Study the effect of Microwaves radiation in the growth of Some isolates fungi

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Abstract

Explained this study the effect of microwaves radiation with a frequency waves (a 2,450 MHz) on five fungal isolates (*Aspergillus flavus, Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum, and alternaria alternate)* .Fungal isolates that cultivated on the Sabouraud dextrose agar (SDA) , at a rate of three frequency for each isolate fungi were exposed to the radiation of microwave oven with four time periods (0 second(control), 5 , 10 , 15 seconds). where the results showed highly growth of colonies of each fungal isolates used in the study that exposed to microwaves of time periods (5 seconds, 10 seconds). While killed all fungal colonies exposed to microwaves for the time period (15 seconds). This study proved the ability of the waves and microwaves period of time (15 seconds) and above to kill the fungal colonies and do not allow for the growth of fungal spores, meaning that the rate of growth of fungal colonies is inversely proportional to the length of time of exposure to radiation of microwaves.

Key words: microwave oven, *Aspergillus , Penicillium , alternaria alternate.*

1- Introduction:-

Microwaves are non-ionizing electromagnetic waves with frequencies between 0.3 and 300 GHz(i.e.,with wave lengths from 1 meter to 1 millimetre ,respectively) [1 ,2].

Mechanism of microwaves action on living organisms, when irradiating living organisms ,microwaves produce two types of effects :thermal and non thermal . Thermal effects are the consequence of absorption of microwave energy by cell molecules causing then vibrate much faster and producing general heating of the cell . The concept of non-thermal effects of microwaves came from experiments in which bacterial cultures were to a large extent destroyed by microwave induced heating than by other heating methods producing the same working temperature and from studies showing an increase in the growth of bacteria induced by microwaves[3].

Microwaves will affect the growth of microorganisms depends primarily on the frequency of the radiation and the total energy absorbed by the microorganisms (absorbed dose). When microwaves are applied at certain frequencies, with high energy and for asufficiently long period of time, their thermal effect is most likely dominant and kills bacterial cells or yeasts. Numerous experiments with microwave irradiation of various cultures of bacteria and yeasts in a wet environment such as a water suspension did not show additional killing of the microbes by microwaves compared to that caused by conventional heating to the same temperature [4, 5, 6]. However , in a dry environment ,the killing effect of microwave radiation was significantly decreased and happened only after a prolonged period of irradiation, most likely due to a lower transformation of microwave energy to heat. Some of the studies even showed that the extent of killing of microorganisms (bacteria and bacteriophages [viruses that attack bacterial]) was correlated with the moisture content of the experimental specimens. In contrast , when microorganisms were irradiated with microwaves at temperatures lower than the thermal destruction level, various effects were observed ,from killing to enhanced growth [7, 8].

Now a days microwave heating is known to inactivate many microorganisms for instance, *Escherichia coli, streptococcus faecalis ,Saccharomyces cerevisiae,conidia of Aspergillus niger and penicillium* sp[9].

Fungi are eukaryotic organisms that exist in two basic forms , yeasts and molds. Yeasts are single cells, wheres molds consist of long filaments of cells called hyphae [10].

Aspergillus is a fungus whose spores are present in the air we breathe, but does not normally cause illness. Aspergillosis is a group of diseases which can result from aspergillus infection and includes invasive aspergillosis, and aspergilloma. Individuals who suffer from asthma and other respiratory diseases are at a greater risk for these infections.[10]

There are many species of *penicillium* , like *penicillium chrysogenum* which is responsible for produced penicillins and cephalosporins, his colonies are rapid growing flat filamentous and velvety, wooly or cottony in texture. The colonies are initially white and become blue ,green, grey, olive grey ,yellow or pinkish in time [11].

Alternaria fungus has about one hundred species which can be found in various places all over the world. Many of them are important pathogens of plants and cause important

economical disease in wide range of hosts. Some of them live saprophytic and are main
part of fungi population in soil and dead or dying plant tissues [12,13,14].

Polyphagous nature and ability of them in producing toxic and carcinogenic
materials indicates that *alternaria* is potentially hazardous to human and animals health . This pathogen has a wide range (more than 380) of hosts including citrus, pistachio, apple, pear, tobacco, tomato, and beens [14]. *Alternaria alternata* has an important place among species of this genus, because of wide range of hosts including garden plants, field crops, vegetables, and ornamentals. This fungus is one of the important pathogens of citrus which cause brown spot by tangerine pathotype, leaf spot by rough lemon pathotype, and black rot of harvested fruits.[14].

