2.1.3.2.1 Rose-Bengal Chloramphenicol Agar (Ottow and Glathe: 1968)

Typical Formula	**gm/liter**
Mycological peptone	5.0
Glucose	10.0
Dipotassium phosphate	1.0
Magnesium sulphate	0.5
Rose-Bengal	0.05
Agar	15.5
pH 7.2 ± 0.2 at Temp. 25°C	

2.1.3.2.2 Yeast extracts broth (Atlas, *et al*., 1995)

The medium (YES) contained 2% yeast extract and 20% sucrose. Distilled water was used throughout the study.

2.1.4 Preparation of reagents, stains, solutions, and buffers

2.1.4.1 Reagents

- **Dinitrosalicylic Acid DNS Reagent**

- Dinitrosalicylic acid: 10 g
- Phenol: 2 g
- Sodium sulfite: 0.5 g
- Sodium hydroxide: 10 g
- Add water to: 1 liter

- **Folin-Ciocalteu's reagent**

Dissolve 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85% phosphoric acid and 10ml concentrated hydrochloric acid. Reflux for 3 hr. Add 15 g lithium sulfate, 5 ml water and 1 drop bromine. Reflux for 15 min. Cool to room temperature and bring to 100 ml with water**.**

2.1.4.2 Stains

2.1.4.2.1 Ready to use stain

- Lacto phenol cotton blue

2.1.4.2.2. Laboratory prepared stain

- coomassie blue

Prepare a staining solution of colloidal coomassie blue as follows:

1. Mix 16ml phosphoric acid in 768ml of distilled water. Add 80gm of ammonium sulfate to this solution.
2. Prepare a solution of 5% CBB G250 in distilled water. Add 16ml of this mixture to the solution prepared in step 1.
3. Immediately before use, slowly add 200ml methanol to the solution to give a final concentration of 0.08% CBB G250/ 1.6% phosphoric acid 8% ammonium sulfate/ 20% methanol.

2.1.4.3 Solutions

- **Sodium potassium tartarate (40%)**

It was prepared by dissolving 40g Sodium potassium tartarate in 60 ml Distilled water and then volume of the solution increased up to 100ml.

2.1.4.4 Buffers (Chandra, 2003)

- **Citrate-Phosphate Buffer; pH range 2.6 to 7.0**

(a) 0.1 M Citric acid; 19.21 g/l

(b) 0.2 M Dibasic sodium phosphate; 35.6 g/l

Mix citric acid and sodium phosphate solutions in the proportions indicated and adjust the final volume to 100 ml with deionized water. Adjust the final pH using a sensitive pH meter.

- **Tris-HCl Buffer, pH range 7.2 to 9.0**

(a) 0.1 M Tris(hydroxymethyl)aminomethane; 12.1 g/l (M.W.: 121.0)

(b) 0.1 M Hydrochloric acid

Mix 50 ml of Tris(hydroxymethyl)aminomethane and indicated volume of hydrochloric acid and adjust the final volume to 200 ml with deionized water. Adjust the final pH using a sensitive pH meter.

2.2 Methods

2.2.1 Isolation and characterization of organism

The fungal species used in this research were isolated from clay soil by serial dilution method. 1 g of the soil sample was suspended in 9 ml of sterile distilled water and was properly mixed. 1ml of the soil suspension was pipetted from original soil suspension and transferred into another 9 ml of sterile distilled water. The soil suspension was further diluted for seven serial dilutions. About 0.1 ml from the 5^{th}-10^{th} dilution was spread on potato dextrose agar plates using a glass spreader, sterilized by dipping in 95% ethanol and flaming. .The plates were incubated at 27°C for 7 days. The growth of fungal colonies was observed after incubation. The individual colonies were then subcultured. Identification was based on cell and colony morphology characteristics (macroscopic and microscopic features) (Atlas, *et al*., 1995).

Fungal species were isolated namely *Aspergillus flavus* - which exhibited a yellowish green coloration. The young colonies of the *Aspergilli* were aseptically picked up and transferred into PDA or SDA slants and incubated at 27°C for 7 days for maximum growth.

Aspergillus flavus planted on the center of (PDA or SDA) and incubated under temperature (27 °C) for seven days, and after a period of incubation, isolated fungi from different dishes then diagnosed according to the key taxonomic.

2.2.2 Toxicity of the organism

The toxogenicity of *Aspergillus flavus* was confirmed by culture the organism on Rose Bengal agar the colonies appeared violet circle around the colony, all of the fungal colonies which used in this research are

locally isolates from corn and obtained from biology department of Baghdad University and it has been supported as toxogenic *Aspergillus flavus* according to (Atlas, *et al*., 1995).

2.2.3 Effect of magnetic poles on the growth of *Aspergillus flavus* on solid media

Aspergillus flavus was influenced by magnetic field energy which applied through a magnet with different forces (5, 7,10,30,50 Gauss).

This application was influenced by three treatments which are:

1. Influence of northern pole
2. Influence of southern pole
3. Influence of both northern and southern poles

And these influences were compared with the control which is not affected by magnetic field energy.

Petri dishes containing the proper media were inoculated with ager disk of 6mm diameter (were made by piercing Cork borer to growing culture of *Aspergillus flavus* 7 days old) and then dishes subjected to impact of the magnetic field, the first dish is to the northern pole (negative) and the second to the southern pole (positive) and the third dish to the influence of north and south poles together, and the fourth dish was left without exposing to any magnetic field to be the control treatment and with three replicates for each treatment .

All the dishes were incubated at (27 °C) for 7 days, after the end of the period the diameter of the colonies were measured in centimeter (cm) for each treatments separately and compared with the control treatment ,As shown in the following Figure (2): -

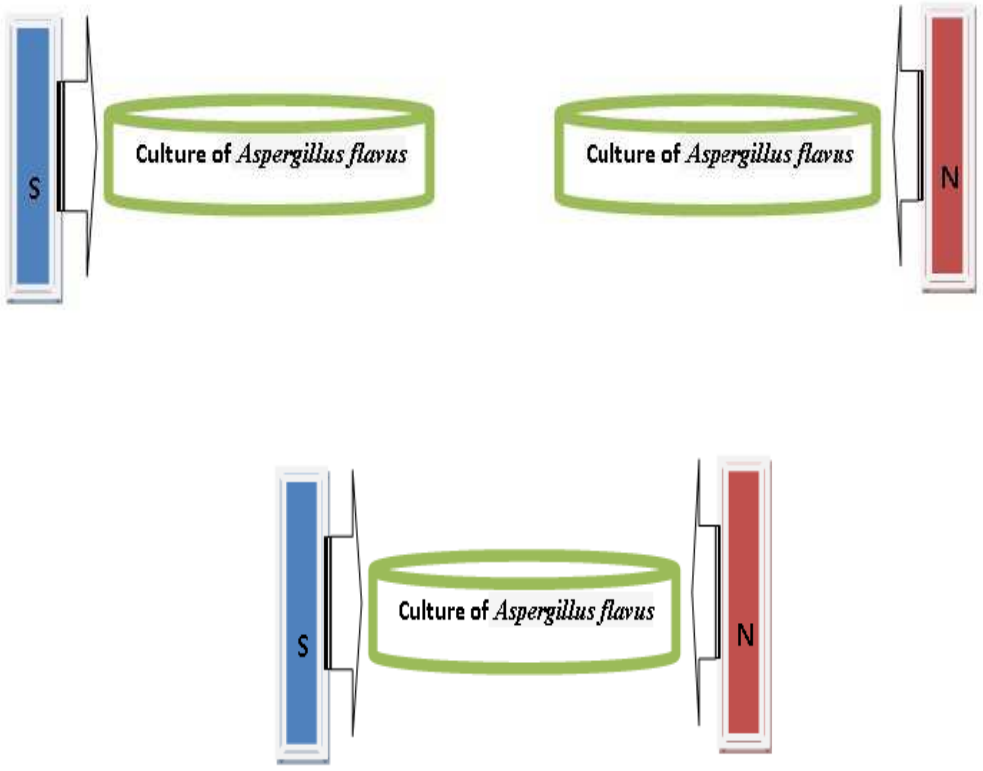


Figure 2. Shows the position of magnetic bar at different treatments (N and S pole) when effected on the growth of *Aspergillus flavus* on solid media

