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Study the effect of ozone gas and ultraviolet radiation and microwave on the degradation of aflatoxin B1 produce by Aspergillus flavus on stored Maize grains

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Abstract: *This study was conducted in the plant protection department / College of Agriculture / University of Baghdad to evaluate the efficiency of physical agents (ozone, ultraviolet radiation , and microwave oven) on inhibiting Aspergillus flavus growth and destroying the mycotoxin that produced in corn seeds . An isolate af A.flavus producing Aflatoxin B1 was obtained from plant protection deptment college of Agriculture University of Baghdad .the results showed that hight significant reduction in A. flavus growth between treated and non-treated cultures for all the agents used was observed . The exposition of A. flavus culture to ozone at 2 and 4 g/min . 5, 10 , 20 mins. induced inhibition pereentages of fungal growth 82.16 , 83.30 , 79.96 % and 78.47 , 81.60 , 78.83 % respectively, to 0.00 % in control treatment . It was found that the more efficient concentration is 2 g / min for to mins . The inhibition percentages of A.flavus growth were found to be 84.73 , 88.50 , 92.53 % and 81.46 , 94.40 , 87.37 % when the fungal culture exposed to Ultraviolet radiation at wave length 240 and 365 nm for 30 , 60 , 120 mins . compared with 0.00 % in control treatment. The best time exposition was found to be 120 mins. at 240 nm and 60 mins. at 365 nm. The biomass weights of A.flavus growth were estimated to be 0.12 ,0.30, 0.42 g and 0.21, 0.38 , 0.44 g when a fungal culture exposed to microwave for 5 , 10 , 20 seconds at 80 C^o and 100 C^o respectively, compared with 0.26 g in control treatment . The best treatment of each agents was chosen for storage experiment to evaluate the ability of these agents to destroy Aflatoxin B1 produced by A. flavus in contaminated corn seeds .*

Keywords*: Aspergillus flavus , Inhabtion , Aflatoxin , Ozone , U.V light , Microwave .*

I. Introduction

The contamination of poultry and animal diet with mycotoxin is one of the most serious problems confronting breeders in several countries in the world (26).

Corn , zea mays , seeds , main constituent of animal and poultry diet , which are suitable for growth of fungi producing mycotoxins were considered the main source of diet contamination with mycotoxins (1) .

The contamination of corn seeds with mycotoxins may happened in the field or during transport and storage through exposition to damage by mechanical or biotic agents forming points for entrance of fungi producing mycotoxins into the seeds . The hight contents of carbohydrates and fats making the seeds suitalele For fungi producing mycotoxin growth especially Aspergillus flavus and producing aflatoxin in the storage where the temperature and humidity were suitalele for its growth (2,19,20) .

Contamination of corn seeds with aflatoxin represent very dangerous problem for animals and humans health that causing growth reduction , reduc production , decrease of enzymatic activity , weaken the tmmune system and causing cancer at law concentrution (3,13) .

Several studies indicated that aflatoxin cause enormous problems for poultry breeding including death of small ducks and chicks , Vulnerability , abortion , diarrhea , nusea , deterioration of eggs production and prepare for disease infection (9,22) .

Several straligies were adopted to avoid the contamination of corn seeds with mycotoxins or minimize its production in different laboratories in the world by using chemicals and biological treatments with some success (12) . This study was conducted to evaluate the activity of ozone , ultraviolet and microwave to restrict the growth of A. flavus in corn seeds and reduce the production of aflatoxin B1 as well as its degradation .

II. Materials and Methods

Fungi isolates

Aspergillus flavus isolats were isolated from corn seeds collected from college of Agriculture – university of Baghdad / Iraq . The seeds were surface sterilized with sodium hypochloride 2% for 3 minutes , washed with distilled water , dried on filter paper and cultivated on potato dextrose agar (PDA) medium amended with anti – bacterial chloromphemicol 250 mg /L in petri plates of 9 cm diameter (5 seeds / plate) . The plates were maintained at 25 ± 2 C^o for 7 days . The fungus was purified and identified as described by (7,17) .