From all of the above the aim of present study is find alternatives way to sterilize culture media and laboratory instruments, as well as foods at home through the use of a microwave oven radiation , which ensures us kill fungi during the heating food process and so we can use the microwave device as the excellent sterilization at home.

2- Materials and methods:-

2 .1. Organism

Aspergillus niger, *Aspergillus flavuse* , *Aspergillus fumigates, Penicillium chrysogenum and Alternaria alternata* were used to evaluate the inactivation effect of microwave radiation in this study. These microbes were supplied by the Biotechnology Division; Department of Applied Science, University of Technology.

All fungal isolates were isolated from soil, bread, and cultivated at 30°C for 4-7 day, on SDA(sabouraud dextrose agar) and then stored at 4°C , until we use. The experiment was performed in various times (0s(control) ,5 s,10s,15s) of exposure to determine the effect of microwave radiation, achieving colony counting method. For the microwave application ,a 2,450 MHz microwave oven (LG) at electrical power of 900 W was used (figure1) .

Microwave oven(LG) . The : Figure 1

The culture media used in this study was Sabouraud dextrose agar (SDA) . The suspension was dissolved by heating, autoclaved for 15 minutes at 121°C, cooled to 50°C, then chloramphenical was added a concentration of (0.05) gm/L, media were poured into sterile petri dishes , left at room tempreture , then kept in refrigerator until use.

The petri dishes containing 15 ml of SDA, cultured with different fungi, each isolate prererd as suspension fungi by taken 10 diameter of fungal colonies(hypha with spores) by loop from their media and but in Sabouraud broth for 1-2 hours and then take 1ml of suspension fungi and poured on (SDA) petri dishes (15). At a rate of three frequency for each isolates and left for 15 minutes at room temperature , so that is absorbed suspension fungi by the (SDA) media , then placed in the center of the microwave oven and exposed to microwaves radiation in various times (0s,5s,10s,15 s) the temperatures achieved by the samples after the microwave exposure are presented in(table 1). After the microwave treatment , each dishes containing fungi were incubated at 30°Cfor 4-7 day and colonies was counted after incubation period .Also , untredated samples were used as a control group of the each tested microorganisms. All the experimental study were carried out aseptic conditions. The number of colonies, usually referred to as Total number count (T.N.C) developed after the incubation time was determined using the colony –countig equipment and by applying the formula:[T.N.C.= C] where C- the sum of the colonies counted in every petri dish.

Table 1.Samples temperature variation during the microwave exposure periods

Time (seconds)	0	5	10	15
Temperature (°C)	25	30	40	50

2.2. Statistical Analysis

The grouped data were statistically evaluated using ANOVA with SPSS/17 software, values are presented as the least significant difference (L.S.D) of the three replicates of each isolates .

3- Results and discussion :-

The values obtained for the number of colonies , after the colonies counting are presented in Table 2.

Table 2.The T.N.C. values obtained for the fungal samples exposed to the microwaves for different periods of time.

Microwaves time exposure of fungi	0	5s	10s	15s
T.N.C. values ,number of colonies	80	60	30	0

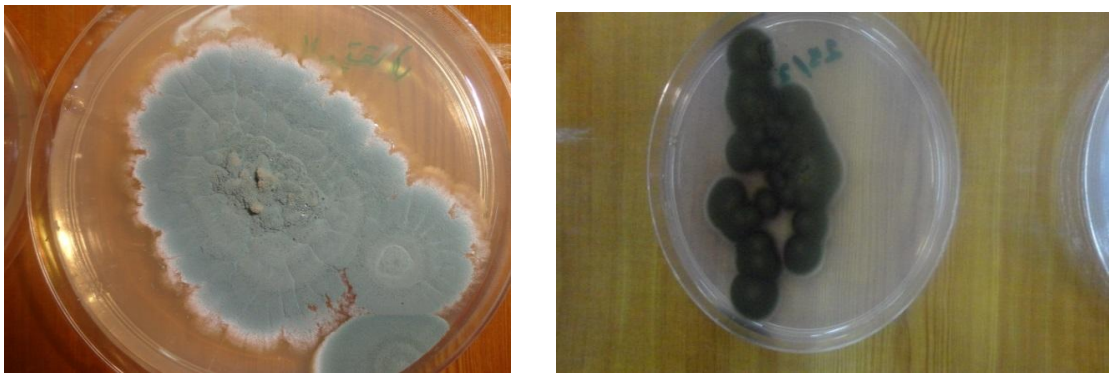
After exposing the isolated fungi to the microwaves and determining the number of colonies with using the colony –countig equipment , one can observe asignificant reduction of the colonies number as the microwave time exposure is increased[16].

