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Zainab abbas

1 Introduction and Literature Review

1.1 Introduction

The fungi include many species of eukaryotic, spore bearing organisms that obtain simple organic compounds by absorption. The organisms have no chlorophyll and reproduce almost by both sexual and asexual means. The fungi are usually filamentous, and their cell walls have chitin. The science which study fungi is called mycology, and fungal diseases which effect human and animal are called mycoses. Together with bacteria, fungi are the major decomposers of organic materials in the soil. They degrade complex organic matter into simple organic and inorganic compounds. In doing so, they help recycle carbon, nitrogen, phosphorous, and other [illegible] diseases and several human diseases (Kirk *et al.*, 2008).

[illegible] Magnetic materials played a prom[illegible] world and in the development of modern technology. The magnetic properties of materials are important to understand them had led to a deep insight into the fundamental structure of materials. The magnetization is one of the basic properties of materials which appear in various forms, but the kinds were studied known as ferromagnetism and ferrimagnetism. Traditionally, only those elements that exhibit ferromagnetism properties are called magnetic. A magnet is an object that has a magnetic field. The word magnet comes from the Greek "magnítis líthos", which means "magnesian stone". Magnesia is an area in Greece where deposits of magnetite has been discovered since antiquity (Peter *et al.*, 2002).

There are two types of magnets: permanent magnets and electro-magnets. Permanent magnets emit a magnetic field without the need for any external source

of power. Electro-magnets require electricity in order to behave as a magnet. There are various different types of permanent magnet materials, each with their own unique characteristics. Each different material has a family of grades that have properties slightly different from each other, though based on the same composition (Carozzi *et al.*, 2009).

Numerous earlier experiments proved that the static or extremely low frequency magnetic fields with small flux density had an effect on various living organisms. A considerable part of the investigations dealt with the effect of electromagnetic fields on macromolecules or cells.

Bioelectromagnetics is the study of the interaction between electromagnetic 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 [illegible] of living cells, tissues, and [illegible] the response of cells to electromagnetic [illegible] energy of fundamental nature and it control the spin of electrons around the nucleus of atoms and cells (Gao *et al.*, 2011).

The impact of the magnetic field energy lies in the stimulus to the events of significant changes in the characteristics metabolism of organisms, these changes take place in the exchange of ions through the cell membrane and in the movement of cells. Living systems are affected by magnetic field (MF) and electromagnetic field (EMF), which are generated from both external (MF and EMF) and internal sources (natural metabolisms of organisms). Several studies concluded that magnetic and electromagnetic fields have different responses for biosystems such as neural and neuromuscular activity, tissue growth and repair, glandular secretion, and cell membrane function. Although electromagnetic field has an impact mostly

on charged units and related metabolisms, magnetic field usually affects biochemical reactions that involve more than one unpaired electron. The effect of an external magnetic field on enzymatic reaction rates can be determined in the same way classical enzyme kinetic parameters are determined. Enzymes with chromogenic substrates or products can be followed spectrophotometrically (Koch *et al.*, 2003).

According to the purpose of the dissertation the current study planned to:

1. Study the effect of magnetic field on the growth of various species of filamentous fungi on solid media and liquid media.

2. Estimate the dry material of the fungal growth, protein concentration, reducing

1.2 Literature Review

1.2.1 Fungi

The kingdom of fungi includes yeasts, moulds, mushrooms and toadstools and comprises of a great diversity of heterotrophic organisms that mostly decompose organic matter for their metabolism. In a mainly saprophytic lifestyle, fungi absorb the necessary nutrients from the de composing material and they are able to utilise water contained in the air. Fungi have a unicellular or a pluricellular filmentous thallus or mycelium and generally display fruiting bodies that produce spores. The filamentous mycelium can be regarded as the whole fungal body with the spore bearing fruiting body or visible mushroom being only a temporary spore dispersal [illegible] occurs. With few exceptions, cell walls of fungi contain chitin, a long carbohydrate polymer also found in insects. Chitin adds structural support and rigidity to the fungal cell [illegible]

The success of *Aspergillus niger* for industrial production is due to the metabolic versatility of this strain. *A. niger* is well known to produce a lot of organic acids, enzymes, plant growth regulators, mycotoxins and antibiotics. The industrial importance of *A. niger* is not only limited to its more than 35 native products but also on the development and commercialization of the new products, which are derived by modern molecular biology techniques. During the past few years numerous studies have been presented on *A. niger*, presumably the most important fungus for production and secretion of protein (Jeenes *et al.*, 1991; Yoon *et al.*, 2010).

Rhizopus species are so named because they have rhizoids, which are root like anchors connected by hyphae called stolons that extend into the medium on

which they are growing. Similar other organisms in the class Zygomycetes include Absidia, Mucor, Apophysomyces, Rhizomucor, Saksenaea, and Cunninghamella. The following can differentiate these genera: the length and location of their rhizoids, the diameter of sporangia, the shape of columellae, and the size, shape and surface texture of sporangiospores and the maximum growth temperature. Of these, only Mucor is without rhizoids. These same characteristics may be further used to differentiate between the Rhizopus spp. (e.g., Rhizopus oryzae, Rhizopus microsporus rhizopodiformis, and Rhizopus stolonifer (Richarson, *et al.*, 2003; Larone, 2002).

Most of the fungi contain chitin in the cell wall which ranges from 22-40% (Muzzarelli, 1977). Its presence together with that of other polysaccharides has been used as a criterion for fungal taxonomy. The polysaccharide microfibrils are different lengths depending on the species and cellular location (Gow and Gooday, 1982).

The chitin content of fungal cell walls is highly variable between species of fungi. The search for alternative sources of chitin has brought to attention the filamentous fungi of the Zygomycetes class, whose cell walls contain native chitin and chitosan, performing protective and supportive functions (Davis and Eveleigh,1984). It appears that the cell wall's chitin content was generally higher in Zygomycetes, as illustrated by the reported chitin amounts in *Mucor mucedo*, *Rhizomucor miehei*, *Rhizopus oryzae*, *Phycomyces blakesleeanus* (Chung *et al.*, 1994) and *Cunningham ellaelegans* (Andrade *et al.*, 2000).

Several simple models had been used to describe chitin production by microorganisms. Investigations on the influence of growth time on the contents of chitin and chitin derivatives by *Mucor* have been reported. However, these studies usually adopt the univariate approach, studying only one variable at a time

(Synowieck and Al-Khateeb, 1997), for example. The alternative multivariate approach, where all factors are considered simultaneously on an equal basis, is becoming increasingly popular for the analysis and optimisation of several experimental systems (Neto *et al.*, 2001).

1.2.1.1 Fungi Important Biotechnology

In recent years the potential of using microorganisms as biotechnological sources of industrially relevant enzymes had stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Akpan *et al.*, 1999; Pandey *et al.*, 2000a and Abu *et al.*, 2005). Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan *et al.*, 1999; Mitchell and Lonsane, 1992) by degrading 1-4 linkage of starch. Besides their use in starch processing, they also find potential application in a number of industrial processes such as in food, baking, brewing, [illegible] advent of new frontiers in biotechnology, its application has expanded into many other fields, such as clinical, medical and analytical chemistry. Although amylases can be obtained from several sources, such as plants and animals, the enzymes from microbial sources generally meet industrial demand (Pandey *et al.*, 2000b). Microbial amylases have successfully replaced chemical hydrolysis of starch in starch processing industries. The use of starch degrading enzymes was infact the first large scale application of microbial enzymes in the food industry (Bennett, 1998 and Pandey *et al.*, 2000b).

Filamentous fungi are important organisms for production of useful enzymes and biological active secondary metabolites. These fungi produce high levels of polysaccharide degrading enzymes and are frequently used for the production of industrial enzymes. Studies on fungal amylase especially in the developing

countries have concentrated mainly on *Rhizopus sp.* and *Aspergillus sp.* probably because of the ubiquitous nature and non-fastidious nutritional requirements of these organisms (Abe *et al.*, 1988).

Protease is an enzyme that breaks the peptide bonds of proteins (Hossain *et al.*, 2006). Protease breaks down peptide bonds to produce amino acids and other smaller peptides. It can be isolated from a variety of sources such as plants, animals and microbia (fungi and bacteria). Its application is very broad and has been used in many fields for years, and is mainly used in food and detergent industries (Yandri *et al.*, 2008). Proteases work best in acidic conditions except alkaline proteases which has its optimal activity shown in alkaline (basic) pH (Hossain *et al.*, 2006). Proteases are one of the most important classes of industrial enzymes and accounts for about 60% of commercial enzymes in the world. They find application in a number of biotechnological processes, viz. in food processing and pharmaceuticals, leather industry, detergent industry, etc. (Na[illegible] and Martins, 2004; Rao and [illegible], 2006). Two thirds (2/3) of the in[illegible] microbial sources (Ellaiah and Adinarayana, 2002). A variety of microorganisms such as bacteria, fungi, yeast and Actinomycetes are known to produce these enzymes (Madan *et al.*, 2002 and Devi *et al.*, 2008). Molds of the genera *Aspergillus*, *Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe (Devi *et al.*, 2008). *Aspergillus clavatus* has been recently identified as a producer of an extracellular bleaching stable alkaline protease (Hajji *et al.*, 2008). Protease is the single class of enzymes which occupy a pivotal position with respect to their application in both physiological and commercial fields (Pastor *et al.*, 2001and Ward, 1985).

Following these major advances in Tow Dimensional gel Electrophoresis (2-DE)-based proteomics, another group reported the use of high-resolution 2-DE to

analyse protein changes during penicillin biosynthesis in three strains of *Penicillium chrysogenum* (Jami *et al.*, 2010). The strains used were the wild type, a strain with a small improvement for penicillin biosynthesis and a strain with a large improvement for penicillin biosynthesis. As a result of the experimental conditions used, the corresponding protein maps generated showed an amazing number of up to a thousand distinct spots per gel, of which 950 proteins could be readily identified. The large number of proteins identified allowed for estimating the main proteome changes between the high producer strains and the wild-type strain. In the high producer strains, a number of pathways were more prominent, like cysteine biosynthesis, enzymes of the pentose phosphate pathway and stress response proteins together, whereas proteins for biosynthesis of other secondary metabolites different from penicillin (cephamycin and isoflavonoid) were reduced. For studies of metabolic processes by proteomics, it is of interest to combine proteomic analysis with metabolic measurements. The process of degradation of aromatic compounds in *Phanerochaete chrysosporium* was follow

be drawn for the process of lignin degradation based on enzyme expression values as assessed by proteomics and the amount of chemical species related to these enzymes, assessed by metabolomics. A second example of an approach combining proteomics with metabolomics comes from the analysis of the effect of lactate and starch on fumonisin B2 biosynthesis in *A. niger* (Sørensen *et al.* 2009). By means of an approach similar to the one adopted by Matsuzaki and co-workers, Sørensen and colleagues were able to show a specific relation between the increase in fumonisin B2 and the enzymes affecting the intracellular levels of acetyl-CoA. From this observation Sørensen and colleagues were able to conclude that fumonisin B2 production in *A. niger* is most likely regulated by acetyl-CoA.

In addition to the study of metabolism, the study of secreted enzymes is an important field in research on filamentous fungi. Many filamentous fungi have evolved to secrete high amounts of specialised enzymes responsible, e.g. for plant cell-wall degradation. For this reason these fungi are applied in various biotechnological processes. In an attempt to disclose the cellulose-degrading system in the model fungus *Neurospora crassa*, Tian and co-workers combined gene expression data with proteomic data from the secretome of the fungus (Tian *et al.* 2009). By applying microarray and shotgun proteomics analysis on strains grown on different media, they were able to identify strong candidate genes involved in cellulose degradation. In addition, some of these candidate genes were further validated by the observation that the corresponding deletion strains grew poorly on cellulose. Another possible approach to identify a given secretome is to separate all secreted proteins, screen for a certain enzyme activity and identify the isolated protein. Following this approach in *A. fumigatus*, the secretome was separated by 2-D PAGE and after fluorescent [illegible] (Kim *et al.*, 2007a). If the purpose of the analysis is to have an overview of the largest number possible of secreted enzymes, then several media must be tested containing different carbon substrates. Two such experimental approaches were carried out in aspergilli grown under varying conditions. Medina and colleagues (Medina *et al.* 2004) were able to identify proteins specifically secreted on medium containing rutin in *Aspergillus flavus*. For this, the genome sequence information of seven different species of *Aspergilli* was used for protein identification to increase the number of identified proteins. In this way, proteins may still be identified even if the corresponding gene model from *A. flavus* would be mis-annoted, e.g. by incorrect intron-exon boundaries or mis-annotated C- or N-termini.

The *A. niger* secretome was subject of several studies the last 2 years. Tsang and co-workers (Tsang *et al.*, 2009) used defined and complex media to increase the spectrum of enzymes secreted by *A. niger*. In this study the analysis of secreted proteins was combined with genome-wide predictions of signal-peptide containing proteins. In this way, secreted proteins could be validated as secreted proteins and not as contaminants resulting from cell lysis. In another study (Jacobs *et al.*, 2009), gene expression data were crossed with 2-DE-based proteomic data from three strains of *A. niger* that are overproducers of lipase, protease and hydrolase. In this work, automated sample processing was used for 2-DE spots, allowing the identification of 898 individual proteins. Protein samples from 2-DE gels were normalised for spot volume and relative expression was estimated. The overproducing strains showed up-regulation of proteins involved in endoplasmic nitrogen metabolism, protein folding and protein degradation. Furthermore, using the information of up-regulated protein folding and protein degradation in overproducing strains, a group was [illegible] involved in protein glycosylation and by simultaneously knocking-out the doaA gene involved in protein degradation.

One fungal organelle of particular industrial importance is the microbody. Microbodies in filamentous fungi can be divided into three categories: peroxisomes, responsible for β-oxidation of long-chain fatty acids; glyoxysomes, which additionally participate in the glyoxylate cycle; and the Woronin body, which functions as a septal plug in case of cell injury. Microbodies have been implicated in the production of β-lactam antibiotics, most notably of penicillin. These organelles were recently analysed in *Penicillium chrysogenum*, the major penicillin producer (Kiel *et al.*, 2009).

1.2.2 Magnate and magnetic field energy

The power of the magnet is one of the most basic powers in nature. We know that magnetism itself was an ingredient in the primordial soup from which the universe and our planet came forth. Magnetism is the force that keeps order in the galaxy, allowing stars and planets to spin at significant velocities. And in a sense, our own planet's magnetic field is responsible for protecting all life on earth (Zdyb and Bauer, 2003).

1.2.2.1 Magnetism and Electromagnetism

usually electrons, but sometimes charged particles called ions. An example of an electric field occurs when you shuffle your feet across a carpet and touch a doorknob. The carpet pulls some electrons from your body and your clothing, leaving you with a deficiency and the carpet with an excess. When you touch the doorknob, it pulls up electrons to satisfy your deficiency, and it balances the electrical charge, creating a spark in the process. Electrical fields are measured in units called volts per meter (vpm) or volts per centimeter. The next side of the three sided coin is the magnetic field. A magnetic field is caused by electrical charges in motion, as opposed to an electric field, which is produced-by electrical charges in different concentrations, more in one place than the other, regardless of whether or

not they are moving. One cannot see the electrical current in a magnet; that delved deeper into the structure of matter. In a static magnet, the electrical current moves in terms of electrons orbiting around the atomic nuclei. An iron body is magnetized when the electrons become aligned to greater degree (Ardhuin, *et al.*, 2000).

1.2.2.2 Magnetic Poles

The effects of magnetism have been known for centuries. Even before 600 B.C., the Greeks had discovered that lodestone, a type of ore containing iron oxide, was able to exert forces of attraction on small iron objects. Also, when pivoted in a horizontal plane and allowed to rotate freely, a needle-shaped piece of lodestone would always come to rest in a north-south position, a fact that led to its widespread use in navigation. Since this type of iron ore was first found near a region in Asia Minor called Magnesia, its effects became known as magnetism.