Ability of A. flavus isolates to produce aflatoxin B1 on rice seeds

Rice seeds (150 g) were added to 100 ml distilled water in 500 flask and autoclaved at 121 C^o at 1.5 kg/cm^2 for 20 min. twice for two successive days. The seeds then were inoculated with 4 discs of 7 mm diameter from A. flavus culture 7 days old on PDA . The flask was agitated for homogenization and incubated at 25 ± 2 C^o for 21 days . the seeds were oven dry at 50 C^o and ground (25) .

Ability of A. flavus isolates to produce aflatoxin B1 in yeast extract succcrose broth (YESB)

yeast extract succcrose broth (20 g yeast extract , 200 guccrose in one litter dustilled water) was distributed in 500 ml flasks for 20 min. The broth in each flask was inoculated with 2 discs of A. flavus isolate from 7 days old culture on PDA and maintained at 25 ± 2 C^o for 14 days (10) .

Aflatoxin B1 extraction from rice seeds

The afla B1 was extraction as described by (6) . Hundered ml of Acitonitral – Water (90:10) mixture were added to 25 g of contaminated rice seeds powder in 500 ml flask . The flask was shaked in electric flask shaker for 30 min. and passed through whatman filter paper No.4 . The filtrate was transferred into 500 ml separating funnel containing 25 ml of hexan and the funnal was agitated for for 30 seconds and let to settle for one minute . twenty five ml destelld Water , 8 ml sodium bicarbonate and 25 ml of chlorofom were added to the lower layer and let to settle for 3 minutes . The upper layer was transferred into 500 ml flask containing 7.5 ml HCL and 10 ml chloroform . The flask was agitated and let to settle for 1 min . The lower layer was passed through whatman filter paper containing hydrated sodium sulfate to eliminate water and the filter paper was oven dried at 50 C^o . The dried material was dissolved in one ml of Acitonitril – Benzen mixture (2 : 98) and conserved in small vials under freezing .

Aflatoxin B1 extraction from (YESB)

YESB containing afla B1 was passed through whatman filter paper No.4 . Twenty five ml of the filtrate were added to 100 ml of chloroform in 500 ml separating funnel . The funnel was agitated and let to settle . The lower layer was taken and passed through whatman filter paper No.2 containing hydrated sodium sulfate and oven dried at 50 C^o . The dried material was dissolved in 5ml chloroform , distributed in small vials and conserved at – 18 C^o (6) .

Delection of afla B1

The delection of afla B1 was done by Enzyme – linked immunosarbent assay (ELISA) test using specific kit purchased from Biotechnology company Ltd Quicing . Fifty microliter of each sample were transferred into each well of ELISA plate previously coated withanti-afla B1 polyclonal antibodies and 50 ml of 0 , 0.1 , 0.3 , 0.9 , 0.27 , 0.81 ppb standard afla B1solution were transferred into other wells for control . Fifty microliter of Enzyme – Linked anti – afla B1 antibiodies were added into each well and left for 30 min . The wells then washed thoroughly with washing buffer diluted with deionized water (1:9) . Fifty microliter of substrate A and 50 ml substrate B contacting chromogen were added into each well and left in the dark at Laboratory temperature for 20 min . The abosobance of ELISA reactions were estimated by ELISA – reader at 450 nm . The standard curve of afla B1 was plotted and the concentration of afla B1 in the samples was calculated by the following equation (21) :

$$\text{Relative absorbance} = \frac{\text{Absorbance standard (or sample)}}{\text{Absorbance zero standard}} \times 100$$

Activity of ozone , Ultraviolet light , and microwave radiation in inhibition of A. flavus

Ozone treatment

A culture of A. flavus on PDA was exposed to ozone gas by wall mound incorporated ozonizer 2 , 4 g/min . for 5 , 10 , 20 min with 3 replications . A culture of the fungus non – treated was used as control (27) . The inhibition percentage was lulculated by the following equation :

$$\% \text{ inhibition} = \frac{\text{colony diameter in control} - \text{colony diam. In treatment}}{\text{colony diam. in treatment}} \times 100$$

U.V treatment

A culture of A. flavus on PDA was exposted to u.v light at 240 and 360 nm for 30 , 60 , 120 min for each with 3 replication non – treated culture was used as control and the inhibition percentage was culculted as befor (4) .