The total number of colonies (T.N.C) we can observed the decrese in number of fungal colonies relatively with perioed of exposure to microwave.

It has been determined that rapid in activation or decress in growing of fungi can observed in the first 15 seconds .This period is consider the period of killing or growing stop of fungal colonies due to high tempreture that affect on the all vital processes[16] . (table 2).

The images of the petri dishes containing the samples after the incubation time are presented in the Figures 2- 4 .

As one can observe in figure 2 , corresponding to the sample that was not treated with the microwaves ,the number of the microorganisms units forming colonies is very high, Comparing with the Figures 3,4 . There can be seen the gradually decreasing of the number of colonies of the petri dishes corresponding with the increasing of the microwave time exposure .This fact was also indicated by the number of colonies counted for every sample using the colony –counting equipment.(Table 2).



A **B**

Figure 2. Samples (A) *Penicillium chrysogenum* , *and*(B) *alternaria alternate*) that were not treated with the microwaves (0 time of exposure).

A **B**

Figure 3 . Samples (A*) Aspergillus .flavus* (B) *Aspergillus .niger,* that were not treated with the microwaves (0 time of exposure).

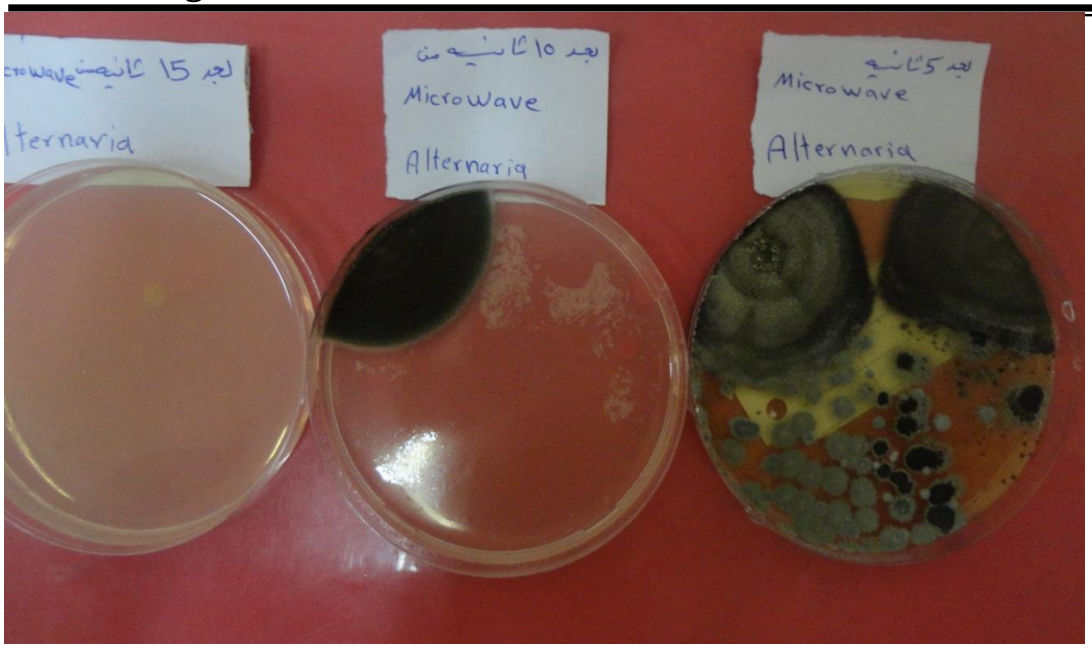


Figure 4 . Show Gradually decreasing of the number of colonies*(Alternaria . alternata*) corresponding with the increasing of the microwave radiation time exposure.