Lodestone is made up of its magnetic ore. Artificial magnets made primarily of steel, nickel, cobalt, and alloys of rare earth metals. When a bar magnet is dipped in iron filings, the filings are attracted to it and accumulate most noticeably around regions at each end of the magnet, called poles. When a bar magnet is allowed to rotate freely, the pole that tends to seek the northerly direction is called the north magnetic pole, or simply the N-pole. The opposite end is called the south magnetic pole, or the S-pole. Magnets can exert forces on each other (Friis-Christensen *et al.*, 2006). By observing how magnets interact with each other, we can state the Law of Magnetic Poles as opposite magnetic poles attract, and similar magnetic poles repel. Another way of saying this is that like poles repel each other, and unlike poles attract. This behaviour is similar to that of like and unlike electric charges. However, it is important to keep one important difference in mind. It is possible to separate positive from negative charges and produce isolated

charges of either kind. In contrast, no one has found a magnetic monopole (an isolated north or South Pole). Any attempt to separate north and south poles by cutting a bar magnet in half fails, because each piece becomes a smaller magnet with its own north and south poles (Newitt *et al.*, 2002).

Biomagnetic nomenclature of identified poles of the magnet, it's just the opposite, Zimmerman (1970) explains. That end of the magnet that points north is labeled the south pole because it's attracted to the north pole of the earth. That end of the magnet that attracts the south pole of the earth is labeled the north pole of the magnet because opposites attract.

The end of the magnet that attracts the arrowhead of the compass needle is the biomagnetic north pole. "Another controversy revolves around the issue when to use the north, or negative, pole and when to use the south, or positive (Peter and Greg, 2002).

[illegible]3 The effect of [illegible]etic field on l[illegible]

The earliest studies on the influence of electromagnetism on organisms date back to the late 19th century, probably beginning in St. Petersburg (Zhadin, 2001). A larger, more general interest arose only some decades later, coinciding with worldwide electrification and telecommunication. Although microorganisms play a major role in the global ecosystem, the number of publications covering magnetoreception in fungi, protists and non-magnetotactic bacteria is small compared to similar reports on humans and animals (Alexander,1962; Bernhardt, 1979; Gould, 1998; Johnsen and Lohmann, 2005; Wiltschko and Wiltschko, 1995); and is perhaps comparable to the state of knowledge in plants (Galland and Pazur, 2005). The magneto orientation of magnetotactic bacteria (Blakemore, 1982), as well as that of migrating birds and insects, belongs to the best understood and most

intensely studied phenomena of magnetoreception (Wiltschko and Wiltschko,1995; Wiltschko and Wiltschko, 2005; Lohmann and Johnsen, 2000). Recently Ritz *et al.* (Zhadin, 2001and Ritz *et al.*, 2004) suggested a light-driven, radical-pair mechanism for the magnetoreception of birds mediated by cryptochrome (Molle *et al.*, 2004 and Mouritsen *et al.*, 2004). There was evidence that even plant cryptochromes are involved in the magnetoreception of Arabidopsis (Ahmad *et al.*, 2007). Bacterial magnetotaxis is based on the magnetoorientation of magnetite crystals; thus representing the only magnetoreception mechanism completely elucidated up to now (Blakemore, 1982; Blakemore, 1975; Walker, 1997 and Schuler, 2004).

Magnetotaxis is defined as movement parallel to the field lines of an external magnetic field. Nevertheless it is not a taxis in the strictest se[illegible]e as the o[illegible]isms are not following the direction of the magn[illegible]tic field itself, but rather utilize the directional information to support other ori[illegible] mechanisms. It was generally [illegible] the inclinat[illegible] a suitable environment within an oxygen (magneto-aerotaxis), or other chemical, gradient; thus reducing search movement in turbid surroundings to just one dimension - up and down (Frankel *et al.*, 1997). The key benefit of magnetotaxis in this process was the enhancement of the bacterium's ability to detect oxygen, not an increase in average speed of reaction (Smith *et al.*, 2006). Movement along a straight path allows for earlier detection of an existing oxigen gradient, and thus enhances the flight from oxygen. A role for magnetosome formation in mediating was responsed to gravity, as magnetosomes and magnetotaxis were shown to be completely absent in prolonged microgravity (Urban, 2000).

In magnetotaxis, polar and axial magnetotactic strains can be discriminated between. Bipolar flagellated cells display axial behavior by swimming back and

forth within a local applied magnetics field. In polar magnetotaxis, the cells follow a preferential direction and swim away when the local field is reversed (Bazylinski and Frankel, 2004). This classification apparently results from cellular morphology, and has no impact on orientation efficiencies in natural environments. The observation that polar magnetotactic cells in the southern hemisphere predominantly exhibited a south-seeking behavior in laboratory tests was taken as support for the importance of the magnetic field line for magnetotaxis (Blakemore *et al.*, 1980). The discovery of seasonally occurring, predominantly south seeking polar bacteria, in populations from the northern hemisphere call this explanation into question. Instead, the oxidation-reduction potential at any given position of a water column seems to influence the polarity of movement (Simmons *et al.*, 2004 ; Simmons *et al.*, 2006).

All magnetotactic cells contain magnetosomes. These organelles consist of a ferrimagnetic crystal surrounded by a [illegible] [illegible] crystals result from the [illegible] magnetite (Fe_3O_4), or greigite (FeS_2) (Mann *et al.*, 1990). A few morphotypes mineralize magnetite and greigite within the same cell, and even within the same crystal aggregate (Bazylinski *et al.*, 1993; Bazylinski *et al.*, 1995).

In both magnetite and greigite, crystal structures follow the spinel type, consisting of two interlocking grid systems with different numbers of grid coordinates (nodes). Magnetite, as well as greigite, contains a mixture of two- and three-valent iron, with each form occupying specific nodes. This leads to the complete extinction of the atomic magnetic dipol moments of Fe^{3+}. The magnetic properties, therefore, are solely attributed to Fe^{2+}. Each morphotype is usually associated with a particular crystalline habit of magnetite, whereas greigite crystals of different shapes may occur simultaneously (Matitashvili *et al.*, 1992 ; Schuler

and Frankel, 1999 ; Lins *et al.*, 2000 ; Daims *et al.*, 2001). Cuboid, bullet-, tooth- and drop-shaped crystals have been described (Sakaguchi *et al.*,1993 ; Lins *et al.*, 2000 ; Thornhill *et al.*, 1994 ; Matsunaga and Sakaguchi, 2000).

Besides eukaryotic microorganisms, magnetite crystals that are similar in appearance and structure to those of bacteria were also found in animal cells ; however no information exists on their origin and biosynthesis. Ferrimagnetic crystals interact in excess of a million times more strongly with magnetic fields than do diamagnetic or paramagnetic materials. If a ferromagnetic nano crystal were fixed to an ion channel an assumption that has not been verified yet - it would generate torque in a weak geomagnetic field that would suffice to alter ion movement across a membrane. Such considerations show that magnetites hold, at least in theory, the potential to directly influence ion transport (Kirschvink, 2005). It has also been pointed out that [illegible] of magnetite may be [illegible] sufficient [illegible] thermal background noise (Kobayashi *et al.*, 1995). Magnetite particles can have dramatic effects on the dynamics of photogenerated free radicals (Scaiano *et al.*, 1997).

For many years, scientists believed that electromagnetic fields (EMFs) of low frequency did not cause any significant biological effects. In recent decades, many scientific studies have verified that electric and/or magnetic fields of extremely low frequency (ELF; <300 Hz) can influence the biological systems.(Muraji *et al.*, 1991, 1992, 1998) reported on the influence of an alternating magnetic field on the growth of the primary root of corn and *Zea mays* seedlings. (Moore, 1979) reported that the stimulation or inhibition of the growth of five bacterial species and yeasts was dependent on the field strength, frequency and types of bacterium. The

stimulation or inhibition of microbial growth was also reported by other authors (Ramon *et al.*, 1987; Crombie *et al.*, 1990; Ivanova *et al.*, 1996; Rao *et al.*, 1997; Fojt *et al.*, 2004). (May *et al.*, 2009) reported the effect of exposure to a static magnetic field on cell growth, viability, and gene expression of *Salmonella enterica*.

The influencing of ELF magnetic fields on the transport of $Ca^{(2+)}$ in a biological system was consisted of highly purified plasma membrane vesicles (Baure´us Koch *et al.*, 2003). (Miyakoshi *et al.*, 2000) reported the effects of ELF magnetic fields on heat-induced expression of heat shock protein 70 (hsp70) at 50mT and 60 Hz in HL60RG cells.

time dependence" of *Escherichia coli*, a parameter that reflects the status of DNA-protein complexes (Binhi, 2001). Interestingly, dose-response curves for this effect showed several minima and maxima. These observations were explained using the framework of the ion interference mechanism, and were linked to the dissociation of ion-protein complexes that rotate at a speed of about 18 revolutions per second. The believed was carring for the rotating, ion-protein complexes is DNA (Binhi, 2001). *E. coli*, *Pseudomonas* and *Enterobacter* display, in a zero-magnetic field, modified resistance to various antibiotics (Poiata *et al.*, 2003; Creanga *et al.*, 2003). Surprisingly, even the extremely low magnetic flux densities generated by the

human body can affect bacteria, because *E. coli* and *Staphylococcus aureus* have altered functional activities (Lekhtlaan-Tynisson *et al.*, 2004).

For fungi and protists no studies on the effect of zero magnetic fields are presently available. Geomagnetic storms can lead to a small increase in geomagnetic fields by some 1−5%.This increase seems to be sufficient to prolong the photobioluminescence of photobacterium (Berzhanskaya *et al.*, 1995). In the slime mold, *Physarum polycephalum*, a weak field of 100 μT elicits a mitotic delay, and decrease of respiration (Marron *et al.*, 1978). At a magnetic flux density of 100 μT, the growth of the phytopathogenic fungi, *Alternaria alternata*, *Curvularia inaequalis* and *Fusarium oxysporum*, decreased by some 10%. At the same time the MF caused an increase of conidia formation in *A. alternata* and *C. inaequalis* by some 68–133% (Nagy and Fischl, 2004).

The exact mechanism of the effect of static magnetic field (SMF) on living organisms is still unclear. Plants cells [illegible] according to many [illegible] magnetic field (MF) and exposure period (Goodman, *et al.*, 1995; Belyavskaya, 2004). It has been reported that external magnetic fields influenced both the activation of ions and polarization of dipoles in living cells (Johnson and Guy, 1972). Response of cells under time varying magnetic fields is contingent not only on the wavelength and amplitude but also on how well the exogenous MF matched the phase of the cell's own oscillators; matched versus unmatched phase gives opposite results (Kindzelskii and Petty, 1997).

The forces induced by magnetic fields may be large enough to affect any process that can change the movement rate of electrons significantly (Goodman and Blank, 2002). Studies on the meristematic cells of plants shown that MF effects

normal metabolisms and has impact on cellular division (Belyavskaya *et al.*, 1992). An optimal external electromagnetic field could accelerate the activation of plant growth, especially seed germination (Morar *et al.*, 1999 ; Moon and Chung, 2000 ; Aladjadjiyan and Ylieva, 2003).

1.2.4 The effect of magnetic field on growth and cell division

Very strong magnetic fields (5.2 − 6.1 T) are able to delay cell death in stationary cultures of *Bacillus subtilis* (Nakamura *et al.*, 1997). A field of (14.1 T) had, however, no substantial effect on the growth of *Shewanella oneidensis*, even though several genes were up- or down regulated (Gao *et al.*, 2005). The latter result shows that growth can be highly inappropriate for evaluating the magnetosensitivity of an organism. Moderate static magnetic field (0.4 – 1 mT) stimulated, both in liquid and solid media, the growth and metabolism of *Pseudomonas fluorescens*, *Staphylococcus albus* and *Aspergillus niger* (Makarevich, 2000). In contrast, three species

weak static field (400 μT, i.e. about 8 times the geomagnetic field) elicits, in *Saccharomyces cerevisiae*, a 30% inhibition of bud formation (Kimball, 1998). Colonial growth of *A. alternata* and *C. inaequalis* decreased by a mere 10% during exposure to weak magnetic fields between (0.1 and 1 mT) (Nagy, 2005). An inhibition of growth was reported for *Anabaena doliolum* for a moderate DC field of 300 mT (Singh *et al.*, 1994). Numerous investigators have reported magnetic effects on development of bacteria, which includes an increase in mass and / or cell division. *Escherichia coli*, for example, when exposed to an AC field (0−2 mT, 16 and 50 Hz), shows a shortened generation time (Aarholt *et al.*, 1981). The dose-response relationships for this effect were complex, they occurred only at certain flux densities between 0 and 2 mT. AC fields (0.8, 2.5 mT, 0.8 and 1 kHz) and

increased the growth of *B. subtilis*, as it caused a growth increase and interestingly also a loss of intercellular cohesion, which is characteristic for cells raised in a geomagnetic field (Ramon *et al.*, 1987).

Whether or not an AC magnetic field exerts an inhibitory or else a stimulatory mode of action depends in a complex manner on the frequency and the field strength. For example, Moore (Moore , 1979) observed elevated or even diminished growth rates for *B. subtilis*, *Candida albicans*, *Halobacterium*, *Salmonella typhimurium*, and *Staphylococci* in dependence of AC frequencies ranging from (0 - 0.3 Hz) and magnetic flux densities of (5−90 mT). In contrast, magnetic square wave signals (0.05–1 mT, 50 Hz) had no effect on the growth of *E.coli* (Del Re, 2004). The viability of *E. coli*, *Leclercia adecarboxylata* and *Staphylococcus aureas* was negatively affected by prolonged exposures to AC fields (1 mT, 50 Hz) (Foit *et al.*, 2004).

In *Tetrahymena pyriformis* AC fields [illegible] a response that was Ca^{2+} specific [illegible] Ca^{2+} blocker. The magnetic treatment also caused alterations in membrane fluidity (Dilnel *et al.*, 1985). *Physarum polycephalum* responds to weak AC fields (0.2 mT, 60, 75 Hz) with a delay in its mitotic cycle (Marron, 1978 ; Marron *et al.*, 1975), exhibited by an increased mitotic cycle length at 0.2 mT and 75 Hz (Goodman *et al.*, 1976 ; Goodman *et al.*, 1979).

The mechanism for magnetotactic effects at ELF-frequencies (e.g. 50, 60, or 75 Hz) is not completely clear, however energy conversion to heat can likely be ruled out because of the low induction of living matter. Conversely higher frequency, long wave band fields (160 mT, 62 kHz) are in fact lethal for *E. coli* (Greenebaum *et al.*, 1982). After an exposure time of 16 h only a small fraction

(10^{-4} organisms) survive. Under these conditions, the dissipation to heat is likely not to be increasingly negligible, and, in general, these results are not comparable with findings for the ELF band in any case (Li *et al.*, 2004).

The dimorphic fungus Mycotyphaafricana can exist in a myceliar or yeast-like form. Weak ELF magnetic fields shift development towards the yeast form (Wittekind *et al.*, 1990). Weak AC fields (0 − 1.2n T, 0.8 − 50 Hz) further increase this germination rate (Broers *et al.*, 2002). Very strong DC fields (5.2−6.1 T) suppress spore formation from vegetative cells of *B. subtilis*, an effect that was paralleled with the diminished activity of alkaline phosphatase (Nakamura *et al.*, 1997).

AC fields (14.6 mT, 60 Hz) can provide protection for *S. typhimurium* from heat stress (Williams *et al.*, 2006). This observation was particularly interesting as earlier studies that magnetic field exposure can induce the heat shock protein HSP70 in eucaryotic cells (Goodman and Bla[illegible] [illegible] effects on the metabolic rates of [illegible] when exposed to an AC field (0.5 μT, 100−200 Hz), responded with a 30% reduction in respiration (Russel and Webb, 1981). *Corynebacterium glutamicum* increases ATP levels by about 30% in an AC field (4.9 mT, 50 Hz) (Lei & Berg, 1998). In the *Cyanobacterium*, *Spirulina platensis*, a DC field of moderate strength (10 mT) enhanced growth, O_2 evolution, and pigment synthesis; at 70 mT however, a repression, rather than stimulation, was observed (Hirano *et al.*, 1998). AC fields (0.1 mT, 60 Hz) caused lower ATP levels in *Physarum polycephalum*, but no decreased in respiration (Marron *et al.*,1986). Reduced respiration was, however, found with 0.2 mT and 60 and 75 Hz (Marron *et al.*, 1975). *Tetrahymena pyriformis* responds to an AC field (10 mT, 60 Hz) with delayed cell division and increased oxygen uptake (Tabrah *et al.*, 1978).

