The Effect of Static Magnetic Field on Growth and Biochemical Indices of Five Fungal Genera

تأثير المجال المغناطيسي الثابت على النمو والمؤشرات البايوكيميائية لخمسة أجناس فطرية

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Abstract

The effect of static magnetic field (MF) on the growth and biochemical indices of five fungal genera were studied. Exposing the above genera to the northern pole, southern pole and both poles and their influences were compared with the control treatment (without MF energy). The static MF of 10 gauss was applied to the above fungal genera for seven days at 28°C. The effect of static MF energy on the growth of fungal genera on solid media Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) was classified as stimulatory, inhibitory and no observable effect on growth of fungal genera. The effects of MF poles (southern, northern and both) inhibited the growth of *Fusarium oxysporum*, while the MF poles stimulated the growth of fungal genera (*Aspergillus niger*, *Alternaria alternate* and *Penicillium chrysogenium*) and had no observable effect of southern pole and both poles 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 MF poles on the biochemical indices of the fungal genera were performed by filtering the growth and measuring the enzyme activity in the filterate. Exposing the fungal genera to the northern pole, southern pole. The fungal genera were influenced by MF energy for 3 days at 28°C and pH6.5 showed increased in the activities of amylase and protease due to northern pole at significant difference (at the 0.05 levels), the northern pole increased amylase activity (U/ml) in the culture medium filteratedof *P. chrysogenum*up to (0.246 U/ml) higher than other mentioned genera, *A. niger*, *F. oxysporum*, *R. oryzae* and *A. alternata* 0.172, 0.146, 0.116, 0.105U/ml respectively. The northern pole increased protease activity (U/ml) in the culture filterate of *P. chrysogenum*up to (0.081 U/ml) higher than other mentioned genera, *A. niger*, *A. alternata* , *R. oryzae* and *F. oxysporum* 0.08, 0.074, 0.056,0.054U/ml respectively and decreased when treated with southern pole however it was higher than the control treatment under optimum condition.

Keyword: Magnetic field, Fungi, growth and Amylase.

المستخلص

تم دراسة تأثير المجال المغناطيسي الثابت على النمو والمؤشرات البايوكيميائية لخمسة أجناس فطريةعائدة لأنواع مختلفة. تم تعريض هذه الأجناس الفطرية الخمسة إلى القطب الشمالي والجنوبي والاثنين معا (على جانبي طبق التنمية)، ثم قورن تأثير أقطاب المجال المغناطيسي على نمو الفطريات مع معاملة السيطرة على الوسط الصلب (Sabauroud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA)ولمدة 7 أيام وبدرجة م. صنف تأثير المجال المغناطيسي على النمو (بالمحفز، المثبط، وبدون ملاحظة أي تغيير واضح على النمو باعتبار قطر النمو على الطبق) للأجناس 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 يلاحظ أي تغييرمعنوي واضح في النمو على الفطر *Rhizopus oryzae* نتيجة لتأثير القطب الجنوبي وكلا القطبين بينما ثبط النمو بتأثير القطب الشمالي للفطر نفسه مقارنة مع معاملة السيطرة. درس تأثير أقطاب المجال المغناطيسي (الشمالي والجنوبي) على الاميليز والبروتييز انزيم للأجناس الفطرية الخمسة من خلال ترشيح النمو وذلك قياس الفعالية الإنزيمية (وحدة/مل) في الراشح. عرضت الأجناس الفطرية الخمسة إلى المجال المغناطيسي لمدة 3 ايام بدرجة حرارة 28م، وأظهرت الدراسة انه الفعالية الإنزيمية للاميليز و البروتييز تزداد بتأثير القطب الشمالي وبفروق معنوية، حيث ازدادت فعالية الاميليز في راشح الزرع *P. chrysogenum* لتصل الى 0.246 وحدة/مل، واكثر من بقية الانواع *A. niger, F. oxysporum, R. oryzae* and *A. alternate* 0.105،0.116،0.146،0.172 وحدة/مل على التوالي. اما الفعالية الانزيمية للبروتييز ازدادت بتأثير القطب الشمالي في راشح الزرع *P. chrysogenum* لتصل الى 0.081 وحدة/مل وأكثر من بقية الاجناس *A. niger, A. alternate, R. oryzae , F. xoysporum*، 0.054,0.056,0.074,0.08 وحدة/مل على التوالي. وقلت بتأثير القطب الجنوبي على الرغم من انه كان اعلى من معاملة السيطرة تحت الظروف نفسها.