Figure 5 . Show gradually decreasing of the number of colonies *(Penicillium. chrysogenum)* corresponding with the increasing of the microwave radiation time exposure.

Comparing Figures (4,5) there can be seen the gradually decreasing of the number of colonies of the Petri dishes corresponding with the increasing of the microwave time exposure. This fact was also indicated by the number of colonies counted for every sample using the colony-counting equipment.(table 2).

Also can observe in (table 3) the growth of fungal colonies growing in the intermediate temperatures (25°C-37°C). we see this dense growth of fungal colonies , because these degrees of heat is considered optimal for the

growth of fungi which do not affect the enzymatic potency of fungi , as well as to not affect the fungal cell proteins and in this, note better growth of fungal colonies with in the rate of heat . While in the rate of tempreture (40-50°C) and above ,we will note, the absence of any growth of fungal colonies, this is because the high temperatures affect the protein that enters in the composition of fungal cell [3]. Cell membrane fungal consists of two layers of protein and during high temperature through exposure to microwave radiation for the time period 15 seconds and the temperature 50 °C this leads to clotting protein and a change in the nature and this will affect the permeability of the cell membrane and thus on all the vital processes leading to the growth of fungus stop and killing fungus[7]. It was also in this study addressed to diameter fungal colonies growth, at a rate of three frequency for each isolates , affected by microwave for three different time periods of exposure . Where the diameter measurement of each fungal isolates by the millimeter ruler. (Table4).

Table 3. Show the growth of fungal colonies during variation time of exposed to the microwaves in different temperature.

microorganisms	**Time of exposure to microwaves**	**0 (control)**	**5s**	**10s**	**15s**
	Temperature °C	**25**	**30**	**40**	**50**
	Growth of fungal colonies				
Aspergillus niger		+++++	++++	++	**-ve**
Aspergillus flavus		+++++	++++	++	**-ve**
Aspergillus fumigates		+++++	++++	++	**-ve**
Penicillium chrysogenum		+++++	++++	++	**-ve**
Alternaria alternate		+++++	++++	++	**-ve**

Results represent the intensity rate of growth in three replicates*

-ve No growth ++ medium growth +++++ Dense growth

The results obtained from these experiments, using continuous power application of 2450 MHz microwaves radiation at the different time of exposure, indicated that the microwaves apparently produced lethal effects on the examined fungi by heat generated during microwave exposure. Lethal effects of tempreture (40-50°C) and above , lead to absence of any growth of fungal colonies .[3,7](table3).

It has been determined that rapid inactivation of each examined fungi were observed in the first 15 seconds. Before this period no considerable change was observed on the colonies total number .

The results demonstrate that the maximum destruction level occurred in a short time.The rapid inactivation process is a significant parameter for large scale application in industrial request. Similar fast bacterial suspension inactivation trend 100 seconds by microwave effect on *Staphylococcus aureus* was reported in aprevious study[17].

The mechanism of destruction of microorganisms through microwaves is controversial. Some have stated that inactivation of microorganisms by microwave is entirely by heat, through the same mechanisms as other biophysical processes induced by heat, such as denaturation of proteins, nucleic acids or other vital components, as well as disruption of membranes . This result agreement with other studies [18]. Microwave destruction of many microorganisms has also been reported, including:
Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, E. coli, Enterococcus, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteridis, Salmonella sofia, Proteus mirabilis and *Pseudomonas aeruginosa, Aspergillus niger[19], Penicillium* and *Rhizopus nigricans* [20]. No pathogen has been reported to be microwave resistant [18].

There was no serious damaging effects were observed when fungal colonies were treated for 5 sec at 30°C and for 10 sec at 40°C. While the treatments for 15 second
at 50- 55°C , using microwave radiation at 2.45 MHz, have had a lethal consequence for all five types of fungal strains.

The duration of exposure to microwave radiation were few, where confined between (5sec , 10sec, 15 sec) for each isolate that caltivated on Sabouraud dextrose agar (SDA) media , the reason is because the tempreture that generated during the period 15 seconds of exposure was very high. Which led to thaw (SDA) media and fusibility the agar material ,so we can not increase the period of exposure more than 15 sec in the microwave oven. (figure6).