Microwave treatment

A culture of A. flavus in czapet broth was exposed to microwave radiation in oven type NM3850DGS , Capacity of 1000 W , at 80 and 100 C^o for 5 , 10 , 20 Sec. with 3 replication for each time . Other cultur non – exposed was used as control . The culture were maintained at 26 ± 2 C^o for 16 days and the biomass of the fungus growth was calculated . In other trial crude afla B1 was exposed to microwave radiation for 5 , 10 , 20 sec. to show the effect of microwave on toxin degradation .

Results and Discussion

Efficiency of A. flavus isolates to produce to produce aflatoxin

It was found that all A. flavus isolates obtained were able to produce aflatoxin as proved by ELISA technique . The rice seeds were found more effective than YESB medium , 1.02 and 0.1 mg / kg respectively . The more effective isolate producing aflatoxin was referred to as H-Lab toxin and used in the next experments .

Activity of ozone on A. flavus growth

Results obtained showed that exposition of A. flavus culture to ozone at 2 g/min. for 10 minutes has exerted high inhibition effect on A. flavus growth with inhibition percentages attaind to 82.16 , 83.30 , 79.96 % for 5 , 10 , 20 minutes of exposition respectively compored with 0.00 % in control . This days of ozone was found more effective than 4 g/min which gave 79.47 , 81.60 , 78.83 % of inhibition for 5 , 10 , 20 min of exposition respectively . Table (1) . Fig(1) .

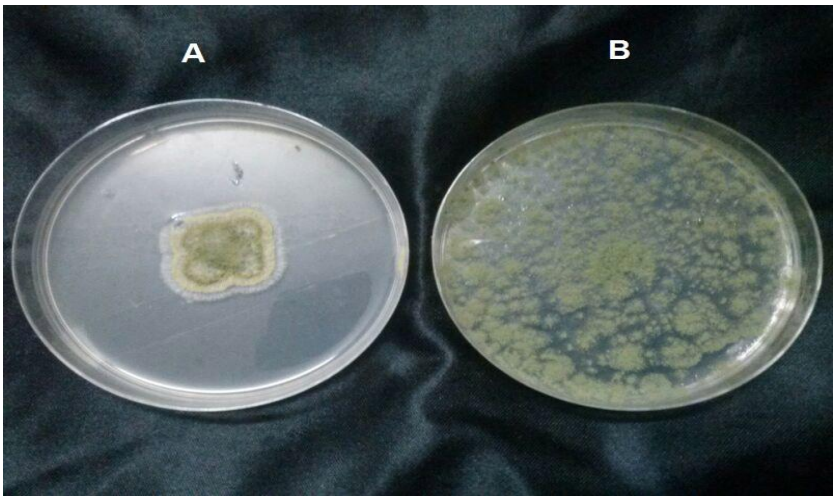


Fig (1) Effect ozone gas at 2 g/min for 10 minutes .

A : Control

B : Treatment with ozone

Table (1) . Effciency of ozone gas on the growth A. flavus

Gas ozone g/min	Time			Mean %
	5	10	20	
2	82.16	83.30	79.96	81.81
4	78.47	81.60	78.83	79.63
Control	0.00	0.00	0.00	0.00
Mean %	53.54	54.96	52.93	…
L.S.D	4.526	4.526	7.839	

P = 0.05

The effect of ozone on fungal growth may became from its effect on cell membrane and other organells in the cytoplasm leading finally to kill the fungal cells . ozone may also interact with DNA and changing its transcription and protin synthesis leading to variations of many metabolism activity and reduce cell division . Several studies indicted that ozone induce breeks in cell membrane and protoplasm leading to inhibition growth of bacteria , virus , parasites and fungi in addition to its effects on DNA , protins and fatty acids (8,14,23,30) .