2.2.4 Effect of magnetic poles on the growth of *Aspergillus flavus* in liquid media, dry weight and Aflatoxin production

Inoculated flasks containing yeast extract broth were subjected by magnetic field energy of poles; this application was effected by three treatments on the flasks containing yeast extract broth media which is:

1. Influence of northern pole
2. Influence of southern pole
3. Influence of both northern and southern poles

And these treatments were compared with the control treatment in which there is no effect of magnetic field energy and with three replicates for each treatment and then incubated at a temperature (27 °C) for 7 days. As shown in the Figure (3):

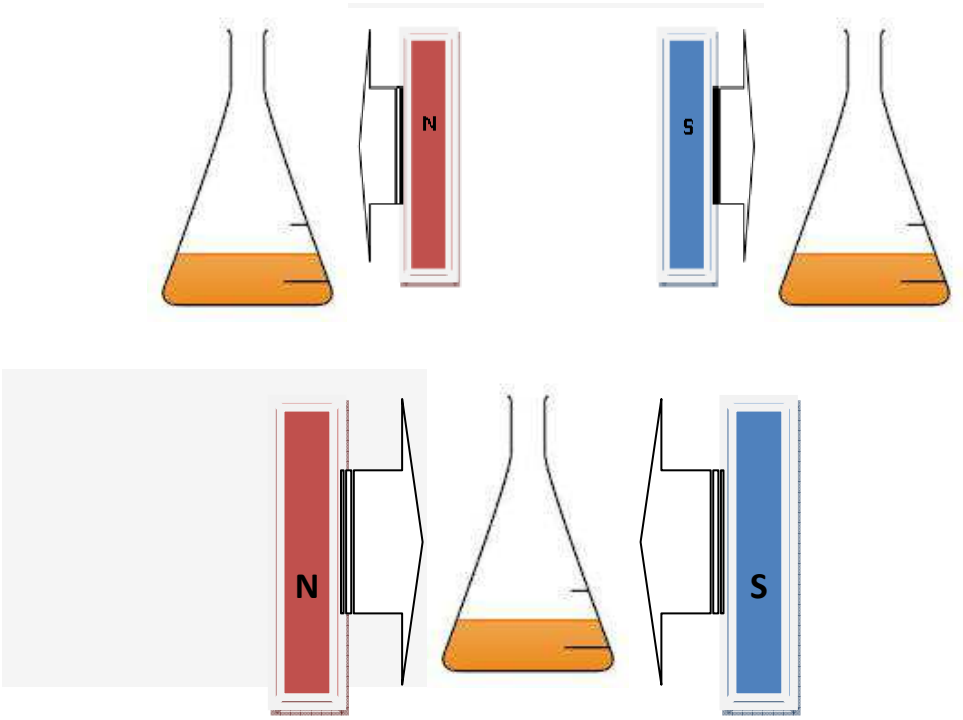


Figure 3. Shows the position of magnetic bar at different treatments (N and S pole) when effected on the growth of *Aspergillus flavus* in liquid media

2.2.4.1 The effect of magnetic field poles on the growth of *Aspergillus flavus* in liquid media

Based on continuous monitoring of changes in the optical density of fungal growth after 48 hr. the growth of *Aspergillus flavus* in liquid media was effected by three treatments which are:

1. Northern pole treatment
2. Southern pole treatment
3. Both northern and southern poles treatment

And these influences were compared with the control which is not affected by magnetic field energy.

The OD_{405} of each growth was read spectrophotometrically and calculated with the following equation:

$$\textbf{Percentage of growth} = \frac{OD_{405} \text{ of Treatment 1, 2 or 3}}{OD_{405} \text{ of the control}} \times 100$$

2.2.4.2 The effect of magnetic poles on the dry weight formed by *Aspergillus flavus*

After incubation for 7 days the cultures were filtered by Whitman filter paper No.4 each filter paper were dried until constant weight then the dry weight of mycelium was determined after drying the mycelia for 12 to 24 hr at 70 °C.

2.2.4.3 The effect of magnetic poles on the Aflatoxin production by *Aspergillus flavus* in broth culture

The filtrate which resulted from the filtration process of yeast extract broth culture was applied to aflatoxin kit procedure to detect toxin concentration, the procedure steps are:-

1. Add 100 µl conjugate to red marked mixing wells.
2. Add 100 µl controls and samples to the red marked mixing
3. Mix. Transfer 100 µl to antibody wells. Incubate at room temperature for 2 minutes.
4. Dump liquid from antibody wells.
5. Wash wells thoroughly5 times with deionized water

6. Tap out water on absorbent paper towel.
7. Transfer 100 µl substrate from reagent boat to antibody wells using 12-
. channel pipette. Incubate at room temperature for 3 minutes.
8. Transfer 100 µl Red Stop from reagent boat to antibody wells.
9. The results were read using a micro well reader with a 650 nm filter.

2.2.5 Biochemical tests

The following biochemical tests were estimated including the proteins concentration, pH, enzymes (amylase and protease) activity and reducing sugars concentration; so in order to start these tests a heavy spore suspension from adding1- 5ml of sterile distill water to slants containing growth of *Aspergillus flavus* for 2-3 weeks which act as an inoculums.

2.2.5.1 Determination of pH

The filtered growth culture that collected from the filtration process of yeast extract broth culture used to estimate the potential of hydrogen by pH meter.

2.2.5.2 Determination of Enzymes Activity

Two types of enzymes were estimated which are amylase and protease in order to study and compare the effect of magnetic field on them.

2.2.5.2.1 Determination of Amylase Activity (Nagwa, *et al*., 2011)

The culture of *Aspergillus flavus* was incubated in 100ml of production media (g\l) the flasks were incubated in a shaker incubator for 72 hr at 27 C^{o}.

After the incubation period the fungal mycelia was removed from enzyme production media by filtration through Whattman filter paper No.1 which the filtrate contain the crud enzyme.

❖ Amylase assay

One ml of enzyme extract added to 1m of 1% starch in citrate-phosphate buffer (pH=6.5) and incubated in water bath at 40 ^{o}C for 30 min.

Blank consisted of 2ml of enzyme extract that was boiled for 20 min (to inactivate the enzyme) and the starch solution was add and treated with the same reagent as the experimental tubes. The reaction was stopped by adding 2ml of DiNitroSalcylis acid (DNS) reagent and boiled for 5 min at 80 ^{o}C, after cooling 20ml of distilled water was added and the absorbance was read at 540nm.

2.2.5.1.2 Determination of protease Activity (Chinnasamy, *et al*., 2011)
Solid state fermented was carried out in 250ml conical flask contains 10g of substrate with 10 ml of salt solution (g/l). The flasks were inoculated with 1.0ml of spore suspension 10^{5} and incubated at 30^{o}C for eight days in an incubator shaker at 125rpm.

At the end of fermentation, cultures were extracted with 100ml of distilled water by shaking for 2hr. The filtrate obtained was centrifuged at 6,000 rpm for 15 min at room temperature. The supernatant used as crude enzyme extract.

❖ Protease assay

One ml of the culture broth was taken in a 100 ml flask and 1.0 ml of phosphate buffer pH 7.0 added to it. One ml of the substrate (2% casein pH 7.0) was added to the buffer-enzyme solution and incubated at 37C^{o}

for 10 minutes in a water bath. At the end of 10 minutes, 10.0 ml of 5N TCA (trichloroacetic acid) was added to stop the reaction.