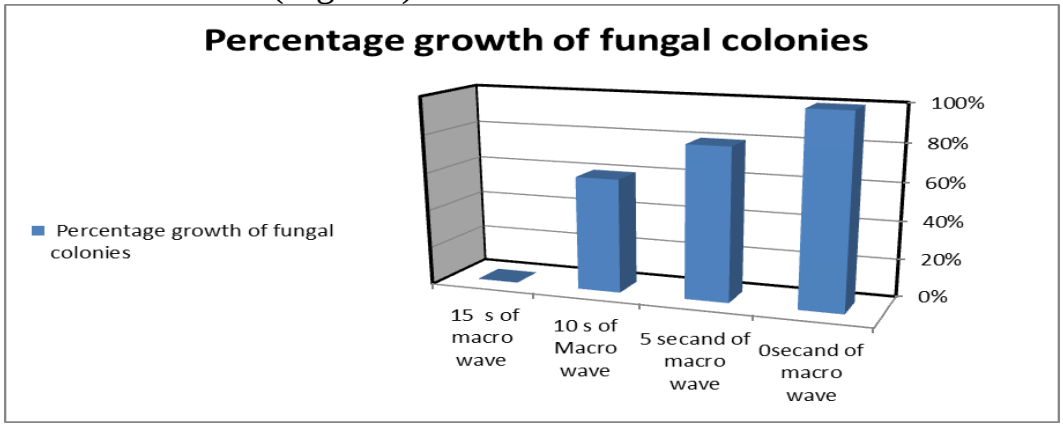


Figure 6 : Show percentage growth of fungal colonies comperative with microwave time exposure.

Table 4. Show the Diameter of growth colonies (mm)

microorganisms	Time of exposure to microwaves	0 (control)	5s	10s	15s
	Temperature °C	25	30	40	50
	Diameter of growth colonies (mm)				
Aspergillus niger		50	50	15	0
Aspergillus flavus		45	40	14.5	0
Aspergillus fumigates		45	40	15	0
Penicillium chrysogenum		*40*	40	14	0
Alternaria alternate		45	40	15	0

Results of statistical analysis showed that there were significant differences($P \leq 0.05$) between microwave time exposure and diameter of the growth colonies. And can observe a significant reduction of the colonies number as the microwave time exposure is increased. .

CONCLUSIONS:- 4-

We can concluded that the microwave radiation can have significant effects on the growth of fungal cultures , which can vary from killing of microorganisms to enhancement of their growth. The nature and extent of the effects depend on the microwave frequency and the total energy absorbed by the microorganisms. It seems that low energy, low frequency microwaves enhance the growth of fungi While high energy, high frequency microwaves, destroy fungi.

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دراسة تأثير اشعة الموجات المايكرويه في نمو بعض العزلات الفطريه

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المستخلص:-

وضحت هذة الدراسه مدى تأثيراشعة الموجات المايكرويه ذات التردد (a 2,450 MHz) على خمسة عزلات فطريه هـــي *(Aspergillus flavus , Aspergillus niger, Aspergillus fumigates, Penicillium chrysogenum, and alternaria alternate*) تـــم تعريض الفطريات المزروعه على وسط السابروبد دكستروز اجار(SDA) بمعـــدل ثلاثـــة مكررات لكل عزله فطريه ، الى موجات فرن المايكروويف المايكرويه وباربع فتـــرات زمنيـــه (٠ثانيه (control) ٥ ،١٠ ، ١٥ ثانيه) . و اظهرت النتائج نمو المستعمرات الفطريه لكل العزلات المستخدمه في الدراسه والتي تعرضت للموجات المايكرويه للفتـــرات الزمنيـــه (٥ ثانيه ، ١٠ ثانيه) . وقتل جميع المستعمرات الفطريه التي تعرضت للموجات المايكرويه للفترة الزمنيه (١٥ ثانيه). وبهذا اثبتت الدراسه مقدرة الموجات المايكرويه وللفترة زمنيه (من ١٥ ثانيه) فما فوق على قتل المستعمرات الفطريه وعدم السماح لنمو الابـــواغ الفطريـــه ، اي ان نسبة نمو المستعمرات الفطريه تتناسب عكسيا مع طول الفترة الزمنيه من التعـــرض لاشـــعة الموجات المايكرويه .