Activity of u.v light on A. flavus growth

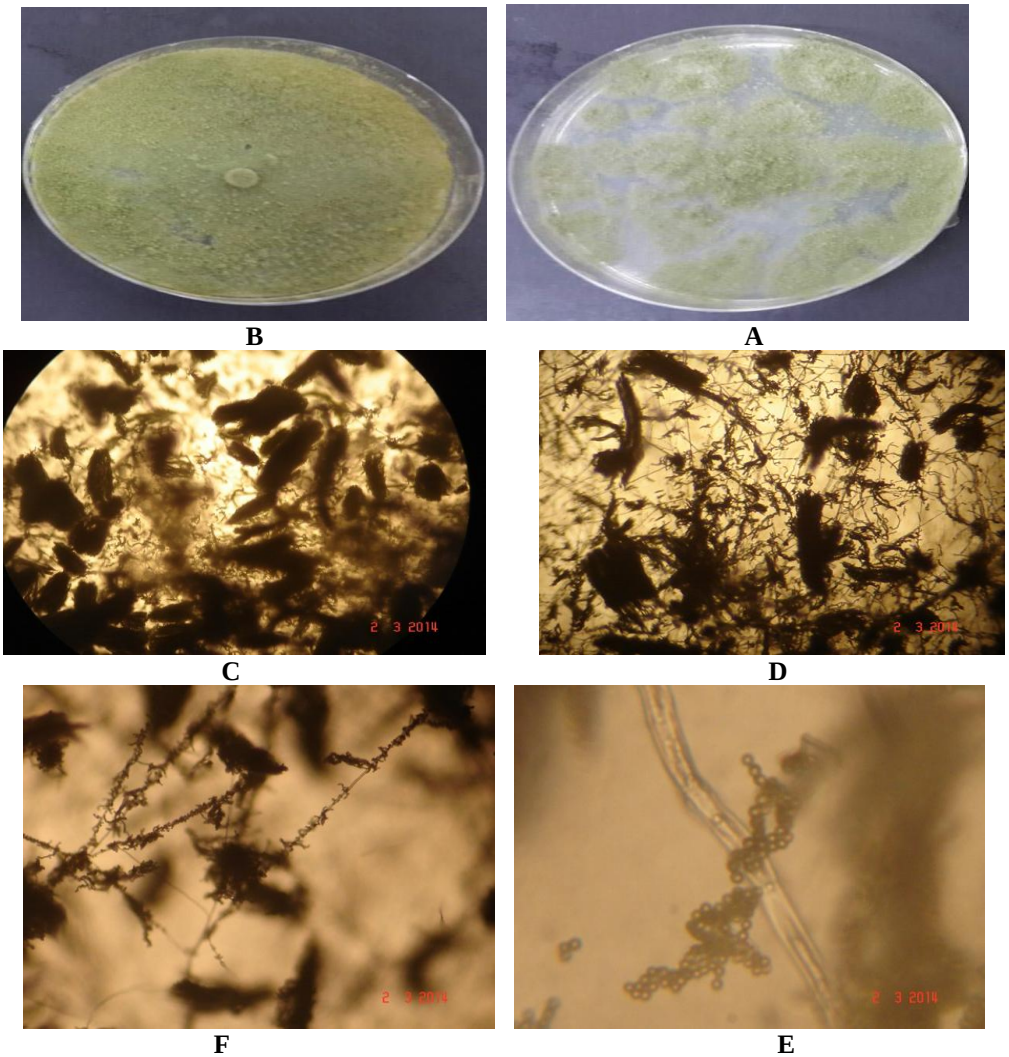
It has been found that exposition of A. flavus culture to u.v light at wave lengh 220 nm and 365 nm caused high reduction in fungal growth with inhibition percentage attained to 84.73 , 88.50 and 90.50 % for 30 , 60 , 120 min. of exposition respectively . Table (2) Fig (2) , (3) .

The activity of U.V light against fungal growth may came from the effect of UV light on nucleic acid (DNA and mRNA) , and proteins (including enzymes) which interfere with cell metabolism and forming compound totic for fungal cells . It was reported that U.V light inhibit fungal spores germination (29) , and disturbed cell metabolism and exerted some effects on mRNA and proteins in the cell (18) . (24) indicated that U.V light interact with compounds containing S-H inducing the formation of complexe causing damage of cell through interfering with certain enzyme work .