The precipitated casein was then filtered off and 5.0 ml of the filtrate were taken in a test tube. To this 10.0 ml of 0.5N NaOH solution and then 3.0 ml of the folin ciocalteu reagent (one ml diluted with 2 ml of distilled water) were added. Final readings were taken in a spectrophotometer at 750 nm. Blanks of the samples were prepared by adding the TCA before the addition of substrate.

2.2.5.3 Determination of protein concentration by Bredford method (Sadasivam, 1996):

After the preparation of standard curve by bovine serum albumin BSA to estimate protein concentration in the samples, which occurred by adding 100µl of the tested sample to the 400µl of Tris-HCl and 2.5 ml of coomasi blue Dye and vortex the tubes for 2 min then read at 595nm.

2.2.5.4 Determination of reducing sugars by DiNitroSalcylic (DNS) Acid Method (Sadasivam, 1996):

1. Add 3 ml of DNS reagent to 3 ml of glucose sample in a lightly capped test tube. (To avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used.)
2. The mixture was heated at 90 ºC for 5-15 minutes to develop the red-brown color.
3. Add 1 ml of a 40% potassium sodium tartrate (Rochelle salt) solution to stabilize the color.

4. After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm.

2.3 Statistical analysis

The results obtained were analyzed for significant differences using ANOVA (analysis of variance) procedure of SPSS software (SPSS Institute,2003).All treatment of significance are based on the probability level of ($P \leq 0.05$), and to compare the differences among treatments Duncan multiple range test was used.

3.1 Identification of fungal isolate

Aspergillus flavus isolate was identified macroscopically on the solid media SDA and PDA as yellow to dark yellowish-green colonies, consisting of a dense felt of conidiophores. While microscopically conidiophores are heavy walled, uncolored, coarsely roughened, usually less than 1 µm in length. Vesicles are elongate when young, later becoming subglobose or globose, varying from 10 to 65 µm in diameter. Phialides are uniseriate or biseriate. The primary branches are up to 10 µm in length, and the secondary up to 5 µm in length. Conidia are typically globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 µm diameter which agree with (Klich, 2007).

3.2 Detection the effect of magmatic field energy on the growth of *Aspergillus flavus* on solid media

The results showed that southern pole, which carries the positive charges had a positive effect on the growth of the fungus under study by increasing the diameter of the colony developing on solid media and the number of spores formed, while for the northern pole , which holds a negative charges had a negative influence on the growth of *Aspergillus flavus*. The colony diameter developed after the southern pole was 73-80 millimeter, however colony developed at northern pole had reach 35-40 millimeter, while the treatment of northern and southern poles together and the control treatment showed diameter 50-60 millimeter (N.B. These results were the average for three replicates for each treatment) as shown in Table (1).

Table 1. Effect of magnetic field energy on the growth *of* *Aspergillus flavus* on solid media

Effect of poles	Mean(cm) ± Stander error	LSD
Control	5.2 ± 0.88	0.77
Northern	3.1 ± 0.66*	0.00
Southern	7 ± 0.12*	0.00
Both	5.4 ± 0.88	0.77

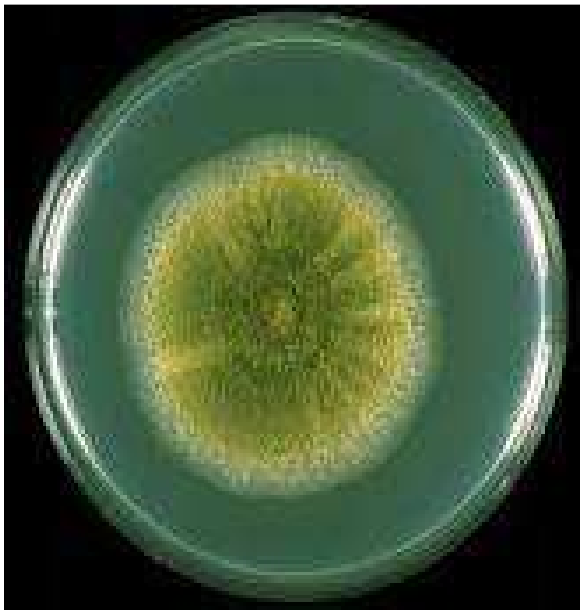
*the mean difference is significant at the 0.05 level

The growth of the examined *Aspergillus flavus* accelerated when exposed to a southern pole of magnetic field while in contrast the effect of northern pole of magnetic field slow down the growth of the fungus, this was the first observation due to the action of the magnetic field on the growth on solid media, as shown in figure (4: a, b, c and d).

The influence of magnetic field on biological activity has aroused great interest, especially in medicine (Ramchand *et al*., 2001; Zhang *et al*., 2002), microbiology and biotechnology (Fiema and Filek, 1998; Seong and Park, 2001).

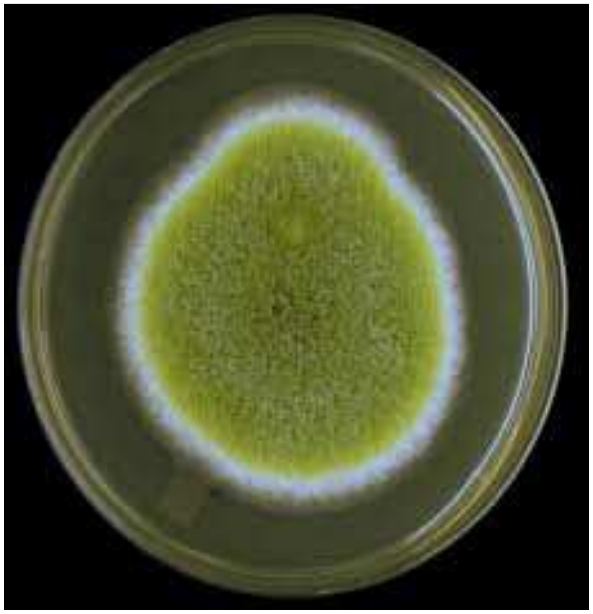
There are many scientific reports on the influence of magnetic or electromagnetic fields on living organisms. Many physiological responses to electromagnetic field pulses have been studied, but not much of this work has addressed growth and morphogenesis (Chrombei, *et al.*, 1990).

4.a. This picture shows there is No effect of magnetic field on the growth of *Aspergillus flavus* on SDA (control)



4.b. This picture shows the effect of northern pole for magnetic field on the growth of *Aspergillus flavus* on SDA

4.c. This picture shows the effect of southern pole for magnetic field on the growth of *Aspergillus flavus* on SDA



4.d. This picture shows the effect of Both poles for magnetic field on the growth of *Aspergillus flavus* on SDA

Figure 4. shows the effect of magnetic field poles on the growth of *Aspergillus flavus* on SDA

Magnetic field-induced acceleration of growth, especially of young seedlings, has been observed . A number of researchers have studied the action of a magnetic field on seed generation or other physiological processes in green plants (Garcia Reina and Arza-Pascual, 2001). In cuttent study on *Aspergillus flavus* showed better and slightly accelerated (in southern pole of magnetic field) growth and germination of spores, however. Inhibition in the growth and germination of spores when influenced by northern pole.

The results suggest that the effect of the magnetic field may have an important environmental factor affecting of the growth as well as the morphology of the examined fungus.

It has been demonstrated experimentally (Lednev, 1991) that the application of a low-frequency, weak magnetic field, both static and time-varying, induces considerable changes in the metabolism characteristic of tested organisms. These changes are manifested primarily in altered ion flow through cell membranes and in the motion of cells. Such results indicate the role of resonance. It had been found that a magnetic field of extremely low frequency affected the fluctuation of cellular calcium content. When cells were exposed to a magnetic field, Ca^{2+} content increased and/or decreased; thus the magnetic field acted as a physiological stimulator. A magnetic field has also been observed to influence calcium signal transduction (Yost and lipurdy, 1992).