1.2.5 The effect of magnetic field on enzyme activity

Several studies have been carried out to investigate the effects of ELF magnetic fields on DNA (Ivancsits *et al*., 2002 ; Nikolai *et al*., 2004), enzyme activity (Blank *et al*., 1995 ; Blank and Soo, 1998) and cells (Chang *et al*., 2005 and Jin *et al*., 1997). Enzymes play a vital role in the biological processes, also cell communication is facilitated by these biocatalysts. Any alteration in the activity of the enzyme may affect these biological processes. The α-amylases are calcium metalloenzymes completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, α-amylase tends to be faster acting than β-amylase. In animals, it is a major digestive enzyme.

A number of studies have shown th[illegible] functions such as growth, cell membrane c[illegible] biosynthesis, enzyme activity, cell reproduction and cellular metabolism but the interaction of such fields with the living cells is still unclear (Tenforde 1996 ; Atak *et al*., 2003).

The biochemically versatile fungus *A. niger* produces a wide array of acids and derivative enzymes to support its absorptive lifestyle. This metabolic diversity and its ability to use a large amount of different carbon sources make *A. niger* a valuable cell factory for applications in many different industrial processes (Bennett, 1997).

1.2.6 Effect of magnetic field on DNA

Weak, static magnetic fields (0−110 μT) affect DNA-protein conformations in *E. coli*. This analysis represents the only dose-response curve for a static magnetic field. The peculiarity of this curve stems from the fact that it has three prominent maxima, a feature that makes it very different from other dose-response curves in nature that often follow rising or decaying exponential functions. The shape of this curve was explained in the context of the ion interference mechanism (Binhi *et al*., 2001).

AC fields (14.6 mT, 60 Hz) have been shown not to cause DNA breaks in a *Salmonella* test system (Williams *et al*., 2006). Various strains of *E. coli*, including DNA-repair mutants, showed no evidence of increased DNA damage when exposed to very strong magnetic fields (0.5 and 3 T) (Mahdi *et al*., 1994).

The transposition frequency of Tn10 in [illegible] enhanced by pulsed square-wave AC fields (Del Re et al., 2004), but is [illegible] Re *et al*., 2003). Increased transposition activity was also obtained for Tn5, after the exposure of *E. coli* to AC magnetic fields (1.2 mT, 50 Hz). Concomitantly, DNA repair was enhanced (Chow and Tung, 2000b), an event that was seemingly mediated by the over expression of DnaK/J (Chow and Tung, 2000a).

AC fields (0.2 mT, 60 Hz) can increase in *S. typhimurium*, azide-induced revertants (Tabrah *et al*., 1994). The enhanced DNA repair of hydroxylamine-mutagenized plasmid pUC8 occurred in *E. coli* in AC magnetic fields (0 − 1.2 mT, 50 Hz) via the induction of heatshock proteins Hsp 70 and Hsp 40 (DnaK and DnaJ) (Chow & Tung, 2000b). Since it is known that DnaK can upregulate UvrA, it is understandable that magnetic field stress causes improved DNA repair. An AC magnetic field also (120 μT, 50 Hz) caused a reduction in the survival of *S.*

cerevisiae after UV irradiation, whilst sustaining no effect on cell cycle kinetics (Markkanen *et al.*, 2001).

AC MFs of moderate flux density (200 − 660 μT, 50 Hz) alter the transcription rate of the lac operon in *E. coli* (Aarholt *et al.*, 1982). Furthermore while a field strength of 300 μT suppresses transcription, a field strength of 550 μT results in a substantial increase. These antagonistic interactions have been attributed to the involvement of different ions, i.e. Ca^{2+} and Mg^{2+} competing for protein-binding sites (Binhi, 1997a and Binhi, 1998).

AC magnetic fields can induce specific sets of genes. In *E. coli* an increase in σ32 mRNA (transcription factor) was found for 1.1 mT and 60 Hz (Cairo *et al.*, 1998). Pulsed square fields (1.5 mT) elicit an increase in the α subunit of RNA polymerase, and also NusA, in *E. coli*. The protein biosynthesis was studied by gel electrophoresis. Thirty proteins were identified, which were up- or down- regulated by approximately a factor of two. Goodman *et al.* (1995) [illegible] in vitro system (Goodman *et al.*, 1993). The translation machinery itself must be magnetosensitive, and was not, for example, dependent on the existance of a biomembrane. Investigations using HeLa cells, though of human origin, generated data that was highly pertinent to the problem of magnetically induced gene expression. The weak, alternating, magnetic fields (8 and 80 μT, 60 Hz) was able to increase the transcription of mouse or human c-myc genes (Lin *et al.*, 1994). This effect was dependent on the presence of specific electromagnetic response elements located between −353 and −1257 bp relative to the promoter (Lin *et al.*, 2001). Similar response elements were also detected in the promoter region of the heat shock gene hsp70 (Goodman and Blank, 2002).

Strong magnetic fields (14.1 T) caused the transcriptional up-regulation of 21 genes, and the down-regulation of 4 genes, in *Shewanella oneidensis*, while at the same time causing no substantial alterations in growth (Gao *et al.*, 2005). No alteration in the profile of stress proteins occurred after exposing *E. coli* to AC fields (7.8 − 14 mT, 5 − 100 Hz) (Nakasono and Saiki, 2000). Furthermore, no changes in differential gene expression (microarray analysis) or protein profile (2-D gel analysis) were obtained with *S. cerevisiae* exposed to AC magnetic fields (10 − 300 mT, 50 Hz) (Nakasono *et al.*, 2003).

2. Material and Methods

2.1 Instruments and Chemicals:

2.1.1 Instruments:

The instruments used in this study are listed in the table (2-1).

Table (2-1): instruments used in this study.

Instruments	Company
Autoclave	Express (Germany)
Shaker Incubater	GLF(Germany)
Magnetic Stirrer	Stuart (England)
Electric Oven	Gallenkamp (England)
Electric Sensitive Balance	Delta Rang (Switzerland)
pH meter	Radiometer (Denmark)
Distillation Apparatus	Merck
Centrifuge	Hera
Water Bath	GLF(Germany)
Incubater	Memmert (Germany)
Cooled centrifuge	Crison (Spain)
Microscope	Olympus (Japan)
Digital camera	Mercury

2.1.2 Chemicals

Table (2-2): chemicals used in this study.

Materials	Company
Sodium acetate	Fluka (Switzerland)
Tween 80	Fluka
Sodium	Fluka
Ethanol	Fluka
Sucrose	Fluka
Maltose	Fluka
Starch	Oxoid (England)
Casein	[illegible]
[illegible]	[illegible]
KH_2PO_4	BDH
$ZnCl_2$	BDH
NH_2PO_4	BDH
NH_4NO_3	BDH
$MgSO_4.7H_2O$	BDH
$FeSO_4.7H_2O$	BDH
$CaCl_2.7H_2O$	BDH
NaCl	BDH

N-Acetylglucosamine	BDH
p-Dimethylaminobenzaldehyde	BDH
Glacial acetic acid	Koch -Light
Sodium potassium tartarate	Sigma (USA)
Sodium tungstate	Sigma
Sodium molybdate	Sigma
Phosphoric acid	Sigma
Hydrochloric acid	Sigma
Bromine	Sigma
Citric acid	Himedia (India)
Dibasic sodium phosphate	Himedia
Tris(hydroxymethyl)aminomethane	Himedia
KCl	Himedia

2.2 Culture Media

2.2.1 Ready to use media:

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

Table (2-3): Ready to be use media

Media	Company	origin

Potato Dextrose Ager	Biolife	Italy
Sabaroid Dextrose Ager		

2.2.2 Yeast Extract Sucrose medium (YES) according to Scott, *et al.*, 1998.

The medium was used to enhance the production of extra cellular secondary metabolites. It contains yeast extract, 20g/l ; Sucrose 150g/l and distilled water 1Liter.

2.3 Buffers and Reagents

All buffer solution and reagents were prepared according to (Chandra, 2003)

2.3.1 Citrate-Phosphate Buffer, pH 0.4

(a) 0.1 M Citric acid; 19.21 g/l (M.W. 192.1)

(b) 0.2 M Dibasic sodium phosphate; 35.6 g/l (dihydrate; M.W. 178.0)
or 53.6 g/l (heptahydrate; M.W. 268.0)

Mixed citric acid and sodium phosphate solutions in the proportions indicated and adjusted the final volume to 100 ml with deionized water than adjusted96584 the final pH using a sensitive pH meter.

2.3.2 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

50 ml of Tris (hydroxymethyl) aminomethane was mixed with hydrochloric acid and completed the final volume to 200 ml with deionized water than adjusted the final pH using a sensitive pH meter.

2.3.3 Phosphate Buffer:

It was prepared according to the instruction on the container of the manufacturing company (Sigma (USA)).

2.3.4 Soluble – starch solution (0.5%):

This solution prepared by dissolving 0.5 g of soluble starch in 25 ml of phosphate buffer (pH 7), the mixture was then heated on hot plate to boiling point until starch was completely dissolved, then it was let to cool at room temperature and the volume was completed to 100 ml in a volumetric flask with phosphate buffer.

2.3.5 Maltose stock solution

This solution was prepared by dissolving [illegible] distilled water and completed the volume to 100 ml.

2.3.6 Bovine Serum Albumin Stock

Protein Bovine Serum Albumin (BSA) stock solution was prepared by dissolving 10 g of BSA in 10 ml of 0.05 M phosphate buffer.

2.3.7 Dinitrosalicylic Acid Solution (DNSA)

This solution was prepared according to (Whitaker and Bernhard, 1972) by dissolving 1g of DNSA in 50 ml of distilled water. Then 20 ml of 2 M sodium hydroxide solution was added followed by adding 30 g of sodium potassium tartarate gradually until it was completely dissolved. The volume was completed to 100 ml in volumetric flask with distilled water and kept in dark bottle.

2.3.8 Folin–Ciocalteu reagent:

It was prepared by dissolving 10 g sodium tungstate and 2.5 g sodium molybdate in 70 ml water. Add 5 ml 85% phosphoric acid and 10 ml concentrated hydrochloric acid. Reflux for 5 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.

2.3.9 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.

2.3.10 N-Acetylglucosamine stock solution:

N-Acetylglucosamine (2g) was dissolved in [illegible] and completed the volume to 100ml distilled water. From this standard solution dilutions which will contain a required amount of substance per ml [illegible] standard solution.

2.3.11 p-Dimethylaminobenzaldehyde reagent:

p-Dimethylaminobenzaldehyde (2g) after two crystallisations from dilute acetic acid is dissolved in 100 ml. of glacial acetic acid containing 50 ml. of hydrochloric acid. The final solution should possess a pale yellow colour. The addition of 1 ml of water to 9 ml of the reagent should not produce an increase in the intensity of the yellow colour. Some preparations of p-dimethyl aminobenzaldehyde have been found to give yellow-coloured acetic-hydrochloric acid solutions which produce bright yellow solutions on the addition of water; these must be avoided. The reagent keeps indefinitely.

2.4 Dyes

2.4.1 Lacto phenol cotton blue:

(According to McGinnis, 1980)

- 20 ml phenol crystals
- 20 ml lactic acid
- 40 ml Glycerol
- 0.05g cotton blue stain
- 20 ml D.W

The cotton blue stain dissolved in D.W. , Phenol, lactic acid and glycerol then added mixed. Stored in 250 ml screw cap bottle.

2.2 Methods

2.2.1 Fungal species

The fungi species *penicillium chrysogenum*, *Rhizopus oryzae* and *Aspergillus niger* were obtained from department of Biotechnology, College of Science, Al-Nahrain University. While *Fusarium oxysporum* and *Alternaria alternata* were obtained from department of biology college of science, university of Baghdad. The isolated fungi were identified after growing on Potato Dextrose Agar (PDA) medium (Lacaz *et al.*, 1991) by observing the growth characteristics (colour, texture appearance and diameter

of the colonies) and microscopic (microstructure) (Baijal and Mehrotra, 1980; Bisset, 1991), . All the culture were maintained on potato dextrose agar slants, stored in refrigerator and sub-cultured regularly at an interval of three months.

2.2.2 Static magnetic field

By using special magnetic bar of thickness (1 cm and 5 cm diameter). Single field strength of 10 Gauss was measured by Gaussmeter. The experimental cultured groups were placed with the magnetic field beside. The magnetic field determination, the north and south poles and it was compared with the control cultured group.

2.2.3 The effect of magnetic field on growth of fungal species on solid media

Two solid media Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were used in the study. Two [illegible] selected fungi was placed individually in the center of the respective medium after it was cut by cork borer (sterile). The inoculated plates were incubated at 28°C for 7 days. Three replicates were maintained. The diameter of the fungal colony was measured following (Daggupati, 1988).

2.2.4 Spore suspension preparation

A slant containing potato dextrose agar (PDA) medium inoculated with fungal isolate were incubated at 28°C.Spore suspension were prepared according to Faraj method (Faraj, 1990), spore were harvested by adding 5 ml of sterilized distilled water containing 0.11% tween 80 to aid wetting and separation of the spores, then the fungal growth was separated by a loop. The suspension was filtered through sterile cotton wool; the filtrate was

centrifuged, and further washes with distilled water. The spore suspension was then centrifuged at 3000 rpm for 5 min. the supernatant was removed and the spores were washed twice by resuspending in sterile distilled water and further centrifuged. Then 5 ml of sterile distilled water was added to the supernatant and mixed vigorously by the vortex for 1 min . A drop of the suspension was added to hemocytometer by Pasteur pipette, spores were calculated under high power (X40) of light microscope using the following equation (Faraj, 1990):

Concentration of spores = $(Z * 4 * 10^6)/n$ spores/ml

Where n: total No. of small squares.

Z: total No. of spores.

2.2.5 The effect of magnetic field on growth of fungal species in liquid media

described by [illegible] following media composition in grams per liter, yeast extract-20g, sucrose-150g, KH_2PO_4-1.4g, NH_4NO_3-10g, KCl-0.5g, $MgSO_4.7H_2O$-0.1g, $CaCl_2.7H_2O$-0.4g, $FeSO_4.7H_2O$- 0.01g, Na_2HPO_4-2.5g,$ZnCl_2$-0.1g, NaCl-0.3g, Casein-20g starch-20g; pH 6.5. The media composition were dissolved in 1000ml of distilled water after which 100ml of the medium was measured into conical flask (250ml capacity each) heated on hot plate to homogenize and then sterilized by autoclaving after which they were removed and allowed to cool.

2.2.5.1 Culture conditions

Spores of the isolated fungi were harvested from 14 days old culture by preparing spore suspension of 2×10^8 spores/ml. 1 ml of the spore suspension was used for inoculating 100 ml of media. All flasks were incubated at 28°C

in a shaking incubator at 150 rpm for 4 days, and were periodically determined at every 24 h. After incubation, culture filtrate was filtered through Whatman No.1 filter paper. Supernatant obtained after 72h was used as the crude enzyme sample for further experiments. Except the *Fusarium oxysporum* were centrifuged at 3000 rpm for 10 min at 4°C in a refrigerated centrifuge before the filteration.

2.2.5.2 Biomass/growth determination of fungal species

The method of Narasimha *et al.*, (2006) was used an applied. The biomass or mycelia growth produced in the liquid culture medium was determined by dry weight measurement Whatman No.1 filter paper was dried to constant weight, and the weight noted. The content of the flask was filtered through the filter paper to separate the mycelial mat and the culture filtrate. The biomass of the culture (residue) was dried until a constant weight was obtained. The growth yield per 100 ml of broth was determined. The filtered contents were washed with water thrice [illegible] hot air oven. The dried content [illegible] the mycelium dry weight.