Fig (2) . Efficiency of ultraviolet light in inhibition of A. flavus growth

Wavelength nm	Time (min)			Mean %
	30	60	120	
220	84.73	88.50	92.53	88.59
365	81.46	94.40	87.37	87.59
control	0.00	0.00	0.00	0.00
Mean %	55.40	60.97	59.97	….
L.S.D	4.274	4.274	7.403	

P = 0.05



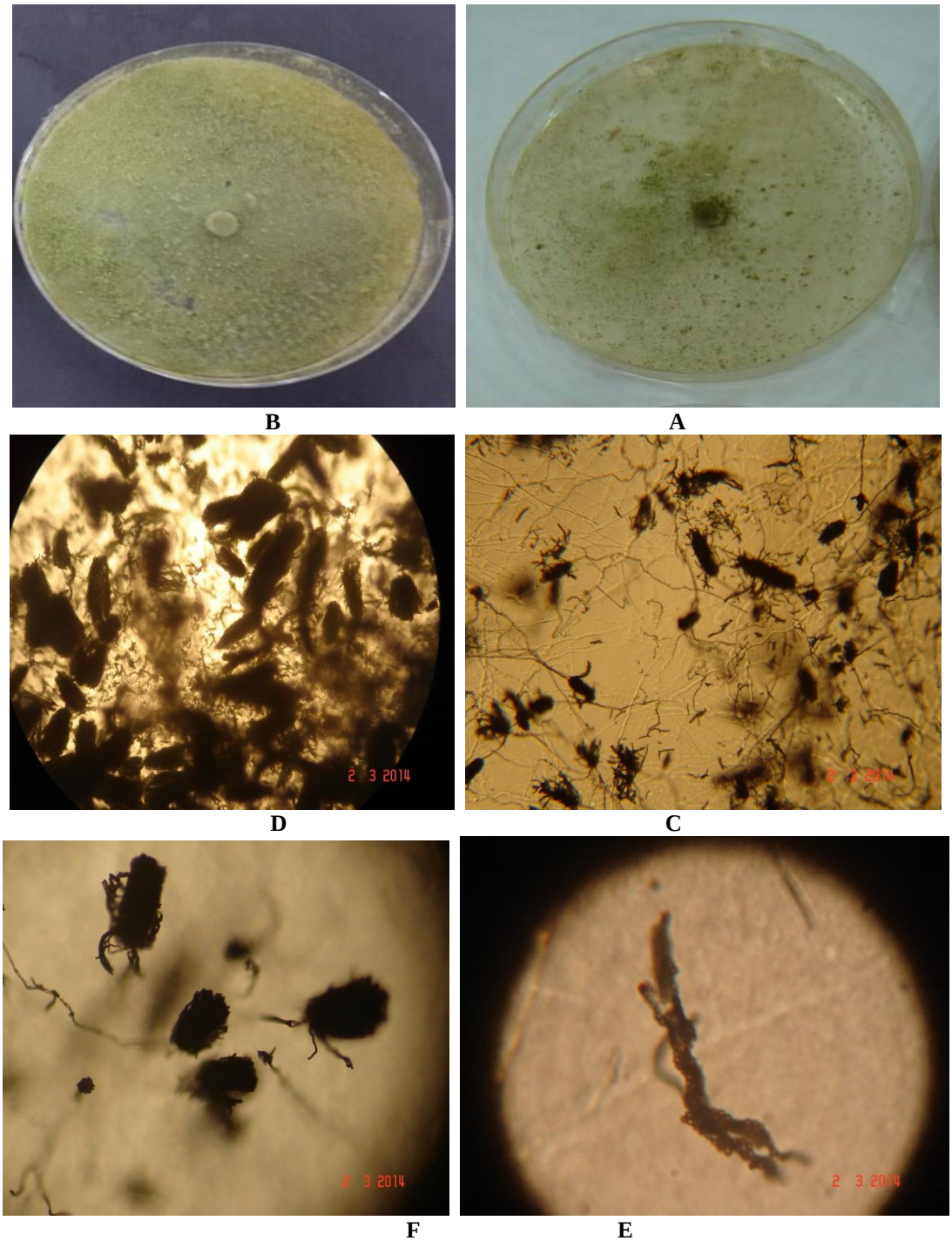
B **A**

C **D**

F **E**

Fig (2). effect of ultraviolet light at 365 nm to fungi A.flavus

A: Control **B: UV light treatment**
C: Control under the microscope 10X **D: UV treatment under a microscope X10**
E, F: UV treatment under a microscope 40X