In order to discuss how magnetic field can influence calcium content of the cell we should take it from two point of view the first is how Ca^{2+} content increased and that can be explained by releasing of calcium from cell membranes through magnetic fields.

While the decrease in Ca^{2+} content is resulted from the leakage of calcium ions bound to the surfaces of cell membranes, which are

important in maintaining their stability. Without these ions, cell membranes are weakened and are more likely to tear under the stresses and strains imposed by the moving cell contents. although the resulting holes are normally self-healing they still increase leakage while they are open and this can explain the bulk of the known biological effects of magnetic fields.

According to the above explanation the southern pole of magnetic field which have the positive charge while induced the calcium signal transduction and such signaling will induce and accelerate the growth of *A. flavus* , In contrasts when influenced by northern pole (which have the negative charge) it will inhibits the calcium signal transduction and so causing inhibition to the growth.

3.3 Detection the effect of magnetic field energy on the growth of *Aspergillus flavus* in liquid media

The Optical Density at 405 $_{nm}$ (OD_{405}) of each Influence after 48 h for *A. flavus* was measured spectrophotometrically. The percentage of growth at each magnetic pole treatment:

1. The control treatment
2. Northern pole treatment
3. Southern pole treatment
4. Both Northern and Southern poles treatment

The growth was increased by the influence of southern pole because the turbidity and so the absorbance of the spectrophotometer while its decreased through the effect of northern pole on the other hand the control (which had no effects) and both poles are the same in optical density.

Table 2 . Effect of magnetic field energy on the on the growth of *Aspergillus flavus* in liquid media

Effect of poles	Mean (nm) ± Stander error	LSD
Control	0.72 ± 0.0177	0.5
Northern	0.4.9 ± 0.0173*	0.02
Southern	1.3 ± 0.152*	0. 04
Both	0.8.1 ± 0.035*	0.03

*the mean difference is significant at the 0.05 level

The main theories that try to discuss the biological effects of MFs were based on the possible effects on the permeability of the ionic channels in the membrane; this can affect ion transport into the cells and result in biological changes in the organism.

The membrane phospholipids are large molecules. One end consists of hydrophobic (water hating) hydrocarbon chains. The other end has a negatively charged phosphate group and is hydrophilic (water loving). In a watery medium, they arrange themselves spontaneously to form double-layered membranes with a central core made from their water hating ends. Their water loving phosphate ends face outwards towards the water.

The affinity that the central hydrophobic parts have for one another helps hold the membrane together but the negatively charged phosphate groups on the outside repel each other and try to tear it apart. Normally, the membrane is stabilized by positive ions that fit in between the negative phosphate groups, so that they do not repel each other. However, not all positive ions stabilize the membrane equally well. Calcium ions

are particularly good because of their double positive charge, but monovalent potassium, with just one charge, is only ordinary.

Therefore, when northern pole of magnetic fields is applied it will swap membrane-bound calcium for potassium, it weakens the membrane (These membranes are only a hundred thousandth of a millimeter thick) and it becomes more prone to accidental tearing and the formation of transient pores. Fortunately, these pores are usually self-healing and the damage to the membrane is not permanent. However, during electromagnetic exposure there will be more tears, slower repair and consequently more overall leakage. In contrast the effect of southern pole of magnetic field is increase the backup of calcium ions in the cell membrane and enhances the cell metabolism. According to the results the influence of magnetic field on the growth in liquid media is very similar to the growth on solid media.

3.4 Detection the effect of magmatic field energy on the dry weight of *Aspergillus flavus* for seven days

According to the recorded results, as for the biomass has reached a dry weight of biomass for the treatment of the Southern (4.1 g) and (0.23 g) of the Northern, (0.81g and 0.8 g) for each treatment of the poles together and the control treatment, respectively. As shown in Table (3).

Table 3 .Effect of magnetic field energy on the dry weight of *Aspergillus flavus* for seven days

Effect of magnetic poles	Mean (gm)± Stander error	LSD
Control	0.81± 0.024	0.947
Northern	0.21± 0.023*	0.00
Southern	4.1± 0.272*	0.00
Both	0.8± 0.031	0.947

*the mean difference is significant at the 0.05 level

These results came in the same rate of those in the effect of magnetic field energy on the growth on solid media , so when we try to explain the decrease and increase in biomass ,it will regarded to the effect of MF on the calcium signal transduction.

According to the above results the southern pole of magnetic field which have the positive charge while induced the calcium signal transduction and such signaling will induce and increase the biomass of *A. flavus* , In contrasts when influenced by northern pole(which have the negative charge) it will inhibits the calcium signal transduction and so causing decreasing to the biomass .

3.5 The effect of Magmatic poles energy on the total Aflatoxine concentration produced by toxogenic *Aspergillus flavus*

Aflatoxins analyses of 12 samples were analyzed by using ELISA (Enzyme-Linked Immuno-Sorbent Assay) technique . Analyses were repeated two times. The influence of magnetic field energy on the

aflatoxin production was observed by the concentration of total aflatoxins in part per billion (ppb) which produced by *Aspergillus flavus* in yeast extracts broth for 7days.

The application of magnetic field on the Aflatoxine production effected by three treatments which are the Northern, Southern pole and both Northern and Southern poles, These influences were compared with the control at which there were no effect of magnetic field energy.

In ELISA analyses, there was evidence about the total aflatoxins. Despite there was no encounter of any of the aflatoxin B_1, ELISA analysis results of total aflatoxin were given in Table (4). According to Product Specifications Lower limit of detection: 0.5 ppb- range of quantization: 1 ppb–8 ppb -Controls provided: 0, 1, 2, 4 and 8 ppb - Antibody cross-reactivity: Total aflatoxins (B_1, B_2, G_1, G_2).

The data shows that concentration of total Aflatoxine production by *A. flavus* react to the magnetic field , Cultures exposed to a magnetic field exhibited changes in Aflatoxine production compared to unexposed cells (control), which was 454.73 ppb (when treated with southern pole) and 25.40 ppb (when treated with northern pole) while the control 212.46 ppb and both poles 88.33 ppb.

Table 4. Influence of magnetic field energy on the Aflatoxine concentration produced by *A. flavus* after 7 days.

Effect of magnetic poles	Mean (ppb)± Stander error	LSD
Control	212.46 ± 13.04*	0.04
Northern	25.400 ± 9.68*	0.00
Southern	454.73 ± 24.55*	0.00
Both	88.33 ± 33.23*	0.04

*the mean difference is significant at the 0.05 level

The main theories that explain the biological effects of MFs are based on the possible effects on the permeability of the ionic channels in the membrane (Galvanoskis and Sandblom, 1998). This can affect ion transport into the cells and result in biological changes in the organism. Apart from its role in maintaining membrane stability, the calcium concentration actually inside cells controls the rate of many metabolic processes, including the activity of many enzyme systems and the expression of genes. 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. This is normally under very close metabolic control so that everything works at the right time and speed. However, when the southern pole of magnetic field exposed to the fungus it increases calcium content of the cell by releasing of calcium from cell membranes proportionally will increase the concentration of total aflatoxine. While the decrease in Ca^{2+} content is resulted from the

leakage of calcium ions bound to the surfaces of cell membranes and while decrease the concentration of total aflatoxine.

Other possible effects are the activity of correlative enzymes entering in the biosynthesis of aflatoxine by the formation of free radicals due to magnetic field exposure. Free radicals are atoms or groups of atoms containing at least one unpaired electron in their orbitals. Once formed these highly reactive radicals can start a chain of reaction, like dominoes. Their main danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs (May, *et al*., 2009).

Most common free radicals are reactive oxygen (ROS) and reactive nitrogen (RNS) species such as: Superoxide (O_2^-), Hydroxyl (OH), Hydroperoxyl (H_2O_2), Carbonate (CO_3), Nitric oxide (NO).