The biomass/growth was calculated as:

Biomass (g/100 ml) = Weight of culture +(filter paper + petri dishes)–(initial weight of filter paper + petri dishes)

2.2.6 Extraction of crude enzyme

Extraction of crude enzyme was done by centrifugation media at 2000 rpm for 5 min after 72 hrs, supernatant collected and filtered off using Whatman No.1 filter paper. The filterate was used as crude enzyme extract (Ali *et al.*, 1998; Oyeleke *et al.*, 2010).

2.2.6.1 Amylase assay

Amylase activity was assayed by measuring the reducing sugars released during the reaction, using starch as a substrate, according to Ramakrishna *et al.* (1982).

Amylase activity was assayed as described by Ramakrishna *et al.* (1982) by pipetting 0.5 ml of culture extract enzyme into test tubes and 1ml of 1% soluble starch in citrate phosphate buffer having a pH of 6.4, the reducing sugars liberated were estimated by the 3,5-dinitrosalicyclic acid (DNSA) method Bertrand *et al.*, (2004). The reaction mixture was incubated in a water bath at 40°C for 30 min. A blank consisting of 1 ml of soluble starch in citrate-phosphate buffer (pH=6.4) was also incubated in a water bath at the same temperature and time with the other test tubes. The reaction was terminated by adding 1 ml of DNSA reagent in each test tube and then [illegible] in a boiling water bath for 5 min after which they were allowed to cool and 5 ml of distilled water [illegible] the [illegible] measured at 540 nm wi[illegible]

Enzyme activity (unit/ml) is defined as the amount of enzyme which produces one micromole (µmole) in a minute under the estimation condition. While specific activity expresses the units activity per each milligram (mg) of a protein.

$$\text{Specific activity (U/mg)} = \frac{Acivity\ (U/ml)}{protein\ concentration\ (mg/ml)}$$

2.2.6.2 Assay of proteases activity

The activity of protease was assayed by the method of McDonald and Chen (1965). To 1ml of the crude filtrate in the test tube, 4.0 ml of 1.0% casein was added. The mixture was incubated at 35°C for one hour. The residual protein

was precipitated by adding 5ml of 5% TCA (Trichloroacetic acid). The precipitates were allowed to settle for 30 minutes. The contents of the tubes were centrifuged at 5000 rpm for 5 minutes. One milliliter of the supernatant was mixed with 5ml of NaOH. Then 1ml of 1N sodium hydroxide was added to make the contents of the tube alkaline. After 10 minutes, 0.5ml of Folin and Ciocalteau reagent was added as a result of which, blue color was produced. The tubes were left for 30 minutes to get maximum development of blue color. The optical density of the mixture was read at 700 nm on spectrophotometer. One unit of protease activity is defined as the amount of enzyme required to produce an increase of 0.1 in optical density under optimal defined conditions according to the following eqution:

2.2.6.3 Determination of protein concentration by Bredford method(1976).

The Bredford method (1976) was used to determine the concentration of protease followed after the preparati

samples, which occurred by adding 1ml of the tested sample to the 0.4ml of Tris-HCl and 2.5 ml of Coomasi blue Dye and vortex the tubes for 2 min. The absorbance for all the test tubes were measured at 595 nm with spectrophotometer.

Table (2-4): preparation of Bovine Serum Albumin Standard Curve:

Tube no	BSA conc. mg/ml	Buffer ml	Total vol. ml
1	0	100	100
2	0.02	98	100
3	0.04	96	100
4	0.06	94	100

5	0.08	92	100
6	0.1	90	100

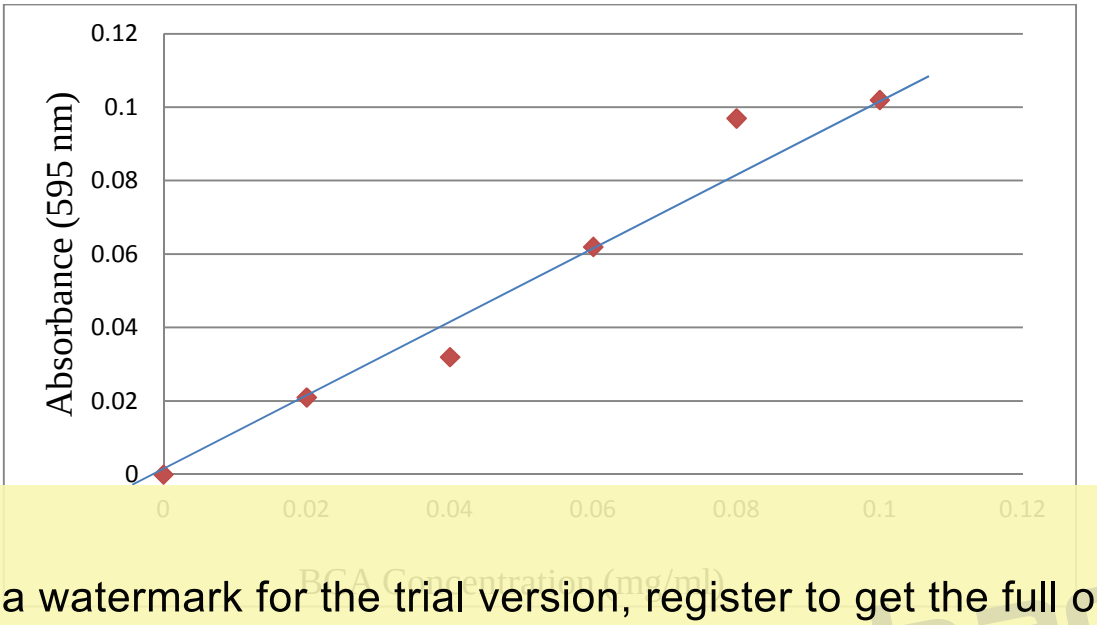


Figure (2-1): Bovine Serum Albumin Standard Curve for determination of

The reducing sugar content, following hydrolyses of substrates by the enzyme extract, was determined using the method of Bertrand *et al.* (2004). The reducing sugar content from hydrolyses of substrates by enzymes activity were assayed by adding 2 ml of 3,5 DNSA reagent to 1 ml of sample. The mixture was heated in boiling water for 5min and then cooled under running tap water. The absorbance at 540 nm of the resulting coloured solution (slight brown) was read in a spectrophotometer against a blank, prepared by substituting the hydrolyzed sample with distilled water. The reaction sugar content was subsequently determined by making reference to a standard curve of maltose concentration.

2.2.6.4.1 Maltose standard curve

Maltose standard curve was prepared by dissolving 0.2g of maltose in 20ml distilled water and completed the volume to 100ml. and as following volumes of maltose standard solution were prepared from maltose stock solution in tests tubes (triplicate for each volume). Then proper volumes of distilled water were added. One ml of DNSA was added to each tube and then immersing tubes were boiled in water bath for 5 minute after which tubes were cooled using tap water and 5 ml of distilled water was added to each tube. The absorbance was measured at 540nm. Tubes number one was used as a blank. A standard curve was plotted between the concentration of maltose and the correspondent absorbance at 540nm as follows in figure(2-2).

Tables (2-5): preparation of maltose standard curve.

Tubes No.	Maltose Sol.(ml)	Distilled Water(ml)	Maltose mg/ml
1	0	1	0
2	0.1	0.9	0.2
3	0.2	0.8	0.6
4	0.3	0.7	1.0
5	0.4	0.6	1.4
6	0.5	0.5	1.6
7	0.6	0.4	2.0
8	0.7	0.3	2.4

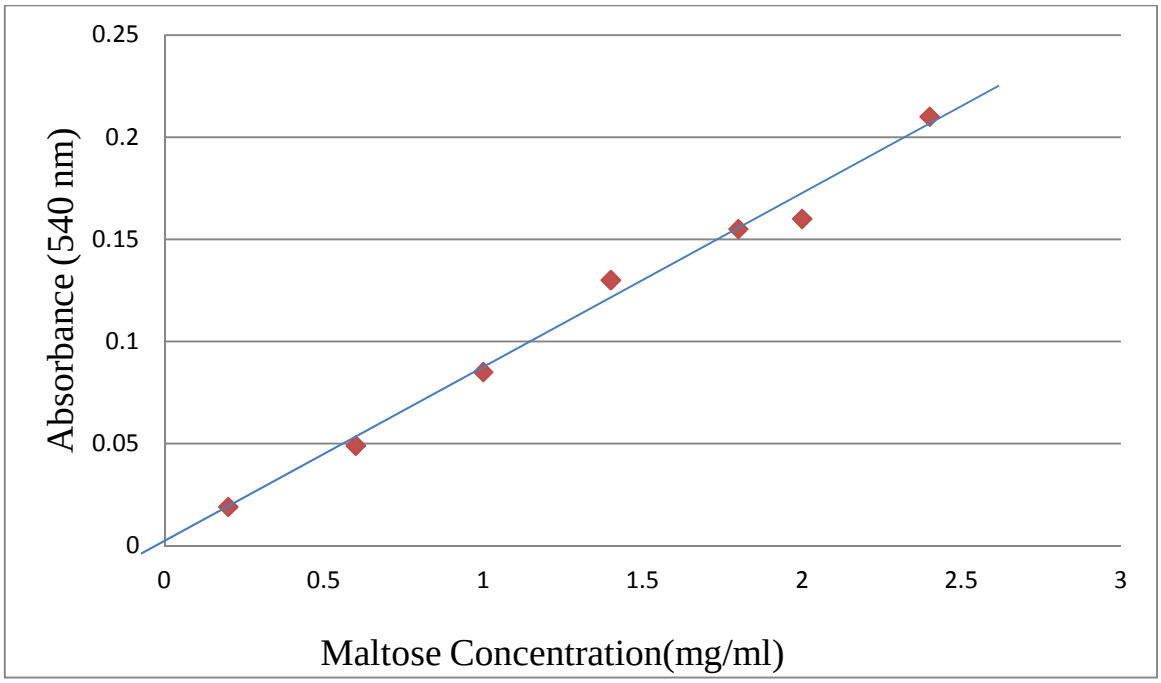


Figure (2-2): Maltose Standard Curve for the Determination of Reducing Sugars

2.2.7 Determination of chitin content in cell wall

Cell wall of *A. bisporus* mycelia was [illegible] dodecyl sulphate. Isolated cell walls were pure and devoid of cytoplasmic contamination as observed under microscope and were noted to contain less than 2% of the mycelial protein associated with them. The samples were stored at 37°C over P_2O_5 till use. Cell walls were hydrolysed with chitinase essentially as per the method of Jeauniaux (1966), except for the use of an acetate buffer in place of the recommended citrate buffer. The sample (1mg) was incubated with chitinase (1 unit) in a total volume of 4ml of acetate buffer (0·4 M, pH 5·6) and digested for 24 hr, at 37°C with constant stirring of the incubation mixture. Aliquots (0·5 ml) were withdrawn at different time intervals for determining the contents of total, free and N-acetyl glucosamines in them (Davidson, 1966). The aliquot, containing up to 0·5 µmol of glucosamine, was treated with 0·1 ml of 1% (v/v) acetic anhydride and 0·5

ml of potassium tetraborate (0·7 M, pH 9·1) in a Teflon-lined screw capped tube for 5 min and then heated in a boiling water bath for 5 min. The solution was cooled to room temperature, mixed with 6 ml of p-dimethyl aminobenzaldehyde (1% (w/v) in 10% (v/v) glacial acetic acid) and incubated at 37°C for 30 min. The absorbance of the solution was measured at 585 nm. Sample values were read from the standard curve of N-acetylglucosamine as followed in tabled (2-6).

Table (2-6): preparation of N-acetylglucosamine standard curve

Tube no.	N-acetylglucosamine Sol. ml	D.W. ml	N-acetylglucosamine conc. (mg/ml)
1	0	1	0.00
2	0.05	0.95	0.005
3	0.10	0.90	0.01
4	0.15	0.85	0.015
5	0.20	0.80	0.02
6	0.25	0.75	0.025
7	0.30	0.70	0.03
8	0.35	0.65	0.035

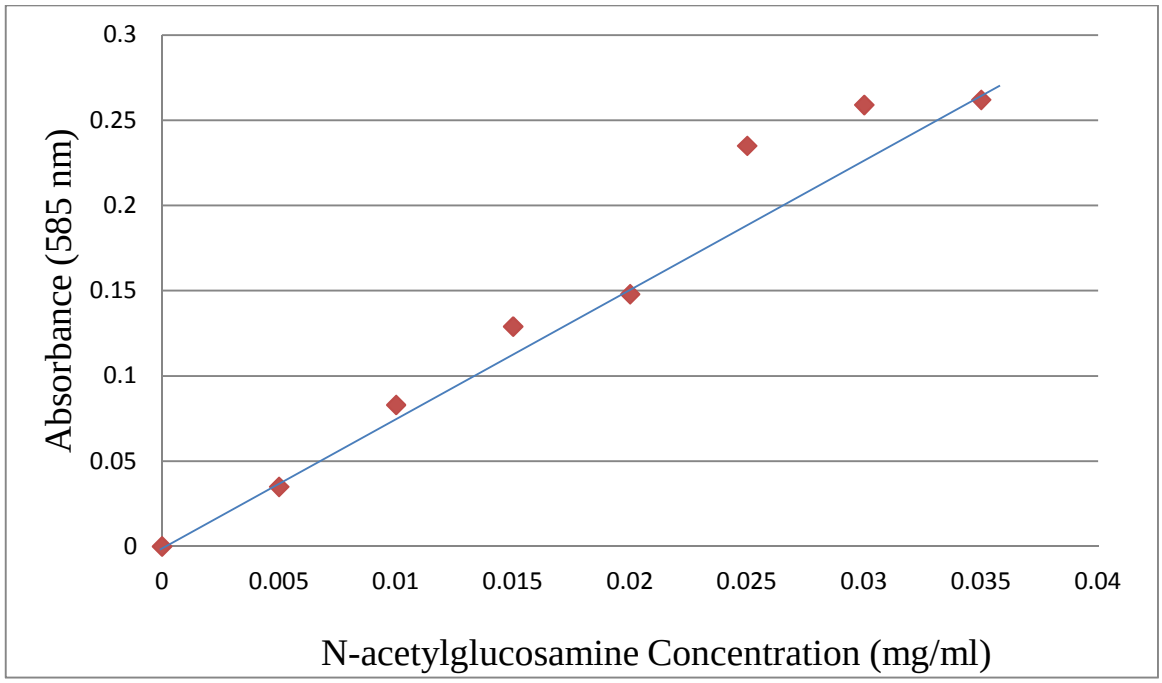


Figure (2-3): N-acetylglucosamine Standard curve for determination fungal chitin content.

Statistical analysis

The results obtained were analyzed for significance using ANOVA (analysis of variance) procedure of SPSS software (SPSS Institute,2005).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 Results and Discussion

3.1 Fungal species

The present study was undertaken to determine the effect of magnetic field on growth and biochemical indices of fungi species. Five species of different fungi were exposed to magnetic field. Based on morphological characters and microscopic observation 5 filamentous fungi were *A. niger*, *A. alternata*, *F. oxysporum*, *P. chrysogenum* and *R. oryzae*. The growth of the different species was studied morphologically on potato dextrose agar at 28°C. The identifying features of *A. niger* are wooly initially white, quickly becoming black with conidial production. Reverse side of petri dish is white to pale yellow and growth may produce radial fissures in the agar (Fröhlich *et al*., 2000). *A. alternata* in culture shows a white growth with profuse aerial mycelium which soon turned greenish grey (Mmbaga *et al*., 2005). *F. oxysporum* colonies are usually fast growing, pale or brightly colored and may have a cottony aerial mycelium. *P. chrysogenum* colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores (Suryanarayanan *et al*., 2000). *R. oryzae* quickly fills a Petri dish (agar surface) with a typically cotton candy like colony, initially white that turns grey to yellowish brown in time. The reverse is white to pale (Caiazzo *et al*., 2006).

3.2 The effect of magnetic field on growth of fungal species

The exposure of magnetic field stimulates or inhibits the growth and proliferation of microorganisms. High intensity magnetic fields can affect membrane fluidity and other properties of cells (Frankel and Liburdy, 1995).