B **A**

D **C**

F **E**

Fig (3). effect of ultraviolet light at 240 to fungi A. flavus

A: Control **B: UV light treatment**

C: Control under the microscope strongly 10X **D: Treatment under a microscope 10X**

F, E:Treatment under a microscope strongly 40X

Activity of Microwave radiation on A. flavus growth

Results showed that exposition of A. flavus culture to microwave at 80 C^o and 100 C^o for 5 , 10 , 20 seconds caused significant reduction in fungal growth as proved by reduction in fungal biomass . The more effective period of exposition found to be 5 min with biomass 0.12 and 0.21 g compared with 0.30 , 0.33 g and 0.42 , 0.44 g for 10 and 20 sec of exposition at 80 C^o and 100 C^o respectively and 0.26 g in control treatment .Table (3) . Fig (4) .

The effect of microwave radiation may came from that , microwave cause increase temperature which effect the transcription of DNA to mRNA and inhibited synthese of proteins , including enzymes , leading to

stope several metabolism activities in the cell , leading finally to cell death . It has been reparted that thermal energy caused by microwave radiation effect enzymes and nucleic acid in addition of damaging cellular membrane (5). (28) indicated that electromagnetic wave cause breakdown the link between amino acids in the protein , other studies reported that microwave radiation is able to ? cell wall and effect fatty acids in cell membrane as well as destroying fungal spores (11,15) .

From the results of this study we concluded that both of ozone , u.v light , and microwave radiation were highly restricted A. flavus growth on culture media which may be promissing to manage the cotamination of cereal seeds with aflatoxin B1 .

Table (3) efficiency of microwave radiation on the biomass of the fungus A. flavus

Microwave radiation /tempture C°	Times / sec	Mean biomass / g
80	5	0.12
100		0.21
80	10	0.30
100		0.38
80	20	0.42
100		0.44
Control		0.26
L.S.D		0.05

P = 0.05

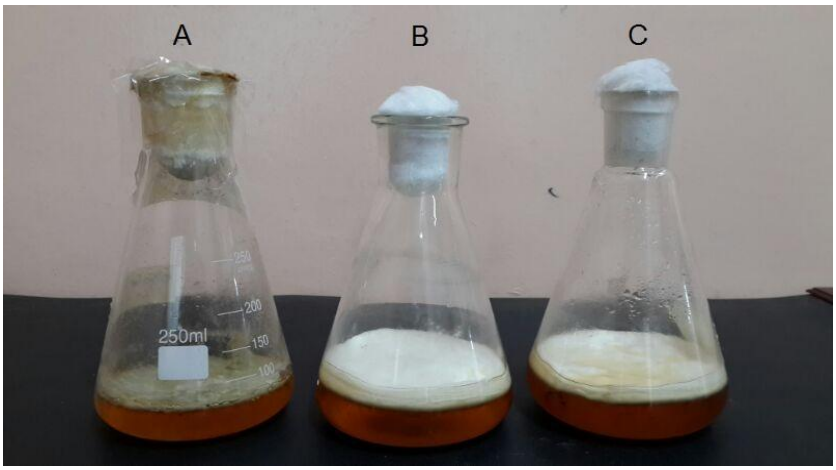
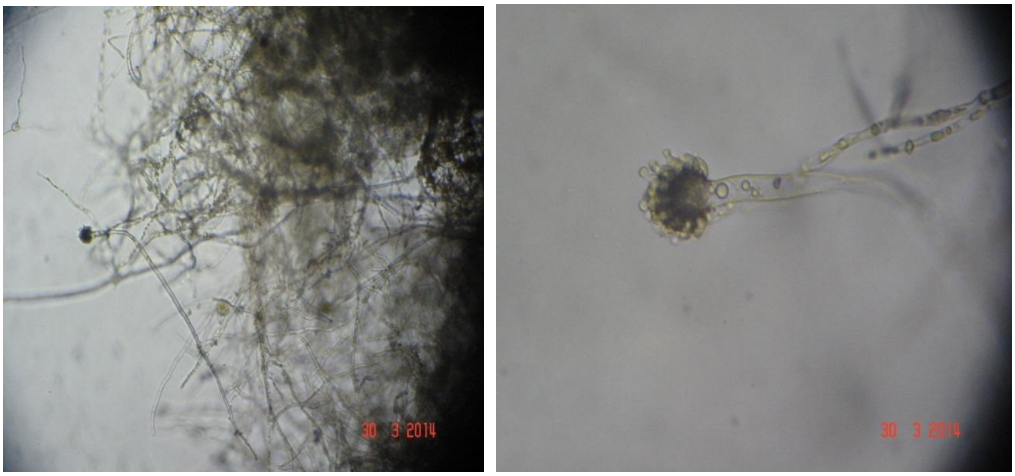
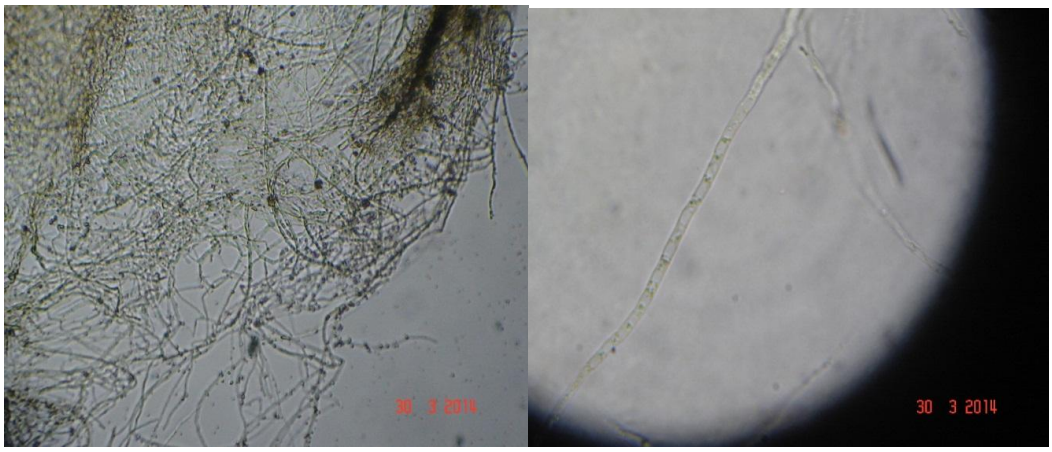