Molecules with even numbers of paired electrons are diamagnetic; i.e., they are slightly repelled by a magnet. Free radicals, however, are paramagnetic (attracted by a magnet) because of the spin of the odd electron, the spins of the remaining paired electrons effectively canceling each other .The spin of the odd electron of a free radical, when placed in a magnetic field, may have two, and only two, orientations, one with and the other against the field. As a result these radicals might effect on the biosynthesis of Aflatoxine by one of the two stages from malonyl CoA, first with the formation of hexanoyl CoA, followed by formation of a decaketide anthraquinone.

A series of highly organized oxidation-reduction reactions then allows formation of aflatoxin several specific enzyme activities associated with precursor conversions in the aflatoxin pathway have been partially , whereas others such as methyltransferases.

Also 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 .

3.6 Biochemical Findings:

3.6.1 Detection the effect of Magmatic field energy on Enzymes Activity

The impact of the magnetic field in the enzymes is concentrated on changing the charge and thus the shape of the active site of enzymes and not on the substrate, because when exposing the substrate alone for a week to magnetic field energy it showed no change in the activity of the enzymes, but when we develop and encourage the organism to produce the enzymes under effect of magnetic field it showed a marked change in the enzymatic activity and difference between the two north and south poles .

The changes of Amylase and protease activity at northern pole and southern pole were detected and it was found in table 5 and 6 respectively the results were screened and can be discussed by the rotating electric field formed by the variable magnetic field. And the last one is the main responsible for the changing in the active site charge and in consequence the shape of enzymes, so that the substrate will not be able to attached to the active site according to the lock and key theory.

3.6.1.1 Amylase Activity

In the primary screening for amylase activity due to the influence of magnetic field energy. at table (5) shows the influences compared with the control and there is an effect of magnetic field energy.

Temperature and pH are most important factors which dose influence enzyme activity to noticeable extent. Optimal pH and temperature are very essential for activity of enzymes.

Changes in pH and temperature may not only affect the shape of an enzyme but may also change the shape or charge properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis.

We inspected whether MF substantially altered the optimal pH and optimal temperature. However, there was a change in OD values when the samples were exposed to magnetic field but there was no significant effect of MF on optimal pH and temperature.

The MF might had no effect on the activities of either integral membrane enzymes such as Ca ATPase, Na/K ATP-ase and succinic dehydrogenase or peripheral membranes (Blank, *et al*., 1995; Blank, 2005).

Table 5. The consequence of magnetic field energy on the Amylase activity

Effect of magnetic poles	Mean ± Stander error (µg/ml/ min)	LSD
Control	31.1 ± 0.176	0.296
Northern	30.2 ± 0.208	0.415
Southern	36.6 ± 0.952*	0.000
Both	30.8 ± 0.470	0.803

*the mean difference is significant at the 0.05 level

3.6.1.2 Protease Activity

Enzyme production by micro organisms is greatly influenced by media components, especially carbon and nitrogen sources, and physical factors such as temperature, pH, and incubation time and inoculums density.

The main observation for the protease activity at the influence of magnetic field energy is shown at table (6).

The fermentation medium was inoculated with the fungal strain and incubated for various time intervals (1-8 days).

The optimum temperature for protease activity produced by *A.flavus* was 30°C (with activity 34.96 µg/ml/min and pH= 6.5) after effecting by magnetic field as shown in table 6. A temperature wasn't changed since the MF in non-thermal method but decrease or increased in protease activity according to the pole to use.

Table 6. Effect of magnetic field energy on the protease Activity

Effect of magnetic poles	Mean ± Stander error (µg/ml/ min)	LSD
Control	34.8 ± 0.491*	0.01
Northern	28.1 ± 0.233*	0.00
Southern	43.3 ± 0.650*	0.00
Both	32.1 ± 0.472*	0.01

*the mean difference is significant at the 0.05 level

Activity of the enzyme in culture was greatly dependant on pH of the fermentation medium. But there wasn't any noticeable change in pH. so

the only explanation for the change in protease activity when we used northern pole and southern pole might be the rotating of electron in the electric field formed by the variable magnetic field.

If we take for granted that the obtained differences in the results at the level of the magnetic field effect on bacteria and fungus are correct, we should look for the cause, taking into account differing conditions when exciting different induction currents in cells.

3.6.2 Detection of Magmatic field energy on protein concentration

The effect of Magmatic field energy on the total protein concentration of *Aspergillus flavus* was publicized in table (7). The measurement of protein concentration was done by using Bradford Method.

This assay is based on the use of a dye, Coomassie Brilliant Blue G-250, to which protein binds, altering the light absorbance properties of the dye. When the dye is prepared as an acidic solution (in 85% phosphoric acid). Addition of protein results in a shift of the dye's absorption maximum to 595 nm. As the protein concentration increases, the absorbance of light at 595 nm increases linearly.

This increase in absorbance can be measured in a spectrophotometer. Although the absorbance of Coomassie blue dye at 595 nm is proportional to the amount of protein bound, it is necessary to establish a correspondence between absorbance values and known amounts of protein. To do this, a series of protein standard curve – dilutions of a protein solution of known concentration such as Bovine Serum Albumin (BSA) was prepared which had shown at figure 3. Once we had measured

the A595 of each standard, we were being able to plot the A595 as a function of the known protein content of each standard. After measuring the A595 of unknown sample, the standard curve then can be used to determine the amount of protein corresponding to the absorbance values measured.

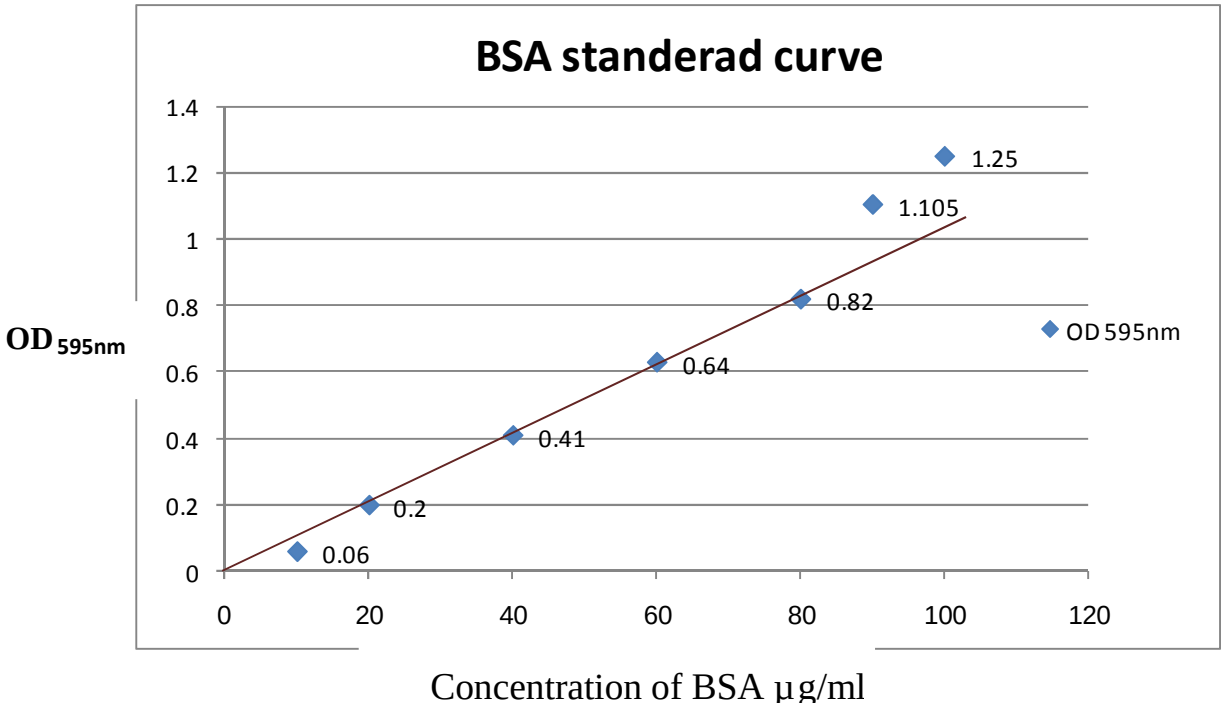


Figure 7 Standard curve for concentration of Bovine Serum Albumin (BSA)

Table 7. Effect of magnetic field energy on the protein concentration

Effect of magnetic poles	Mean ± Stander error (µg/ml)	LSD
Control	30.1 ± 0.328	0.257
Northern	25.5 ± 0.924*	0.00
Southern	41.1 ± 0.145*	0.03
Both	28.6 ± 1.083	0.263

*the mean difference is significant at the 0.05 level

Potenza, *etal*. (2004) reported that the protein synthesis by *E. coli* was stimulated under the effect of MFs with a six fold increase in the protein concentration. We can conclude that a similar effect occurs with *A. flavus* based on the data in table 7.