الكلمات المفتاحية: المجال المغناطيسي، فطريات، النمو، الاميليز

Introduction

Scientists believed that electromagnetic fields (EMFs) of low frequency did not cause any significant biological effects and studied have verified that electric and/or magnetic fields of extremely low frequency (ELF; <300 Hz) can influence the biological systems. The influence of an alternating magnetic field on the growth of the primary root of corn *Zea mays* seedlings has reported [1]. In addition to the stimulation or inhibition of the growth of five bacterial species and yeasts was dependent on the field strength, frequency and types of bacterium [2]. The stimulation or inhibition of microbial growth was also reported by other authors [3]. Effect of exposure to a static magnetic field on cell growth, viability, and gene expression of *Salmonella enterica* were also indicated [4].

The dimorphic fungus *Mycotypha africana* can exist in a mycelium or yeast-like form. Weak ELF magnetic fields shift development towards the yeast form [5]. Weak AC fields (0 − 1.2n T, 0.8 − 50 Hz) induce further increase the germination rate [6]. Very strong DC fields (5.2−6.1 T) suppress spore formation of vegetative cells of *Bacillus subtilis*, an effect that was paralleled with the diminished activity of alkaline phosphatase [7].

Several studies have been carried out to investigate the effects of low frequency electromagnetic fields on DNA [8,9], enzyme activity [10] and cells [11]. 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 that MFs influences a variety of cellular functions such as growth, cell membrane characteristics, gene expression, protein biosynthesis, enzyme activity, cell reproduction and cellular metabolism but the interaction of such fields with the living cells is still unclear [12].

The biochemically versatile fungus *A.niger* produces a wide array of acids and degradative 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 [13].

This research describes the effect of static magnetic field on the growth of various species of filamentous fungi on solid media and estimates the amylase and protease activities (U/ml) to the five fungal genera in filtrate.

Materials and Methods

Fungal isolates

The isolated fungi *Penicillium chrysogenum*, *Rhizopus oryzae* and *Aspergillus niger* were obtained from the department of Biotechnology, College of Science, Al-Nahrain University. While *Fusarium oxysporum* and *Alternaria alternata* were obtained from the department of Biology College of Science, University of Baghdad. The isolated fungi were identified after growing on Potato Dextrose Agar (PDA) medium [14], by observing the growth characteristics (color, texture appearance and diameter of the colonies) and microscopically (microstructure) [15]. All the culture was maintained on PDA slants, stored at 4°C in refrigerator and sub-cultured regularly at an interval of three months.

Static magnetic field

By using a special magnetic bar of thickness (1cm and 5cm diameter), Single field strength of 10 Gauss was measured by Gauss meter. 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.

The effect of magnetic field on the growth of fungal isolate on solid media

Two solid media Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were used for the study. For single petri dish twenty ml of autoclaved solid media were poured into a sterilized Petri plates. Actively grown culture of the 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 replications were maintained. The diameter of the fungal colony was measured following [16].

The effect of magnetic field on enzyme activity

The media were prepared as described as in [17] by weighing the 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 was dissolved in 1000ml of distilled water after which 100ml of the medium was measured into a conical flask (250 ml capacity each) heated on a hot plate to homogenize and then sterilized in an autoclave at 121°C (1.08Kg/cm^2) for 15 min after which they were removed and allowed to cool.