Fig (4). The effect of microwave radiation on the fungi A. flavus

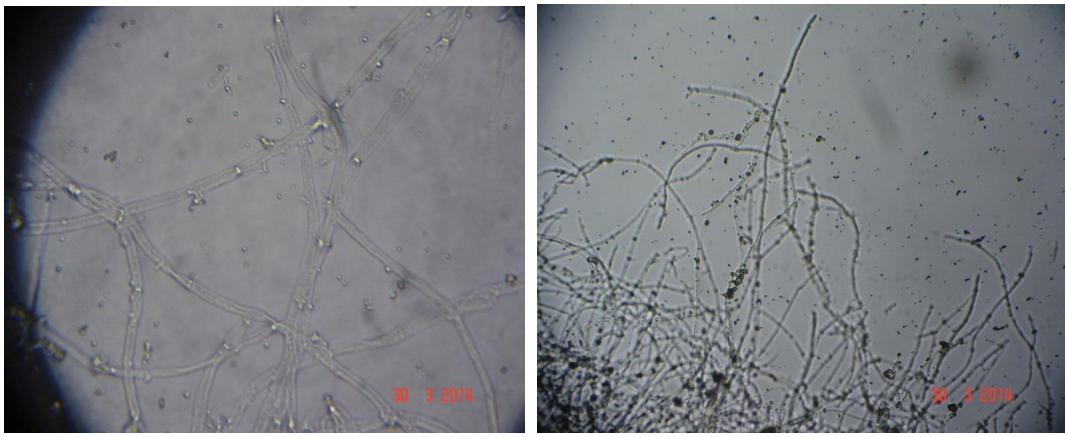
A: Control
B: Treatment of microwave 100 C° for 20 sec
C: Treatment of the microwave 80 C° for 20 sec



B **A**



C **D**



F **E**

Fig (5) Effect of microwave radiation on the fungi A. flavus

A: Control 5 X **B: Control 40X**
C: Treatment of 80 C^o 5 X **D: Treatment 80 C^o 40 X**
E: Treatment of 100 C^o 5 X **F: Treatment of 100 C^o 40 X**

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