The mechanism of MF action in biological systems can be examined by its interaction with moving charges and enzymes activities rates in cell-free systems increasing (or decreasing) transcript levels for specific genes. It is likely. However, MF also interacts directly with electrons in DNA to affect protein biosynthesis (Blank and Goodman, 2000; Gao *et al*., 2011).

3.6.3 effect of Magmatic field energy on reducing sugar concentration

The effect of Magmatic field energy on reduced carbohydrates was revealed in table 8 by dinitrosalicylic colorimetric method which need glucose standard curve that shown in figure 8 to extract glucose concentration.

This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions:

Because dissolved oxygen can interfere with glucose oxidation, sulfite, which itself is not necessary for the color reaction, is added in the reagent to absorb the dissolved oxygen.

One mole of sugar will react with one mole of 3,5-dinitrosalicylic acid. However, it is suspected that there are many side reactions, and the actual reaction is more complicated than that previously described. The type of side reaction depends on the exact nature of the reducing sugars. Different reducing sugars generally yield different color intensities; thus, it is necessary to calibrate for each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reactions such as the decomposition of sugar also competes for the availability of 3,5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color.

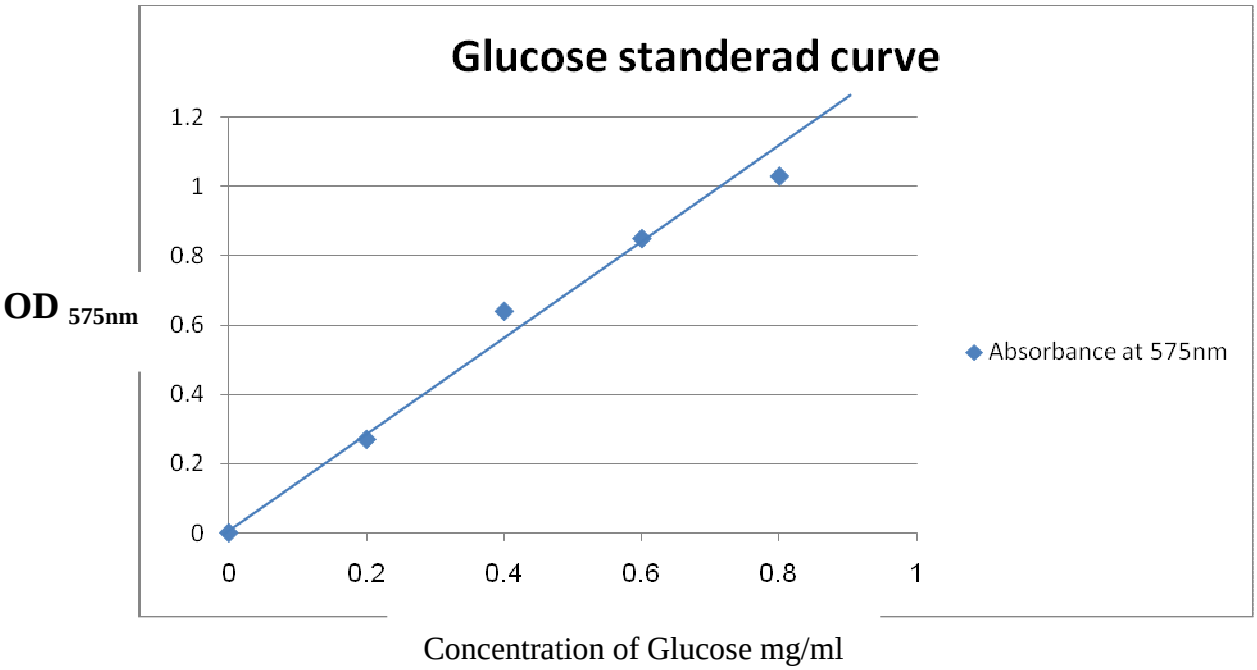


Figure 8. Standard curve for glucose concentration

Table 8. The effect of magnetic field energy on the reducing sugar

Effect of magnetic poles	Mean ± Stander error (mg/ml)	LSD
Control	0.29 ± 0.005	0.120
Northern	0.32 ± 0.008*	0.02
Southern	0.23 ± 0.008*	0.002
Both	0.28 ± 0.023	0.866

*the mean difference is significant at the 0.05 level

Depending on the results above we note that the concentration of reducing sugars directly proportional to the growth rate in the case of exposure to the magnetic poles.

As the northern pole, which working to reduce the growth of *Aspergillus flavus* in the culture of solid and /or liquid media , had increased the concentration of reducing sugars due to lack in the effect of magnetic

field on the substrate which are carbohydrates and this was confirmed by exposing the substrate of the magnet when measuring the effectiveness of enzyme activity to both amylase and protease, while for the southern pole, which accelerate the growth and the concentration of reducing sugars had been reduced.

The concentration of reducing sugars for control is not equal to the effect of both poles, although the growth rate was very close so we would not noticed any difference with respect to growth in liquid and solid media.

3.6.4 Effect of Magmatic field energy on potential of Hydrogen (pH)

There was no effect of magnetic field energy at different treatments of the northern, the southern pole and both northern and southern poles on the pH and that had been confirmed by the results in table (9).

Table 9. Effect of magnetic field energy on pH

Effect of magnetic poles	Mean ± Stander error	LSD
Control	5.1 ± 0.088	0.002
Northern	4.4 ± 0.176	0.195
Southern	4.6 ± 0.033	0.004
Both	5.3 ± 0.120	0.342

*the mean difference is significant at the 0.05 level

Depending on the results shown, we find no effect of the magnetic field poles on the pH and the results came in strict accordance with a number of research included the impact of the magnetic field on the metabolism of certain organisms.

These were also confirmed by measuring the enzymes activity of amylase and protease, so no change in the proportion of pH and thus had not changed the Iso-electric point of the metabolic reaction of *Aspergillus flavus*

4.1 Conclusions

- The most important conclusion that had been observed was the effect of magnetic field poles on the increase of total aflatoxines concentration produced by *A. flavus* when treated with southern pole and decreased when treated with northern pole while the control is not equal to the both poles.

- The effect of magnetic field poles was observed on the growth of *Aspergillus flavus* on solid media. when the southern pole had a positive effect on the growth of *Aspergillus flavus* by increasing the diameter of the colony developing on solid media, while for the northern pole has a negative influence on the growth of *A. flavus*, on the other hand, treatment of northern and southern poles together and the control (which had no effects) approximately the same.

- Magnetic field poles was detected on the growth of *Aspergillus flavus* in liquid media, which increased by the influence of southern pole and its decreased through the effect of northern pole while the control and both poles are almost the same in the percentage of the growth.

- The biomass of *Aspergillus flavus* was studied when the effect of magnetic field poles applied which decreased through the effect of northern pole and increased by the influence of southern pole while the same dry weight for each treatment of the poles together and the control.