Figure (4) and table (7) showed that the mean of *A. niger* growth diameter at 10 gauss static magnetic field, there was stimulation in fungal growth

comparing to the control group. The growth diameter with northern magnetic pole (5 cm) was more than southern magnetic pole (4.1 cm), both poles (4.5 cm) and controls (3.5 cm).

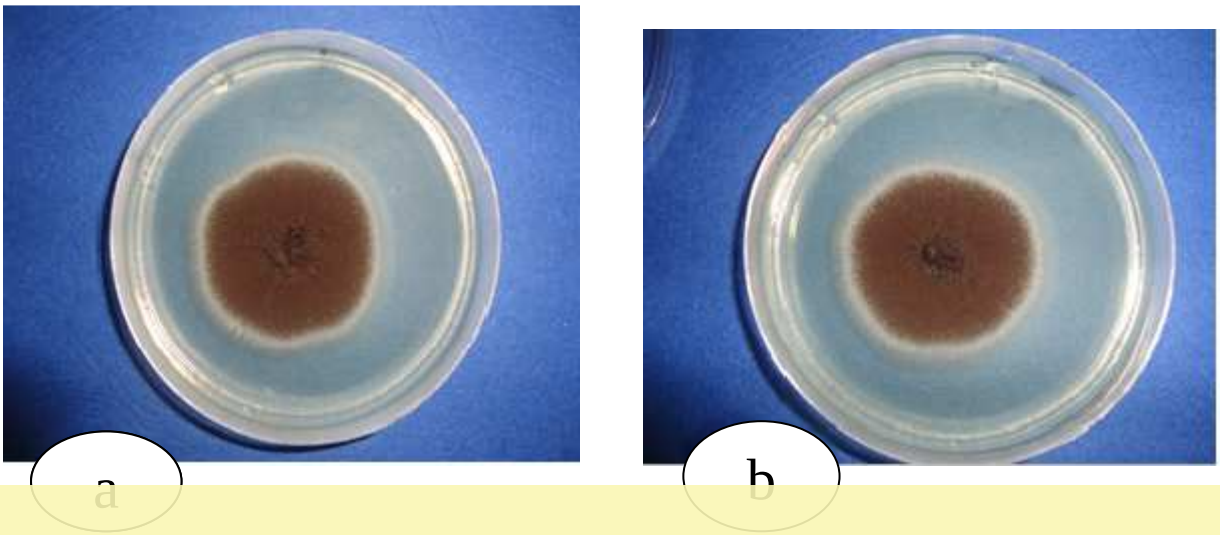


Figure (3-1) Effect of static magnetic field poles on the growth of *Aspergillus niger* on solid media. Panels, a. control, b. southern, c. northern after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Table (3-1): Effect of static magnetic field on growth rate of *Aspergillus niger* after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Effect of poles	Mean Growth diameter (cm)	LSD
Control	3.5 ± 0.88*	-
Southern	4.1 ± 0.166*	0.04
Northern	5 ± 0.145*	0.00
Both	4.5 ± 0.288*	

*The mean difference is significant at the p≤ 0.05 level.

The results obtained from the fungal growth in figure (5) and table (8) showed the relation of the growth of *A. alternata* on solid medium and affected by magnetic field, it can be ascertained that the growth diameter was increased significantly when exposed to the southern pole (5 cm), northern pole (5.1 cm) and both poles (5.3 cm) as compared with control (4 cm).

a

b

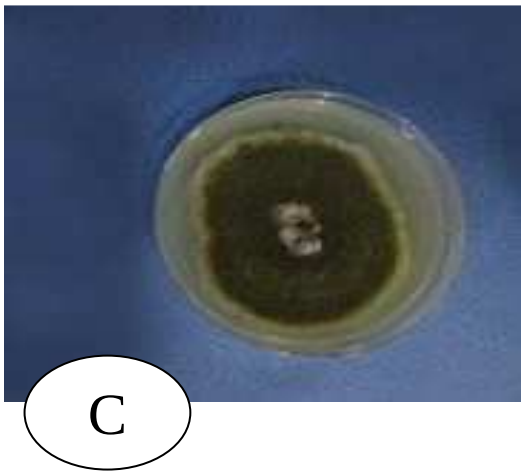


Figure (3-2) Effect of static magnetic field poles on the growth of *Alternaria alternata* on solid media. Panels, a. control, b. southern, c. northern after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Table (8): Effect of static magnetic field on growth of *Alternaria alternata* on solid media (SDA) after incubation for 7 days at 28°C.

Effect of [illegible]	Mean ± SE Growth diameter (cm)	LSD
[illegible]	[illegible]	[illegible]
Southern	5 ± 0.664*	0.00
Northern	5.1 ± 0.878*	0.00
Both	5.2 ± 0.135*	0.00

*The mean difference is significant at the p≤ 0.05 level.

From tables (3-1) and (3-2) which showed that static magnetic field has slight stimulatory effect on the growth of *A. niger* and *A. alternate*, this stimulatory effect may due to increase in the metabolic activity of the fungal cell and increase in the rate of replication of cell DNA. This result agree with Sadauskas *et al*. (1987), who examined the effect of 200 mT flux density on static and pulsating magnetic field on the different species of fungi. According to

their examination, morphological changes were observable on the conidia of *Aspergillus puniceus* and *A. alternata.*

Figure (3-3) and table (3-3) showed inhibitory effect on growth rate of F. oxysporum with 10 gauss static magnetic field. Table (8) shows significant differences in growth diameter (cm) as compared with control (4.1cm) as exposed to the southern pole (3.5), northern pole (3.7 cm) and both poles (3.5 cm) on solid medium. From these results a conclusion was made that magnetic poles inhibits the F. oxysporum replication rate and increase the metabolic activity.

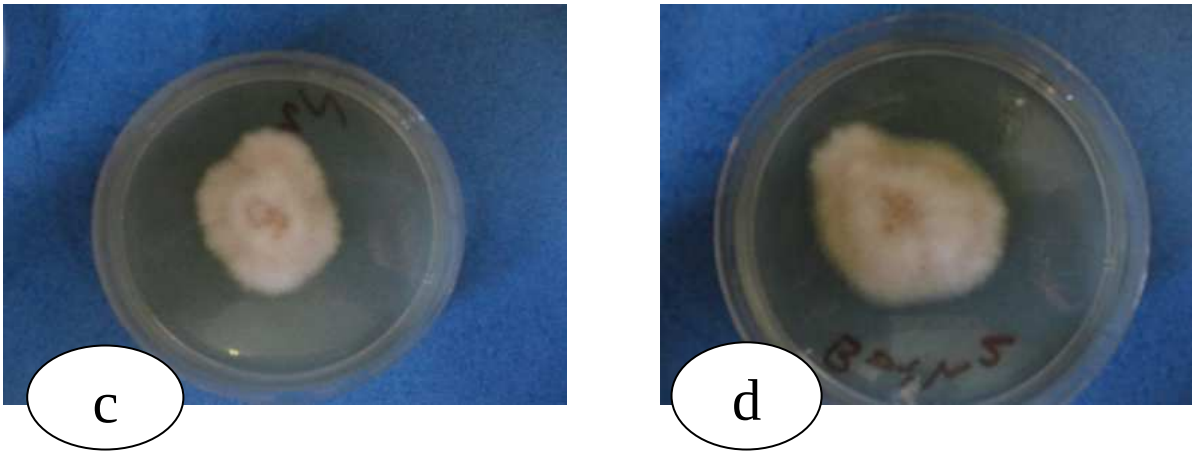


Figure (3-3) Effect of static magmatic field poles on the growth of *Fusarium oxysporum* on solid media, panels a. control, b. southern, c. northern and d. both after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Table (3-3): Effect of magnetic field on growth of *Fusarium oxysporum* after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Effect of poles	Mean Growth diameter (cm)	LSD
Control	4.1 ± 0.88*	-
Southern	3.7 ± 0.145*	0.02
Northern	3.7 ± 0.332*	0.02
Both	3.5 ± 0.115*	0.03

*The mean difference is significant at the p≤ 0.05 level.

Table (3-4): Effect of static magnetic field on growth of *Pencilliumm chrysogenum* expressed by colony diameter after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Table (3-4) showed results on the effects of static magnetic field on growth of *P. chrysogenum* on solid medium. The magnetic field accelerated growth by increasing in growth diameter (2.3, 2.1, and 2.8) cm when exposed to the southern pole, northern pole and both poles respectively and compared with the control (1.6 cm) cultured under identical conditions. The results suggest that the action of magnetic field may be an important environmental factor affecting the function of the biological clock as well as the morphology of the examined *P. chrysogenum*.

It has been demonstrated experimentally (Lednev, 2001) that the application of a low-frequency, weak magnetic field, both static and time-

varying, induce considerable changes in the metabolism of cells. These changes are manifested primarily on altered ion flow through cell membranes and in the motion of cells. Other investigated of magnetic fields include those on the activity of ion channels (Galt *et al.*, 1993) and ion transport in cells (Garcai-Sancho and Jawier, 2004).

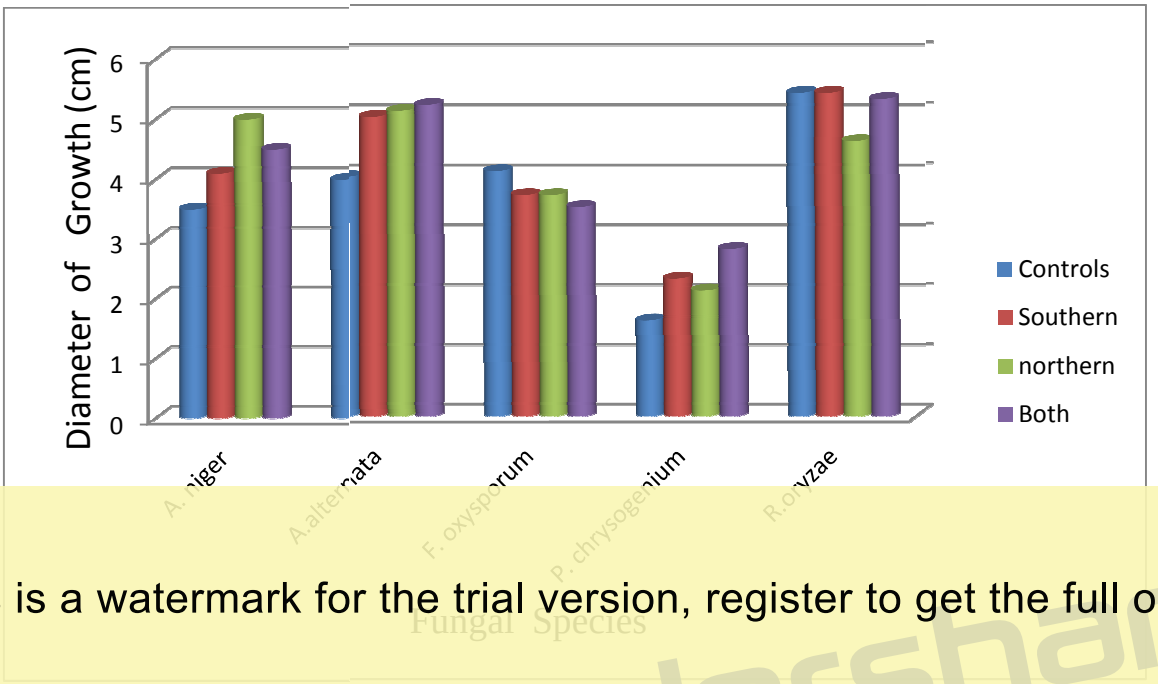
Table (3-5): Effect of static magnetic field on growth of *Rhizopus oryzae* after incubation growth (SDA) or (PDA) medium under magnetic field for 7 days at 28°C.

Effect of poles	Mean Growth diameter (cm)	LSD
Control	5.4 ± 0.664*	-
Southern	5.7 ± 0.208	0.89
Northern	4.6 ± 0.202*	0.01
Both	5.7 ± 0.202	

magnetic field as it polarized and depolarized with each cycle of the current which lead to same effect on magnetic element or minerals in the cell according to Domain theory (Colin, 2008) and this may have effect on cell metabolism and replication, which agreed with (Kate Melville, 2006 ; Bokkon, 2008).

From the mentioned results in figure (3-4) the five fungal species when exposed to static magnetic field (southern pole, northern pole and both poles) were stimulate the growth of *A. niger, A. alternate* and *P. chrysogenum* and inhibited the growth of *F.oxysporum*, but has no effect on southern pole and both poles on growth of *R.oryzae* and inhibited growth of *R. oryzae* when exposed to the northern pole as compared with control group. This indicates that the polarity

has significantly affect the interrelationship between magnetic field and microorganisms and indicates that the magnetic field effectively influenced the formation of conidia of the examined fungal species.



3.3 The effect of magnetic field on growth of fungal species in liquid media

The basic concept of magnetic field assisted living organisms was to force or decelerate their growth and biomass production in liquid media. (Yoshimura, 1989) classified the effects of magnetic fields on microbial growth and reproduction as (1) inhibitory, (2) stimulatory and (3) none observable.

Results in figure (3-5) showed increasing in biomass of *A. niger*. when exposed to the southern and northern poles magnetic field as compared with controls. The main theories that try to explain the biological effects of electromagnetic field 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 on the organism.

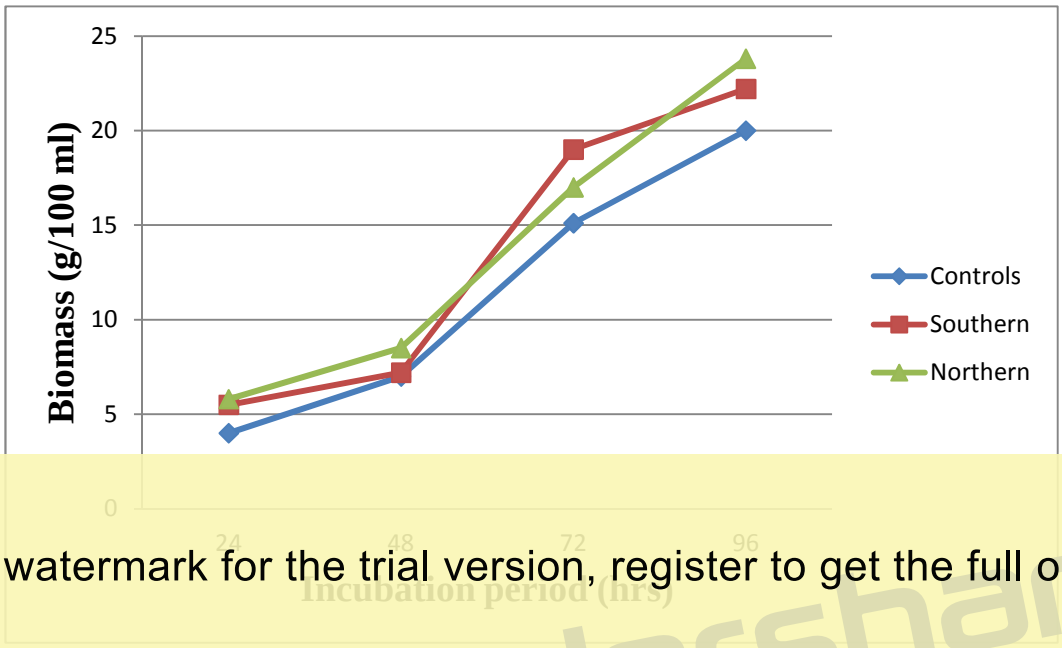


Figure (3-5) Effect of static magnetic field on growth of *Aspergillus niger* in liquid medium after incubation period (hrs)

Figure (3-6) displays the growth of *A. allternata*. in liquid medium (g) it was decreased and the biomass production due to the effect of southern and northern poles of magnetic field as compared with controls. These results indicated that the magnetic field decelerated growth rate of *A. alternate* by effect on the growth of mycelcia.