Culture condition

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 3 days, after incubation, culture filtrate was filtered through Whatman No.1 filter paper. Supernatant obtained after 72hrs was used as the crude enzyme sample. Except the *Fusarium oxysporum* were centrifuged at 3000 rpm for 10 min at 4°C in a refrigerated centrifuge before the filtration.

Extraction of crude enzyme

Extraction of crude enzyme was done by centrifugation of the fermented media at 2000 rpm for 5 min after 72 hrs; supernatant collected and was filtered off by using Whatman No.1 filter paper. The filterate was used as crude enzyme extract [17,18].

Assay of amylase activity

Amylase activity was assayed as described by [19], 0.5 ml of culture extract enz yme added to the 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 [20].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 immersing the tubes in a boiling water bath for 5 min after which they were allowed to cool and 5 ml of distilled water was added. The absorbances for all test tubes were measured in 540 nm with spectrophotometer.

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.

Assay of proteases activity

The activity of protease was assayed by the method of McDonald and Chen, 1ml of the enzyme extract was added tothe 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 alkaline reagent, 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 the maximum development of a blue color. The optical density of the mixture was read at 700 nm on spectrophotometer [21]. 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.

Results and Discussion

Fungal isolates

The present study was undertaken to determine the effect of magnetic field on growth and biochemical indices of some fungal isolates. Five species of different genera 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 different species were studied morphologically on potato dextrose agar at 28°C. 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 [22]. *A. alternata* in culture shows a white, growth with profuse aerial mycelium which gradually turned greenish grey [23]. *F. oxysporum* colonies are usually fast growing, pale or brightly colored and may have a cottony aerial mycelium. The color of the thallus varies from white to yellow, brown, pink, red or lilac shades. *P. chrysogenum* colonies are usually fast growing, in shades of green, sometimes white, mostly consisting of a dense felt of conidiophores [24]. *R. oryzae* quickly fills a Petri dish (agar surface) with a typically cotton candy like colony, initially white that turns grey to yellow brown. The reverse is white to pale [25].

The effect of magnetic field on the growth of fungal species

The exposures of magnetic field stimulate or inhibit the growth and proliferation of microorganisms. High intensity magnetic fields can affect membrane fluidity and other properties of cells [26].

Table (1): Effect of static magnetic field on growth rate of *Aspergillus niger*

Effectof poles	Mean Growth diameter (cm)	Stander error	LSD
Control	**3.5**	**0.88***	**-**
Southern	**4.1**	**0.166***	**0.04**
Northern	**5**	**0.145***	**0.00**
Both	**4.5**	**0.288***	**0.06**

***The mean difference is significant at the .05 level.**

Table (1) showed 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 of north magnetic pole 5 cm was more than south magnetic pole 4.1 cm, both poles 4.5 cm and controls 3.5 cm.

Table (2): Effect of static magnetic field on growth of *Alternaria alternate*.

Effect of poles	Mean Growth diameter (cm)	Stander error	LSD
Control	**4**	**0.575***	**-**
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 .05 levels.**

The results obtained from the fungal growth in table (2) 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, north pole 5.1 cm and both poles 5.2 cm as compared with control 4 cm.

Tables (1,2) showed that a 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 [27] 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*.[27].

Table (3): Effect of magnetic field on growth of *Fusarium oxysporum*.

Effect of poles	Mean Growth diameter (cm)	Stander error	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 .05 levels.**

Table (3) showed inhibitory effect on growth rate of *F. oxysporum*with 10 gauss static magnetic field. Table (3) 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 conclusion was made that magnetic poles inhibits the DNA replication of *F. oxysporum* and increase the metabolic activity.

Table (4): Effect of static magnetic field on growth of *Pencillium mchrysogenum*.