- The southern pole of magnetic field which induced the calcium signal transduction and such signaling will induce and accelerate the growth of *A. flavus* , In contracts the northern pole will inhibits the calcium signal transduction and so causing inhibition to the growth

- The influence of magnetic field poles on the activity of some *A. flavus* enzymes such as amylase and protease are affected because the southern pole, while for the northern pole had a negative influence enzymes activity. The impact of the magnetic field on the enzymes was concentrated on changing in the charge and thus the shape of the active site of enzymes and not on the substrate, the enzymes under effect of magnetic field showed marked change in the enzymatic activity and difference between the two, north and south poles.

- The influence of Magnetic field poles on the protein concentration in *A. flavus* was affected hence the southern pole which had a positive effect on the protein concentration while the northern pole had a negative influence on protein concentration.

- The effect of magnetic field poles cause reducing sugars concentration which decreased in the treatment of the southern and increased in the northern.
- There is undeniable proof that magnetic field can remove bound calcium ions from cell membranes. Which consequently increase temporary pore formation under the mechanical stresses from pressure differences within the cell and abrasion by its moving contents.

4.2 Recommendations

- Studying the effect of magnetic fields poles on the production of Aflatoxine B_1 produced by *Aspergillus flavus*

- Studying the effect of magnetic fields poles on gene which responsible for Aflatoxin production in *Aspergillus flavus*

- Studying the effect of magnetic fields poles on the Aflatoxine B_1 *in vivo*

- Studying the effect of magnetic fields poles on the contaminated wheat, corn, rice and barley by *Aspergillus flavus* .

Committee Certification

We, the examining committee certify that we have read this thesis entitled **"Effect of Magnetic Field Energy on the Growth and Aflatoxin Production by *Aspergillus flavus*"** and examined the student **"Aieman Muhammad Ahmad"** in its contents and that in our opinion, it is accepted for the degree of Master of Science in **Biotechnology**.

Signature:
Name: Dr. Khulood Al-Samarrai
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Name: Dr. Abdul Wahid Sh. Jabir
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(Member and Supervisor)

I hereby certify upon the decision of the examining committee.

Signature:
Name: Dr. Khulood Al-Samarrai
Scientific Degree: Professor
Title: Dean of College of Science
Date:

Dedication

To my love, my strength and my refuge after Allah

My Mother Jenan

To my mother who provided me with tenderness
My Mother soul

To the man who is the best supporter to me in my life
My grandfather

To the candle that burned to enlighten my way in life My Father soul

To my dearest friend & mentor who was always helping me
Ismail

To kind pure hearts to Basils my life
My Brothers
Abdo & Mysa

To the people who were always encourage me in my life
My family
Adel, Ahmad, Hanan, Amal, Iman and Zahraa

Aiman

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List of Abbreviation

Abbreviation	Meaning
µg/ml	Microgram per milliliter
µg/ml/ min	Microgram per milliliter per minute
AFB_1	Aflatoxine B_1
AFB_2	Aflatoxine B_2
AFG_1	Aflatoxine G_1
AFG_2	Aflatoxine G_2
AFL	Aflatoxicol
AFM_1	Aflatoxine M_1
AFM_2	Aflatoxine M_2
ATP	Adenosine tri-phosphate
Ca ATPase	Calcium Adenosine tri-phosphatase
Ca^{+2}	Calcium
cm	Centimeter
CYP_S	Cytochrome P450 enzymes
DNA	Deoxyribonucleic acid
DNS	Dinitro-sylcilic acid
ELISA	Enzyme linked immunosurbent assay
EMF	Electromagnetic failed
G	Gauss
gm	Gram
LSD	Least significant differences
MF_S or MF	Magnetic failed
mg/ml	Milligram per milliliter
N	Northern pole
Na/K ATPase	Sodium – potassium Adenosine tri-phosphatase

nm	Nanometer
OD	Optical density
PDA	Potato dextrose agar
pH	Potential of hydrogen ions
ppb	Part per billion
BSA	Bovine serum albumin
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S	Southern pole
SDA	Sabaroid dextrose agar
UV	Ultra violate light
CAST	Center for Applied Science Technology
IARC	International Agency for Research on Cancer

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Summary

The study was conducted to conclude the effect of the magnetic field poles on aflatoxins produced by toxogenic *Aspergillus flavus*. The fungus had been exposed to the northern pole, southern pole; both poles and their influences were compared with the control at which the fungus is not affected by magnetic field energy. *A. flavus* was subjected to magnetic field energy which applied through a magnet at different forces (5, 7,10,30,50 Gauss) for seven days at temperature of 27^{o}C and for three replications.

The use of magnetic fields poles as new detoxification method that did not cause heating of tissues or damage to the nutritive value of the crops, but only inhibit organisms living cells or their secondary metabolites. Magneto-biological effects have unique features that obviously distinguish them from thermal effects. Also, they are dependent on simultaneously static magnetic fields and their poles. The quality of this effect is the same but the quantity of the effect is dependent on the chemical structure of metabolites.

The effect of magnetic field poles was observed on the growth of *Aspergillus flavus* on solid media. The southern pole had a positive effect on the growth of *A. flavus* by increasing the diameter of the colony (7.3-8 centimeters) developing on solid media, while the northern pole had a negative influence on the growth of *A. flavus*, with (3.5-4 centimeters) diameter of the colony, while the treatment of northern and southern poles together and the control treatment is (5-6 centimeters) respectively.

A similar result was obtained, when the effect of magnetic field poles was detected on the growth of *A. flavus* in liquid media, which increased by the influence of southern pole, because of the turbidity and

so the absorbance of the spectrophotometer was also increased and the percentage of the growth was (173.2 %). While it decreased through the effect of northern pole (65.5 %), on the other hand the control (which had no effects) and both poles are the same in the percentage of the growth (98-100 %).

The investigation on the effect of magnetic field poles on the biomass that had reached a dry weight of biomass for the treatment of the southern (4.3 g) and (0.23 g) for the northern, (0.81g and 0.83 g) for each treatment of the poles together and the control treatment, respectively.

The most important conclusion that had been observed was the effect of magnetic field poles on the concentration of total aflatoxin produced by *A. flavus*, which was 454.73 ppb (when treated with southern pole) and 25.40 ppb (when treated with northern pole) while the control 212.46 ppb and both poles 88.33 ppb by using ELISA technique.

The southern pole of magnetic field which had the positive charge while induced the calcium signal transduction and such signaling induced and accelerate the growth of *A. flavus* , in contrasts when influenced by northern pole (which had the negative charge) it inhibited the calcium signal transduction and so causing inhibition of the growth.

Magnetic field may be able to remove bound calcium ions from cell membranes. There was also no doubt that bound calcium ions were essential for the stability of these membranes. Consequently, their loss would increase temporary pore formation under the mechanical stresses from pressure differences within the cell and abrasion by its moving contents. This very simple conclusion can account for virtually all of the known biological effects of electromagnetic fields, including changes in metabolism and genetic damage.

Also the influence of magnetic field poles on the activity of some *A. flavus* enzymes such as amylase and protease were affected because the southern pole which had a positive effect on the enzymes activity, while for the northern pole had a negative influence enzymes activity however the treatment of northern and southern poles together and the control had the same value.

The impact of the magnetic field on the enzymes were concentrated on changing the charge and thus the shape of the active site of enzymes and not on the substrate, because when we develop and encourage the organism to produce the enzymes under the effect of magnetic field there would be a marked change in the enzymatic activity and difference between the two, north and south poles.

The influence of magnetic field poles on the protein concentration in *A. flavus* was affected hence the southern pole which had a positive effect on the protein concentration, while the northern pole had a negative influence on protein concentration .however, the treatment of northern and southern poles together and the control has the same amount.