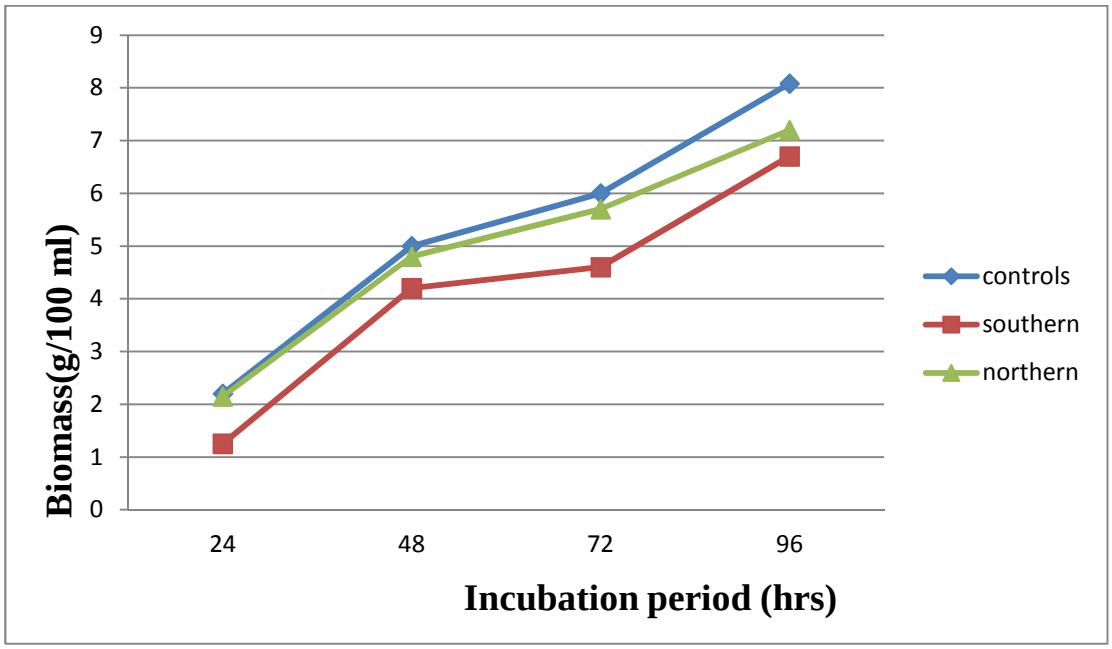


Figure (3-6) Effect of magnetic field on growth of *Alternata alternata* in liquid medium after incubation growth (YSE) medium under magnetic

Figure (3-7) represent the effect of magnetic field on growth of *F. oxysporum*. As compared with control which indicates a decrease in the growth [illegible]. These results [illegible] in her study on exposure of the fungus *F. oxysporum.* to a static magnetic field of 0.3T inhibited mycelia growth and was accompanied by morphological and biochemical changes. Germination of asexual fungal spores and cell viability were also reduced. The effect appeared to be through calcium-dependent signal transduction pathway. Perturbation of these pathway by adding different compounds (ie calcium chloride, porbal 12-myristate 13-acetate, neomycin and lithium chloride) to the medium, suggested that exposed asexual spores were unable to mobilize calcium from intracellular stores.

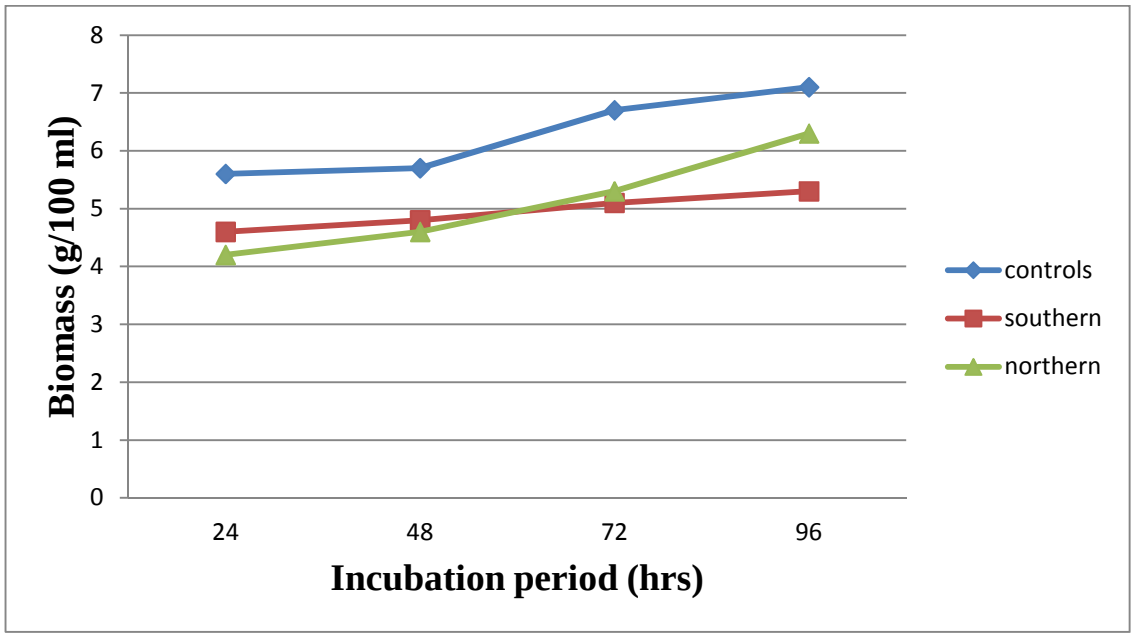


Figure (3-7) Magnetic field effect on growth of *Fusarium oxysporum* in liquid medium after incubation growth (YSE) medium under magnetic field every 24hrs for 96 hrs at 28°C, pH 6.5 and 150 rpm.

Figure (3-8) illustrates that growth rate was increased in the examined *P. chrysogenum* exposed to a magnetic field after 96 hrs, this increase were related to the effect of magnetic field on growth by inducing the positive and negative poles as compared with the controls (without magnetic field).

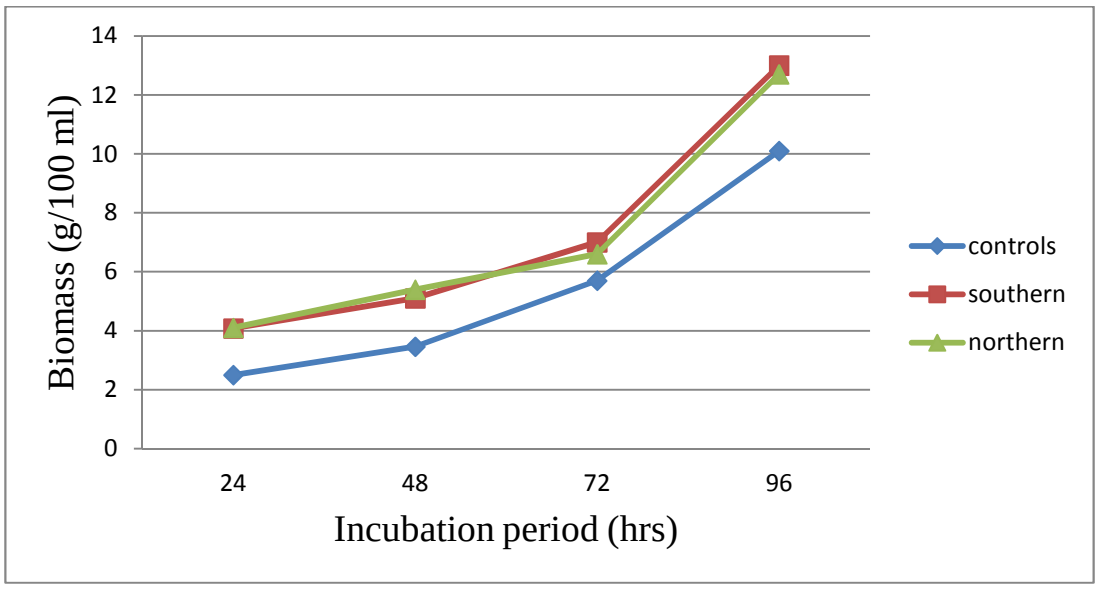


Figure (3-8) Magnetic field effect on growth of *Penicillium chrysogenum* in liquid medium after incubation growth (YSE) medium under magnetic field every 24hrs for 96 hrs at 28°C, pH 6.5 and 150 rpm.

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 had addressed growth and morphogenesis (Celestino *et al.*, 1998).

Figure (3-9) shows the effect of the magnetic field on the biomass production of *R. oryzae* growth. The static magnetic field inhibited the growth rate may be due to the larger surface area of *R. oryzae*. biomass for exposure, as mycelium of *R. oryzae* in the form of suspended growth, As sexual reproduction produce two type of sporangiophores (+,-) and the magnetic field imposed

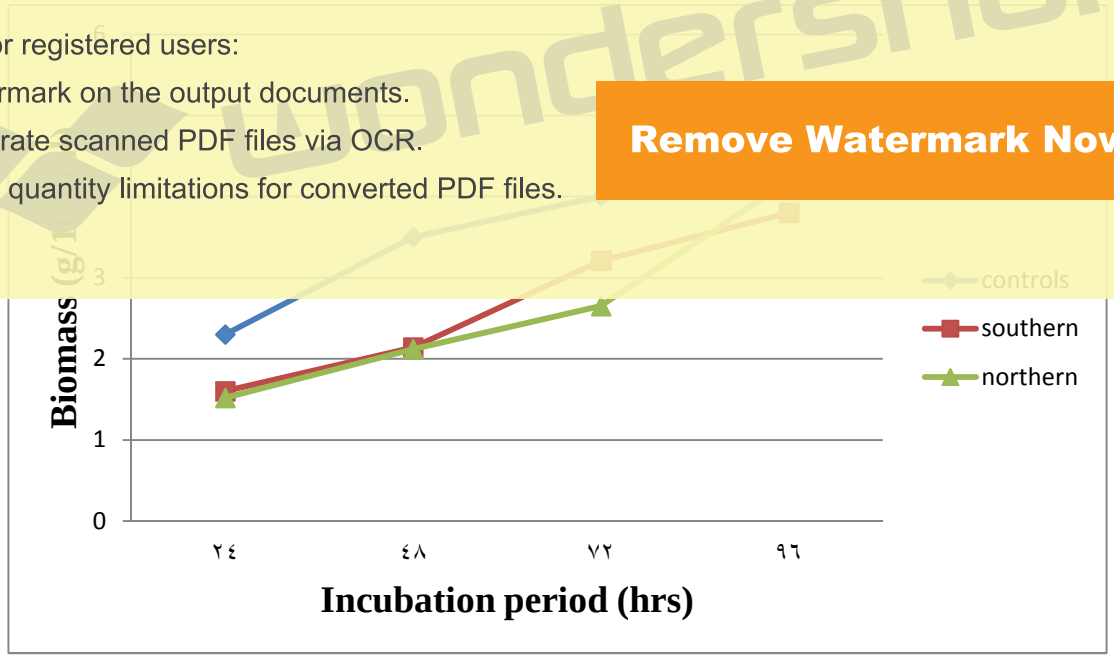


Figure (3-9) Magnetic field effect on growth of *Rhizopus oryzae* in liquid medium after incubation growth (YSE) medium under magnetic field every 24hrs for 96 hrs at 28°C, pH 6.5 and 150 rpm.

3.4 Effect of magnetic field on enzyme activity

The impact of the magnetic field on the enzymes is concentrated on changing the charge and the shape of the active site of enzymes and not on the substrate, so when exposing the substrate alone for a week to magnetic field energy we do not notice a change in the activity of the enzymes, but when we develop and encourage the organism to produce the enzymes under effect of magnetic field be a marked change in the enzymatic activity and difference between the two northern and southern Poles .

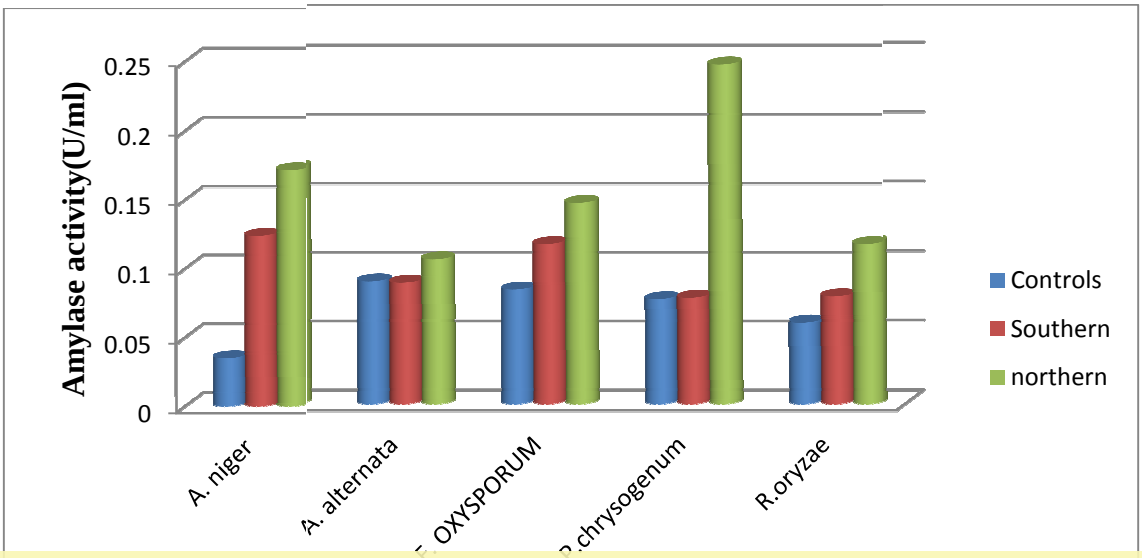
The changes of Amylase and protease activity and specific activity of enzymes at northern pole and southern pole were detected and results are presented in figures (3-(10.11,12,13)) respectively and can be discussed by the rotating electric field formed by the variable magnetic field. And the last one was the main responsible reason 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

3.4.1 Effect of magnetic field on amylase activity

Amylase enzyme had a very important role in the hydrolysis of starch to sugars which provide energy for growth (Dehghanpour *et al.*, 2011). Amylase (endo 1 and 4 -D-Glucagon Gludehydrolase) is found in all living organisms. α-Amylase catalyzes the endo-hydrolysis of 1,4- alpha-D-gylcosidic linkages in polysaccharides containing 3 or more 1,4- α-linked glucose units (Demirkan, 2011).

Figure (3-10) and (3-11) represent the effect of magnetic field on activity and specific activity of amylase of fungal genera growth at optimal pH (6.5) and temperature 28°C. The northern pole increased amylase activity of all fungal

species higher than when exposed fungal species to the southern poles and the two poles increased activity higher than controls(without MF).



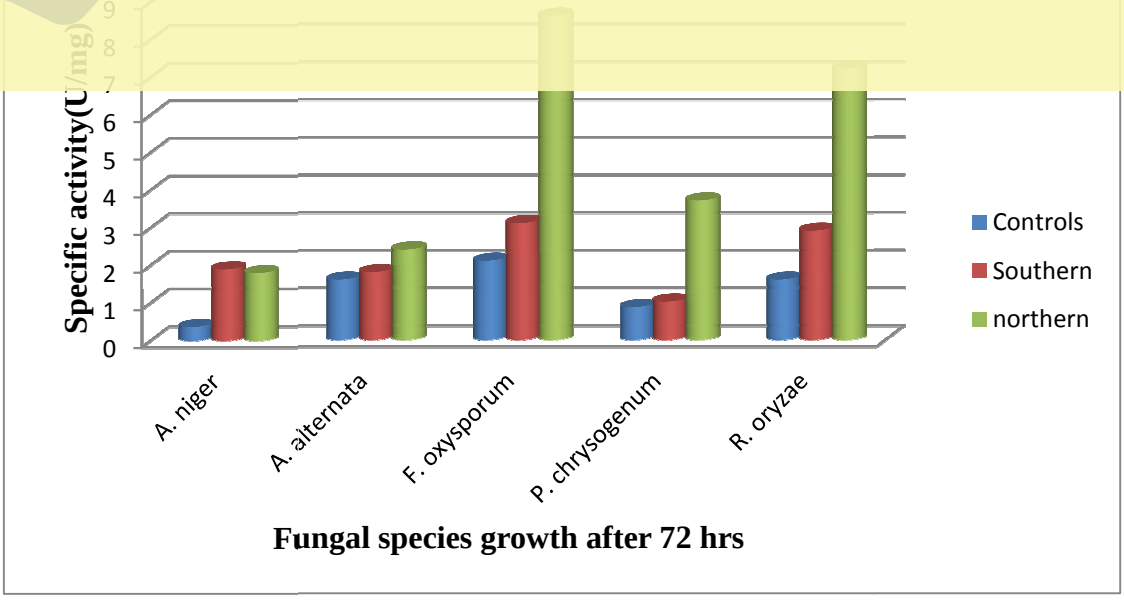


Figure (3-11): Effect of magnetic field on Amylase specific activity for fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C, pH 6.5 and 150 rpm.