Effect of poles	Mean Growth diameter (cm)	Stander error	LSD
Control	1.6	0.88*	-
Southern	2.3	0.332*	0.00
Northern	2.1	0.575*	0.002
Both	2.8	0.88*	0.00

***The mean difference is significant at the .05 levels.**

Table (4) showed 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, 2.1,2.8) cm when exposed to the southern pole, northern pole and both poles respectively and compared with the control (without magnetic field) 1.6 cm cultured under identical conditions. The results suggest that the action of the magnetic field may be an important environment factor affecting the function of the biological clock as well as the morphology of the examined *P. chrysogenum*.

It has been demonstrated experimentally that the application of a low-frequency, weak magnetic field, both static and time-varying, induce considerable changes in the metabolism of cells [28]. These changes are manifested primarily on the altered ion flow through cell membranes and in the motion of cells. Other investigation of magnetic fields include those on the activity of ion channels [29] and ion transport in cells [30].

Table (5): Effect of static magnetic field on growth of *Rhizopus oryzae*.

Effect of poles	Mean Growth diameter (cm)	Stander error	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	0.7

*** The mean difference is significant at the .05 level**.

Table (5) showed no effect on growth of *R. oryzae* at 10 gauss in South Pole and both poles but there was slight inhibitory effect on the North Pole as compared with the control group. This effect may be due to more potent effect of 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 [31] and this may have effect on cell metabolism and replication, which agreed with [32,33].

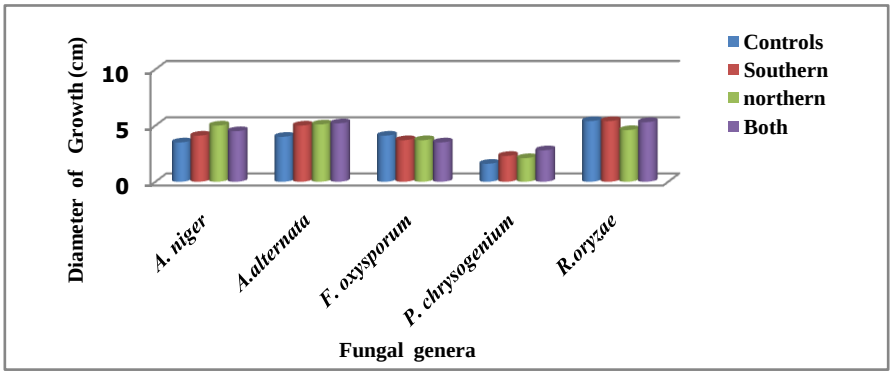

Fig. (1): Effect of magnetic field on the growth of some fungal genera on solid media.

Figure (1) showed the five fungal 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 both poles of growth of *R.oryzae*and stimulated of *R. oryzae*when exposed to the north pole as compared with the control group. This indicates that the polarity has significantly affected the interrelationship between magnetic field and microorganisms and indicates that the magnetic field effectively influenced the formation of conidia of the examined fungal genera.

Effect of magnetic field on enzyme activity

The impact of the magnetic field on the enzymes is concentrated on changing the charge and thus 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 see change in the activity of the enzymes, but when we develop and encourage the organism to produce the enzymes under the effect of magnetic field, a marked change in the enzyme activity and difference between the two north and south Poles .

The changes of Amylase and protease activity at north and southern pole were detected and the results are presented in figures (2,3) 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 attach to the active site according to the lock and key theory [34].

Amylase enzyme had a very important role in the hydrolysis of starch to sugar which provide energy for growth [35]. Amylase (Ando 1 and 4 -D-Glucagon Gludehydrolaz) 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 [36].