The effect of magnetic field poles was studied on the reducing sugars concentration which decreased in the treatment of the southern (0.23 mg/ml) and increased up to (0.32 mg/ml) in the northern, while (0.28 mg/ml and 0.29 mg/ml) for each treatment of the poles together and the control treatment, respectively. the northern pole, which reduced the growth of *A. flavus* in the culture of solid and /or liquid media , it will increase the concentration of reducing sugars due to lack in the effect of magnetic field on the substrate which are carbohydrates, while for the southern pole, which accelerate growth and lead to decrease the reducing sugars in the organism .

بسم الله الرحمن الرحيم

وَلَو أَن ما فِي الأَرضِ مِن شَجَرَةٍ أَقلامٌ وَالبَحرُ يَمُدُّهُ مِن بَعدِهِ سَبعَةُ أَبحُرٍ ما نَفِدَت كَلِماتُ اللَّهِ إِنَّ اللَّهَ عَزيزٌ حَكيمٌ

صدق الله العظيم

سورة لُقمان - الآية ٢٧

الملخص

أجريت هذه الدراسة لتحديد تأثير أقطاب المجال المغناطيسي على سموم افلا التي ينتجها الفطر *Aspergillus flavus*. تعرض الفطر إلى القطب الشمالي، القطب الجنوبي، كلا القطبين وقورنت تأثيراتهم مع معاملة السيطرة التي لا يتأثر الفطر بطاقة المجال المغناطيسي. تأثر *A. flavus* بطاقة المجال المغناطيسي التي تم من خلال تعرضه لمغانط مختلف القوى (٥، ٧، ١٠، ٣٠، ٥٠ غاوس) لمدة سبعة أيام و في درجة حرارة ٢٧ درجة مئوية وبواقع ثلاثة مكررات. إن استخدام أقطاب المجال المغناطيسية كطريقة جديدة لإزالة السموم لا تسبب تسخين للأنسجة أو اضرار تلحق بالقيمة الغذائية للمحاصيل، وانما فقط تثبط خلايا الكائنات الحية أو نواتج تفاعلاتها الثانوية. التأثيرات المغناطيسية للاحياء تعتبر ذو مميزات فريدة من نوعها بحيث يمكن تفريقها عن التاثيرات الحرارية. كذلك، فهي تعتمد على المغناطيسية الساكنة أو المجالات الكهربائية و الاقطاب المغناطيسية .

لوحظ تأثير المجال المغناطيسي للقطبين في نمو *Aspergillus flavus*على الاوساط الصلبة، حيث كان للقطب الجنوبي ذو تأثير إيجابي على نمو *A. flavus* من خلال زيادة قطر المستعمرات النامية على الطبق (٧,٣- ٨ سم) ، في حين كان القطب الشمالي ذو تأثير سلبي على نمو *A. flavus* (٣,٥ - ٤ سم) كقطر للمستعمرات النامية على الطبق، في حين كانت معاملة القطبين الشمالي والجنوبي معا ومعاملة السيطرة (٥-٦ سم).

تم الحصول على نتائج مشابهة عند الكشف عن تأثير أقطاب المجال المغناطيسي على نمو *Aspergillus flavus* في الاوساط السائلة، حيث زدادت نسبة النمو عند تأثير القطب الجنوبي الى (١٧٣,٢٪). في حين انخفضت خلال تأثير القطب الشمالي (٦٥,٥٪)، و من جهة أخرى كانت معاملة السيطرة وكلا القطبين هي نفس النسبة المئوية للنمو (٩٨-١٠٠٪).

اما بالنسبة لتأثير المجال المغناطيسي على الكتلة الحيوية فقد وصل الوزن الجاف من الكتلة الحيوية عند المعاملة بالقطب الجنوبي الى (٤,٣ غرام) و(٠,٢٣ غم) للقطب الشمالي و (٠,٨١غم - ٠,٨٣ غم) لكلا القطبين معا ومعاملة السيطرة، على التوالي.

اهم استنتاج لوحظ عند تأثير أقطاب المجال المغناطيسي على التركيز الكلي لسموم افلا و المنتجة من قبل *A. flavus* كانت ٤٥٤,٧٣ جزء في البليون (عند تاثير القطب الجنوبي) و ٢٥,٤٠ جزء في البليون (عندما معاملة القطب الشمالي)، في حين معاملة السيطرة ٢١٢,٤٦ جزء في البليون وكلا القطبين ٨٨,٣٣ جزء في البليون باستخدام تقنية ELISA .

هناك احتمال لأمكانية المجال المغناطيسي على إزالة أيونات الكالسيوم المتجهة من أغشية الخلايا. و لا يوجد اي شك في أن ربط أيونات الكالسيوم ضرورية لاستقرار هذه الأغشية. وعلى هذا الاساس فأن فقدان ايونات الكالسيوم من الغشاء الخلوي ستؤدي لتشكيل ثقوب مؤقتة . وهذا استنتاج بسيط جدا يمكن أن تمثل تقريبا كل الآثار البيولوجية المعروفة من المجالات الكهرومغناطيسية ، بما في ذلك التغيرات في التمثيل الغذائي والضرر الوراثي.

ان القطب الجنوبي من المجال المغناطيسي الذي يولد الشحنة الموجبة سوف يستحث نقل الإشارة الكالسيوم ومثل هذه الإشارات تؤدي الى تسريع نمو *A. flavus* ، و بالعكس عند تتأثر القطب الشمالي (يشع بالشحنة السالبة) والتي تحول دون ذلك مما يسبب تثبيط النمو.

بالاظافة الى ذلك فان فعالية بعض الإنزيمات *A. flavus* مثل الأميليز والبروتيز تتغير بتأثير أقطاب المجال المغناطيسي حيث ان القطب الجنوبي الذي كان له أثر إيجابي على فعالية الانزيمات، اما بالنسبة للقطب الشمالي اكان له اثر سلبي على فعالية الانزيمات ، و من ناحية اخرى كانت معاملة كلا القطبين والسيطرة على نفس القيمة.

تركزت تأثير المجال المغناطيسي على فعالية الانزيمات خلال تغيير الشحنة وبالتالي شكل الموقع النشط من الانزيمات وليس على المادة الفعالة للانزيم ، لأنه عند تنمية وتشجيع الكائن الحي لإنتاج الإنزيمات تحت تأثير المجال المغناطيسي سيكون هناك تغير ملحوظ في النشاط الأنزيمي .

تأثرت تركيز البروتين في *A. flavus* بتأثير أقطاب المجال المغناطيسي حيث ان القطب الجنوبي الذي كان له أثر إيجابي على تركيز البروتين، في حين أن القطب الشمالي كان له اثر سلبي على تركيز البروتين لكن معاملة أقطاب الشمالي والجنوبي معا والسيطرة اعطت نفس القيمة.

تمت دراسة تأثير أقطاب المجال المغناطيسي على تركيز السكريات المختزلة والتي انخفضت في معاملة الجنوبية (٠,٢٦ ملغ / مل) وزيادة تصل إلى (٠,٣١ ملغ / مل) في شمالي، في حين (٠,٢٤ ملغ / مل و٠،٢٩ ملغ / مل) لكل معاملة القطبين معا ومعاملة السيطرة، على التوالي. القطب الشمالي الذي قلل من نمو *Aspergillus flavus* فعندة تنميته على الاوساط الصلبة و / او السائلة، فإنه سيتم زيادة تركيز السكريات المختزلة بسبب قلة بتأثير المجال المغناطيسي على المادة الفعالة التي هي الكربوهيدرات، بينما القطب لجنوبي ، الذي سريع وتيرة النمو ادى لتخفيض تركيز السكريات المختزلة.

Chapter One

Introduction and Literature review

Chapter Two

Materials and Methods

Chapter Three

Results and Discussion

Chapter Four

Conclusion and Recommendation

References

Republic of Iraq
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Effect of Magnetic Field Energy on the Growth and Aflatoxin Production by *Aspergillus flavus*

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رسالة

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وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

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