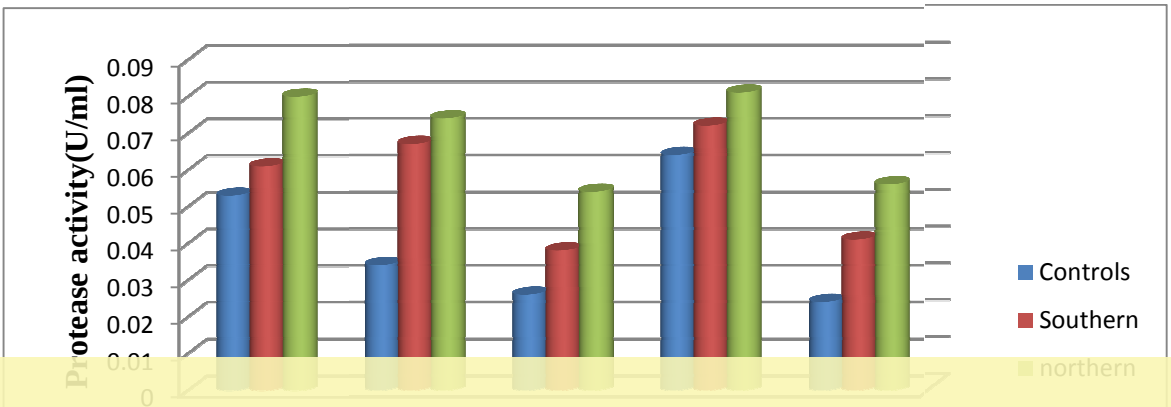
The northern pole increased amylase activity (U/ml) in the culture filterate of *P. chrysogenum* (0.246 U/ml) higher than other mentioned species, *A. niger*, *F. oxysporum*, *R. oryzae* and *A. alternata* (0.172, 0.146, 0.116, 0.105)U/ml respectively and decrease when treated with southern pole however it was higher than the control treatment.

The action of magnetic field on enzyme activity seems to switch the enzyme to a state of an increase in activity. 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. Inspection whether ELF, EMF substantially altered the optimal pH and temperature. However, there was a change in OD values when the samples were exposed to EMF at different pH and temperature which indicates there was alteration in the enzyme activity. But there was no significant differences between ELF, EMF at optimal pH and temperature. This [illegible] a [illegible] increase of α-[illegible] considering the Ca^{2+} ions present in the enzyme structure, which mediate the EMF action at the cell level (Karabakhtsian *et al.*, 1994).

3.4.2 Effect of magnetic field on protease activity

Fungi elaborate a wide variety of enzymes than do bacteria and proteases are among the most important enzymes produced by fungi. Fungi produce a variety of proteolytic enzymes however, most of these are usually acidic in nature (Fernandez-Lahore, *et at.*, 1998). The filamentous fungi have been a potential to grow under varying environmental conditions such as time course, pH and temperature, utilizing a wide variety of substrates as nutrients and to produce proteases (Tremacoldi and Eleonora, 2005).

Figure (3-12) and (3-13) represent the effect of magnetic field on activity and specific activity of protease of fungal genera growth at optimal pH (6.5) and temperature 28°C. The northern pole increased protease activity of all fungal species higher than when exposed fungal species to the southern poles and the two poles increased activity higher than controls.



fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C, pH 6.5 and 150 rpm.

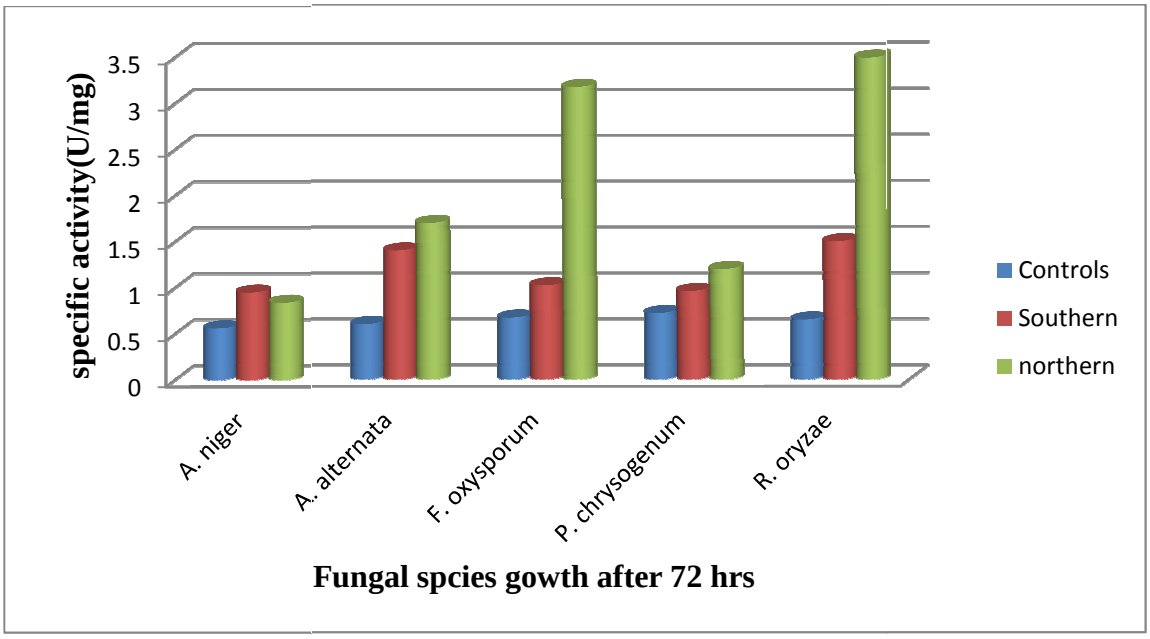


Figure (3-13): Effect of magnetic field on protease specific Activity for fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C, pH 6.5 and 150 rpm.

The northern pole increased protease activity (U/ml) in the culture filterate of *P. chrysogenum* (0.081 U/ml) higher than other mentioned species, *A. niger*, *A. alternata*, *R. oryzae* and *F. oxysporum* (0.08, 0.074, 0.056,0.054) U/ml respectively and decreased when treated with southern pole however it was higher than the control treatment.

Activity of the enzyme in culture is greatly dependent 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.

The mechanism of MF action in biological systems can be examined by its [illegible] and enzyme [illegible] (or decreasing) transcript levels. However, MF also interacts directly with electrons in DNA to affect protein biosynthesis (Goodman and Blank, 2002).

The theory stated that a "weak" OMF could loosen the bonds between ions and proteins. Many proteins vital to the cell metabolism contain ions. In the presence of a steady background magnetic field such as that of the earth, the biological effects of OMF are more pronounced around particular frequencies, the cyclotron resonance frequency of ions (Coughlan and Hall 1990).

The simplest explanation of Blank and Soo (1998) concerning the close link between effects of electric and magnetic fields and enzyme activity is based on the mobile charge interaction model. The model suggests magnetic field interaction with moving charges, and hence the basal enzyme activity as the

result of the field interaction with charge movements during enzyme function. Blank and Soo (1998) also referred to probable effects of Lorentz forces that clearly indicate the complete misunderstanding in the interpretation about the observed phenomena, because the field was somewhat weak to create any effective ponder motive forces.

The macroscopic ordering displayed in living systems is an "emergent" property arising from a collective behavior of the elementary microscopic components (Fröhlich and Kremer, 1983). The low-frequency internal motions in protein molecules play a key role in biological functions where it was suggested that there was a direct relationship between low-frequency motions and enzymatic activity (Merlino and Sica, 2007). The symmetry of protein molecules is also a very important factor in understanding its structure and functions, which depends on stability, number of subunits and their efficiencies that limits the functionality of the protein. The functionality requirement of symmetry has mainly guided the evolution of proteins to [illegible] point group

3.5 Effect of magnetic field on reducing sugar:

The effect of Magmatic field energy on reduced carbohydrates was revealed in figure (3-14) by Dinitrosalicylic colorimetric method.

Depending on the results in figure (3-14) we note that the concentration of reducing sugars directly proportional to the growth rate in the case of exposure to the magnetic poles. Which mean 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.

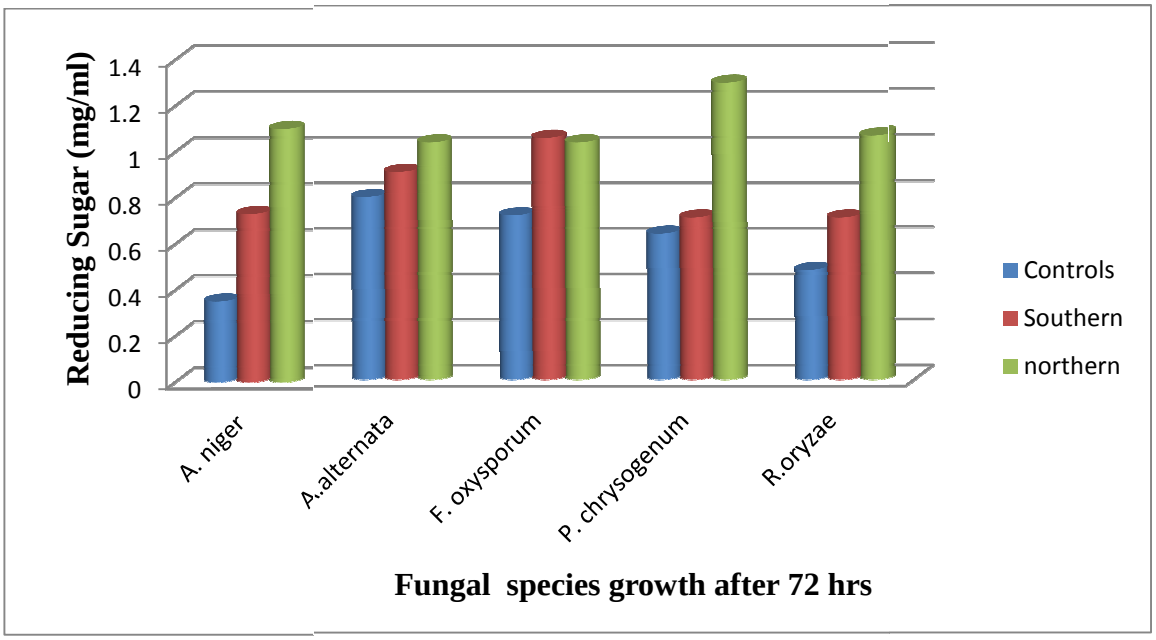


Figure (3-14): Effect of magnetic field on reducing sugar for fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C, pH 6.5 and 150 rpm.

3.6 Effect of static magnetic field on protein concentration in liquid medium

The effect of static magnetic field on protein concentration of five fungal species (figure (3-15)) revealed that the effect of north magnetic pole due to increase in protein concentration of 3 fungal species (*A. niger*, *A. alternate* and *F. oxysporum*) more than south pole but due to decrease in protein concentration of *P. chrysogenm* and *R. oryzae* more than north pole compared with control group.

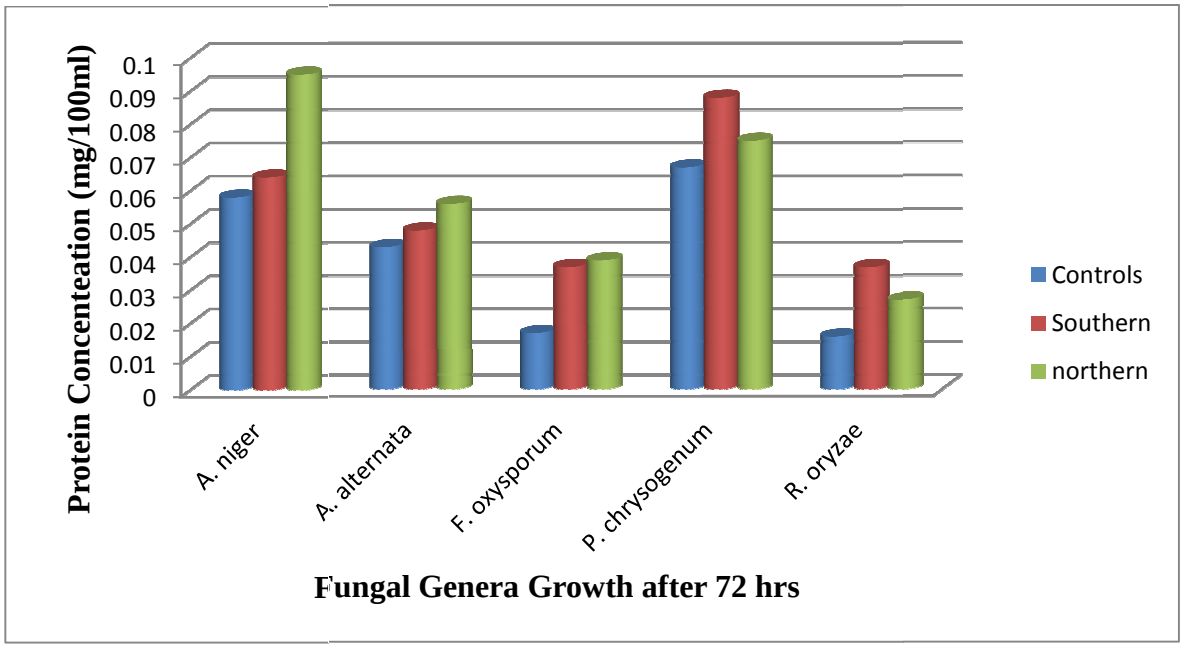


Figure (3-15): Effect of static magnetic field on protein concentration for fungal species growth after incubation growth medium under magnetic field for 72 hrs. SMF: Static magnetic field.

Potenza, *et al*. (2004) reported that the protein synthesis by *E. coli* was stimulated under the effect of MFs with a six-fold increase in the protein synthesis rate including th[illegible] fungal species based on the result in figure (3-15).

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).

3.7 Effect of magnetic field on chitin content in cell wall

Chitin and chitosan are copolymers of N-acetyl-Dglucosamine and D-glucosamine linked by β-(1-4) glycosidic bonds. Chitin is a substance found naturally in the exoskeletons insect, in the shell of crustaceans, such as crab, shrimp and crawfish and in fungal cell walls. Chitin is widely distributed in

fungi, where it was component of the cell walls and structural membranes of mycelia, stalks, and spores (Roberts, 2002).

Figure (3-16) shows the effect of magnetic field (southern pole and northern pole) on the chitin concentration in liquid medium showed a decrease in chitin (a polymer of N-acetylglucosamine) of *(A. alternata, F. oxysporum and R. oryzae*) and increased in the concentration of chitin of *(A. niger* and *P. chrysogenum*) in treatments of MF compared with controls (without treatment).