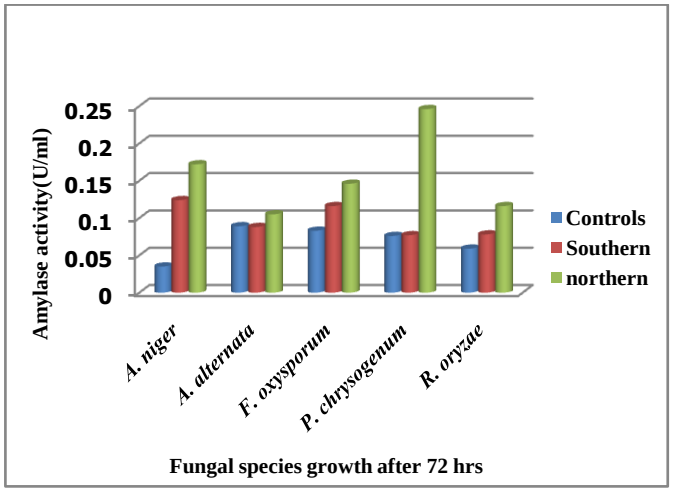

Fig. (2): Effect of magnetic field on Amylase activity

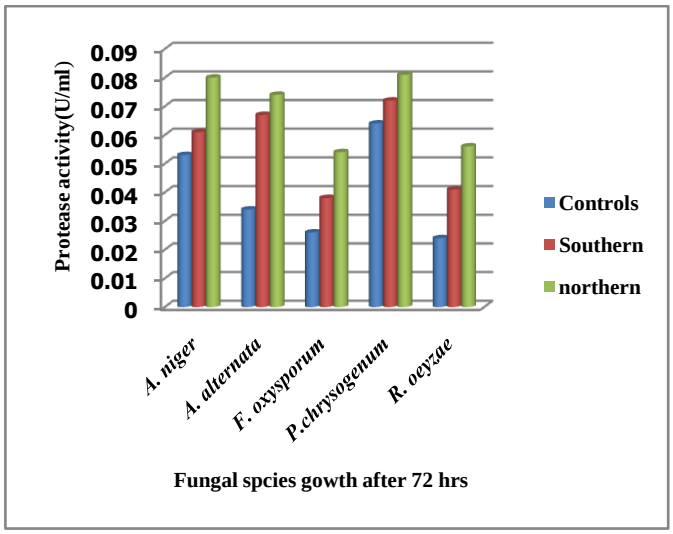

Fig. (3): Effect of magnetic field on the protease Activity.

Figure (2,3) represent the effect of magnetic field on amylase and protease activity of fungal genera growth at optimal pH 6.5 and temperature 28°C respectively. The north pole was increased amylase activity of all fungal genera higher than when exposed fungal species to the southern poles and the two poles increased activity higher than controls (without MF).

The northern pole increased amylase and protease activity (U/ml) in the culture filtrate of *P. chrysogenum* (0.246 U/ml) higher than other mentioned genera, *A. niger*, *F. oxysporum*, *R. oryzae* and *A. alternata* (0.172, 0.146, 0.116, 0.105)U/ml respectively, while protease activity (U/ml) in the culture filterate of *P. chrysogenum* (0.081 U/ml) higher than the others mentioned genera, *A. niger*, *A. alternata* , *R. oryzae* and *F. oxysporum* (0.08, 0.074, 0.056,0.054)U/ml respectively.

The action of the magnetic field on enzyme activity seems to switch the enzyme to a state of an increase in the activity. Optimal pH and temperature are very essential for the 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 either the substrate cannot bind to the active site or it cannot undergo catalysis. Inspection whether extremely low frequency, electromagnetic fields 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 were no significant differences between ELF, EMF on optimal pH and temperature. This fact could be the cause of increase of α-amylase activity determined by EMF, considering the Ca^{2+} ions present in the enzyme structure, which mediate the EMF action at the cell level [37]. 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. However, MF also interacts directly with electrons in DNA to affect protein biosynthesis [38].

Conclusion

Magnetic field poles 10 gauss stimulated the growth of 3 fungal genera *A. niger*, *A. alternetaand* *P. chrysogenum*, on the other hand it inhib the growth of *F. oxysporum* while no effect on the growth of *R. oryzae*, compared with controls by measuring the diameter growth (cm) on solid medium.North pole had a positive effect on the enzymes activity while the south pole has a negative influence enzymes activity as compared with control under the same condition at significant difference.

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