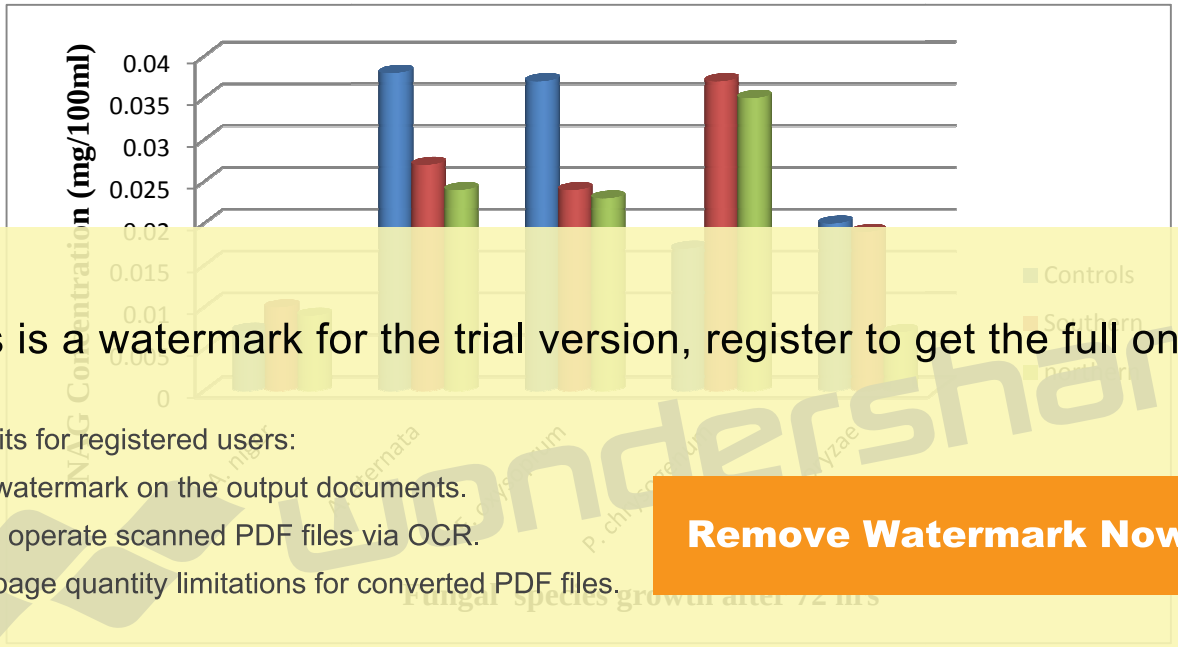


Figure (3-16): Effect of magnetic field on chitin concentration for fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C, pH 6.5 and 150 rpm.

The effect of magnetic field on cell membrane may increase membrane permeability under ion cyclotron resonance and increased circulation and selective enhancement of ion flow may affect the rate of biochemical reactions.

The magnetic field alter the rate of binding of calcium ions to enzymes or receptor sites in membrane and change distribution of protein and lipid domains, and conformational changes in lipid-protein associations and also Change internal molecular distribution of electronic charge inside lipid molecule in the membrane bilayer .

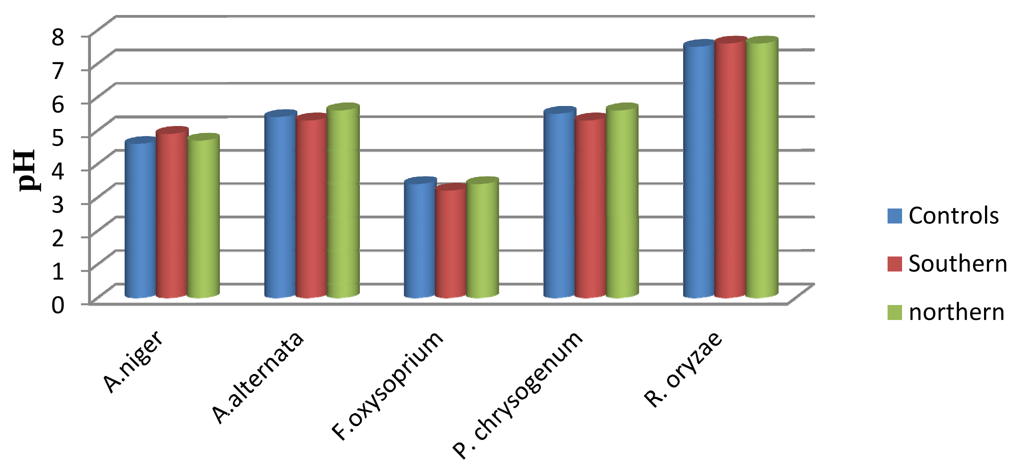
Non irradiated chitin reflects “substrate exhaustion”. In this particular case, it was more of a case of all accessible bonds in the chitin particles having been hydrolyzed. Hence, it was understandable that microwave treatment generated more accessible bonds and the hydrolysis continued with a linear response to enzyme concentration.

3.8 Effect of magnetic field on medium pH

Optimum pH represents one of the important parameter which effect enzyme activity. It was found that most enzymes remain active within a wide or narrow range of pH. However most of enzymes appear to be active at the optimum pH of the microorganism itself (Reed, 1975).

To investigate the magnetic field effect on optimum medium pH for growth and enzyme activity of [illegible] fungal genera with pH value [illegible].

[illegible] figure (3-17) showed that There was [illegible] effect of magnetic field at different treatments of the northern and [illegible] fungal species, compared with control and [illegible] with a number of research included the impact of the magnetic field on the metabolism of certain organisms.



Fungal species growth after 72 hrs

Figure (3-17): Effect of static magnetic field on pH for fungal species growth after incubation growth medium under magnetic field for 72 hrs at 28°C and 150 rpm.

In the context of the pH effects, Yavuz and Celebi (2000) reported that there was no correlation between the microorganisms growing rates with and without magnetic field applied.

The fundamental characteristics of extracellular amylases are expected to reflect the pH and temperature of the environmental in which they were grown and that means the optimum pH for the α- amylase and pH of the growth were the same (McTigue, *et al.*, 1994). Nevertheless many researchers have indicated that the pH of growth and pH of α- amylase production was not necessarily compatible (Volesky and Loung, 1985).

Conclusions

- Magnetic field poles (10 gauss) stimulated the growth of 3 fungal genera (*A. niger*, *A. alterneta* and *P. chrysogenum*) and inhibiting the growth of *F. oxysporum* while had no effected on the growth of *R. oryzae* compared with controls by measuring the colony diameter growth (cm) on solid medium.
- Magnetic field inhibits the growth of (*A. alternata*, *F. oxysporum* and *R. oryzae*) and stimulate the growth of (*A. niger* and *P. chrysogenum*) in liquid medium by measuring the dry weight (g/100ml).
- The northern pole had a positive effect on the enzymes activity while the southern pole has a negative influence enzymes activity as compared with control under the same condition.
- Magnetic field poles effect on the N-acetylglucosamine concentration, which decreased the concentration of N-acetylglucosamine in (*A. alternata*, *F. oxysporum* and *R. oryzae*) and increased the concentration in (*A. niger* and *P. chrysogenum*) compared with

Recommendations

- Study the effects of MF poles (both) on the growth of 5 fungal genera of different species in liquid medium.
- Study the effect of MF poles on the repair system of 5 fungal genera.
- Study the effect of magnetic fields poles on the production of different toxins by important toxigenic fungi.

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LIST OF ABBERVIATION

Abbreviation	Meaning
T	Tesla
Vpm	Volt per meter
Ms	Millisecond
Vpc	Volt per centimeter
Cps	Cycle per second
Ssf	Solid state fermentation
mg/ml	milligram per milliliter
Smf	Submerged fermentation
Ca^{+2}	Calcium
Cm	Centimeter
CYP_S	Cytochrome P450 enzymes
DNA	Deoxyribonucleic acid
DNS	Dinitro-sylcilic acid
EMF	Electromagnetic field /fields
G	Gauss
LSD	Least significant differences

MF	Magnetic failed
mg/ml	Milligram per milliliter
Na/K ATPase	Sodium – potassium Adenosine tri-phosphatase
Nm	Nanometer
PDA	Potato dextrose agar
BSA	Bovine serum albumin
SDA	Sabouraud dextrose agar
UV	Ultra violette
Wa	Water activity
DC	Direct current
AC	Alternating current
SFE	Supercritical fluid extraction
Hsp	Heat shock protein
Hz	Hertz
kHz	Killohertz
Tn	Transposition
Lac	Lactose operon
BDC	Static magnetic field
BAC	Alternating field
DnaK/J	Chaperone protein

OMF	Oscillating Magnetic Field
NusA	N(phage Lambda protein
mb	Megabase
µm	micrometer

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Summary

The effect of static magnetic field on the growth and biochemical indices of five fungal species were studied (*Aspergillus niger*, *Alternaria alternate*, *Fusarium oxysporum*, *Penicillium chrysogenium* and *Rhizopus oryzae*). Exposing the above species to the northern pole, southern pole and both poles and their influences were compared with the control treatment (without magnetic field energy). The static magnetic field of 10 gauss was subjected to the above fungal species separatly for seven days at 28 °C, The effect of static magnetic field energy on the growth of fungal species on two solid media (Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA)) was classified as stimulatory, inhibitory and no observable effect on growth of fungal species. The effects of magnetic field poles (southern, northern and both) inhibited the growth of *Fusarium oxysporum*, while the magnetic field poles stimulated the growth of fungal [illegible] *niger*, *Alternaria alternata* and *Penicillium chrysogenium*) and [illegible] no observable effect on growth of *Rhizopus oryzae*, while the northern pole inhibited the growth of *R. oryzae* compared with control group by measuring the growth diameter (cm). The effects of magnetic field poles on mycelia growth of the mentioned fungal species in liquid medium (Yeast Extract Sucrose (YES) broth) was determined by the dry weight measurement (g/100cm^3) every 24 hr for 4 days, the content of flask was filtered through Whattman No.1 fiterpaper to separate the mycelial mat and culture filterate, and the fungi were effected by northern pole, southern pole only, The magnetic field poles were inhibited the growth of three species (*A. alternata, F. oxysporum* and *R. oryzae*) and increased in the growth of *A. niger* and *P. chrysogenium as* compared with controls at the same condition. The effects of magnetic field poles on the biochemical indices of the fungal species

were performed by filtering the growth and measuring the enzymes activity, protein and chitin concentration in the filterate, The fungal species were influenced by magnetic field energy for 3 days at 28°C, pH (6.5) showed increased in the activities of amylase and protease due to northern pole and decreased when treated with southern pole however it was higher than the control treatment under optimum condition at significant difference (at the .05 level), The northern pole increased amylase activity (U/ml) in the culture filterate of *P. chrysogenum* (0.246 U/ml) higher than other mentioned species, *A. niger*, *F. oxysporum*, *R. oryzae* and *A. alternata* (0.172, 0.146, 0.116, 0.105)U/ml respectively. The northern pole increased protease activity (U/ml) in the culture filterate of *P. chrysogenum* (0.081 U/ml) higher than other mentioned species, *A. niger*, *A. alternata*, *R. oryzae* and *F. oxysporum* (0.06, 0.054, 0.046, 0.05)U/ml respectively, while protein concentration increase under the effect of magnetic field poles of 5 fungal species. The chitin concentration in liquid medium showed decrease in chitin concentration (a polymer [illegible] and *P. chrysogenum*) and chitin of (*A. niger* and *P. chrysogenum*) in treatments of magnetic field compared with controls (without treatment) at the same condition.

الإهـــداء

سهر العيون لغير وجهك باطل

بكائهن لغير فقدك ضائع

الى من وهبني كل شئ "الله عز وجل"

يا هذه الدنيا اصيخي واشهدي، إنا بغير محمد لا نقتدي..

الى سيد الثقلين حبيب الله تعالى محمد عليه الصلاة والسلام

إلى سَندي ومُتكأي.. الذي رَحَل على حِينِ غفلة

والـدي الحَبيـب

أُهدي لِروحِهِ ثَمرة جُهدي المتواضع

الى منبع الحنان والطيبة .. الى مصدر اصراري و عزيمتي.. "امي الغالية"

الى من اسأل لهم التوفيق والسداد .. عائلتي حفظهم الله من كل سوء.

الى رفاق الدرب... زميلاتي و زملائي.

زينب عباس علي

بسم الله الرحمن الرحيم

(لَقَدْ أَرْسَلْنَا رُسُلَنَا بِالْبَيِّنَاتِ وَأَنْزَلْنَا مَعَهُمُ الْكِتَابَ

صدق الله العظيم

[الحديد: ٢٥]

الخـــــــــــلاصـة

لقد تمت دراسة تأثير المجال المغناطيسي الثابت على النمو والمؤشرات البايوكيميائية لخمسة أنواع فطرية عائدة لأجناس مختلفة. *Aspergillus niger, Alternaria alternate,* ولقد *Fusarium oxysporum, Penicillium chrysogenum* and *Rhizopus oryzae.* تم تعريض هذه الأنواع الفطرية الخمسة منفردة إلى القطب الشمالي والجنوبي والاثنين معا (على جانبي طبق التنمية)، ثم قورن تأثير أقطاب المجال المغناطيسي على نمو الفطريات مع معاملة السيطرة التي لم تعرض على الوسط الصلب (Sabauroud Dextrose Agar and Potato Dextrose Agar) ولمدة ٧ أيام وبدرجة ٢٨°م، قسم تأثير المجال المغناطيسي على النمو الى ثلاث أنواع (بالمحفز، المثبط، وبدون ملاحظة أي تغيير واضح على النمو على اساس قطر النمو على الطبق) للأنواع المذكورة، وأظهرت الدراسة أن المجال المغناطيسي (الشمالي والجنوبي والاثنين معا) ثبطا النمو للفطر *Fusarium oxysporum* وحفزا النمو للفطريات *Aspergillus niger, Alternaria alternate* and *Penicillium chrysogenum* ولم تلاحظ أي تغيرات واضحة على النمو للفطر *Rhizopus oryzae* نتيجة تعريضه للقطب الشمالي للفطر نفسه مقارنة مع معاملة السيطرة بينما تم تثبيط النمو بتأثير القطب الشمالي للفطر نفسه مقارنة مع معاملة السيطرة بقياس قطر النمو (cm) للأنواع المذكورة للمجال المغناطيسي (الشمالي والجنوبي والاثنين معا) (Yeast Extract Sucrose Broth) وتمت ملاحظة تثبيط النمو بتأثير اقطاب المجال المغناطيسي للفطريات *A. niger* and وزيادة النمو للفطر *A. alternate, F.oxysporim* and *R. oryzae* *P.chrysogenum* مقارنة مع معاملة السيطرة تحت الظروف نفسها. وتمت دراسة تأثير أقطاب المجال المغناطيسي (الشمالي والجنوبي) على المؤشرات الكيموحيوية للأنواع الفطرية الخمسة من خلال ترشيح النمو وقياس الفعالية الإنزيمية وتركيز الكايتين في الراشح. وتم تعريض الأنواع الفطرية الخمسة إلى المجال المغناطيسي لمدة ٣ ايام بدرجة حرارة ٢٨°م، وأظهرت الدراسة ان الفعالية الإنزيمية للاميليز و البروتييز تزداد بتأثير القطب الشمالي وبفروق معنوية، حيث ازدادت فعالية الاميليز في راشح الزرع *P. chrysogenum* لتصل الى (٠,٢٤٦ وحدة/مل) ، واكثر من بقية الانـــــواع *A. niger, F. oxysporum, R. oryzae* and *A. alternate* (٠,١٤٦،٠,١١٦،٠,١٠٥،٠,١٧٢) وحدة/مل على التوالي. اما الفعالية الانزيمية للبروتييز ازدادت بتأثير القطب الشمالي في راشح الزرع *P. chrysogenum* لتصل الى (٠,٠٨١ وحدة/مـل) وأكثرمن بقيـــة الأنـــواع *A. niger, A. alternate, R. oryzae*

F. oxysporum and (٠,٠٥٤،٠,٠٨،٠,٠٧٤،٠,٥٦ وحدة/مل على التوالي، وقلت بتأثير القطب الجنوبي على الرغم من انه كان اعلى من معاملة السيطرة تحت الظروف نفسها. أما تركيز الكايتين في الوسط السائل فقد قل للفطريـــات *A. alternate, F. oxysporum* and *R. oryzae,* وازداد للفطر *P. chrysogenum* and *A. niger* بتأثير المجال المغناطيسي تحت نفس الظروف مقارنة مع معاملة السيطرة.

Chapter One

Introduction

Literature review

Chapter Two

Materials And Methods

Chapter Three

Results and Discussion

Conclusions

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تاثير المجال المغناطيسي الثابت على النمو والمؤشرات الكيموحيوية لبعض الفطريات

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