The role of *Fusarium* and *Alternaria* in the bio-degradation of round up pesticide

The present study aimed to investigating the role of soil fungi in the biodegradation of the round up pesticide . The study focused on two of the fungi , *Alternaria* and *Fusarium* which were isolated from soil . The used concentrations of round up were (0, 30,40,50,60,) part per million added to samples of soil tainted with the fungal inoculums . The samples were then incubated at 27 cº for (0,3,6,9) days at the end of which the pesticide was extracted and the residues of it was estimated through the Gas chromatography method .

The study also investigated the influence of some other factors on the two fungi mentioned above and its role in the biological degradation of round up . The factors included temperature , pH , amount of fungal inoculums used and the effect of pesticide on the fungi at solid media. Results showed that the two fungi have significant ability in degradation of the pesticide especially when using 40 ppm in of the *Fusarium* and 30 ppm for *Alternaria* .The two concentrations resulted in the percentages of degradation (%19.25 and %13.33) respectively . The optimal temperature for degradation was 25c° and pH was 7 while the amount of fungal inoculums correlated positively with the two fungi s ability in enhancing pesticide degradation .It was also found that the two fungi are

able to withstand the low concentrations of pesticide using it as food source while high concentrations lead to the prohibition of the growth of the two fungi .

Introduction

Weeds pesticides are chemical compounds to eliminate harmful weeds which compete with plants for food source hence causing economic loss . A century and a half ago , Petroleum , some organic acid and copper were used as pesticide to eliminate needs from railways and street and with the passage of time new more effective pesticides were found[(1)] .

Round up is a world wide weeds Pesticide used to eliminate both wide and narrow leaves needy especially those with deep roots such as reed and halfago , and it is absorbed by the plant and transmitted to other parts . This pesticide is also called Glyphosate, its (C3 H8NO2) N-Isopropylamin-N-Phosphonomethyl –Glycine , and its one the amino phosphorous organic compounds . This pesticide is characterized by its toxic effect on digestive tract and its ability to aggregate and deposit in duodenum [(1,3)]. The USA was the find to produce this pesticide and when makes largest consumption of it . This pesticide is prohibits enzyme responsible for the synthesis of aromatic amino acids such as Tyrosine ,

Tryptophan and phenylalanine at the cellular level though there are some plants which resists this effect[1] .

A prerequisite feature of any good pesticide its ability to degradation into non- toxic material quickly and easily after it performs its purpose so that it would not hurt other untargeted beings and plants . Otherwise an pesticide would have catastrophic consequence on environment which might last for long time due to its gene transition[2] .

Fungi has a great effect in the biological breaking up of pesticide (round up) into carbon and the mineral element (phosphor) . This process depends on the structure of pesticide , temperature , moisture, ph , and the general environmental condition supporting the optimal growth of fungi . The species *Aspergillus , Fusarium ,Penicillium , Alternaria* Can break down many of Pesticide , [14]Ouhib *et al*.(2001) states that *A .niger* fungus can degenerate Malathion diacid and Malathion monoacid , while [2]Al-amry(2001) states that *penciillium* , *Fusarium* and *Rhizopus* can break up 2,4 – Dihlorophenoxy acetic acid pesticide . Researcher Schewette(1998) [11] gave a detailed account of the trend taken by the round up during the biological degradation .

The importance of this subject emanates from this need to protect environment and prevent hazards of pollution by round up using *Alternaria* and *Fusarium* .

The present study including the Isolating the two fungi *Alternaria* and *Fusarium* from soil ,testing the effect of round up on the growth of the two fungi invivo, studying the effect of *Fusarium* and *Alternaria* in the degradation if the round up using different concentration and in different times , studying the effect of temperature and pH on the degradation of the round up and studying the effect of the living mass of the two fungi on the degradation of the pesticide.

Material and Methods Material and Method

Soil samples collection

Soil samples were Collected from a farm in AL- Diwaniya at 5 different places randomly and in depth (0 -10) cm ,the Samples were then mixed in a plastic container and 1 kg amounts of each type were taken as replicates and placed in plastic sacs (polyethylene) to be tested in the lab[(13)] .

Fungus Isolation

The two fungi were isolated through the dilution method one gram of soil was solved in 99 ml water , then 1ml of it was added to 99 ml of distilled water and so until we reached the dilution 1×10^{4} .

One ml was taken from each dilution and added to the culture medium Sabouroud Dextrose Agar (SDA) which located in conical flask , already processed with 0.25 Chloramphenicol to prevents bacterial growth ,the antibiotic and dilution were added to the medium after cooling it to 45c°. The mix was then poured in plastic plates (9 cm diameter) which were marked and incubated under 27c° for seven days . The plates were inspected daily until colonies appear . The colonies were then purified by taking parts of them and growing them in new plates containing (SDA) .

Estimation of round up in soil Samples

100 grams soil Samples were sterilized by hot steam then cooled and processed with fungi spores inoculums already prepared by adding one ml distilled water to plates containing fungal colonies . The plates were then shaken well and filtrated via medical lawn the spores concentration was fixed to $1x10^{6}$ spores/ ml ,mixed with the fungal pesticide starting from 30 parts per million to 90 part per million. They were finally incubated under 72c° and stored at (2,4,6,8,10) days

respectively[8] and the pesticide was extracted through the following steps .

- **Extraction From Soil Sample**

The method in [10] was employed as follows:

1- The Soil was drayed in an oven under 35c° for one hour . 2- Fifty grams of the Soil was processed with 75 g of non aqueous Na_2SO_4 and then 100 ml . of acetone was added to the mix and the whole was put in an incubator cooled in 27° and cetrifuging in 200 round per minutes . 3- the solution was filtrated by using Whatman n.1 filter paper into a glass conical flask4- The deposit was subjected into a re-processed with Acetone and the process was repeated one more time ,the final filtrate received in conical flask . 5- The process was repeated adding 50 ml of acetone . 6- The last step was repeated again to get 300 ml of the final filtrate .

- Cleaning

This process aims at removing dyes and other material sticking on the deposit by making a chromatographic column ,its apipette (100 ml size) contain glass wool to prevent the passage grains of florisil from the lower hole of the pipit ,the next layer was 10 gram layer of florisil the

volume of each particle about 150 cubic ml . followed by a layer of activating coal to remove dyes . these layers were packed by pressing and shaking to achieve the necessary cohesion. A fine layer of glass wool was finally added . The completed column was then saturated by adding acetone (50m) in a funnel in the upper end of pipette. The solution from the extraction process In the previous item was passed through the aforementioned column and the filtrate was collected in a glass flask[2] .

- Condensing

The filtrate was condensed by using the vacuum rotary evaporator . the filtrate was put in this device under 40c^{o} water bath and 200 rotation degree and an unstable pressure until the last drop . the flask was finally washed by benzene (10ml) then concentration by the rotary evaporator device and the resultant matter was put in a well locked container under -4 c^{o} waiting for next test[10].

- **Estimating the amount of Round up through the gas chromatography.**

The amount of remaining pesticide was estimated by gas chromatography provided by pickering labs company according to method mentioned in Dorzad and Novak(2009) [8]. 0.5 microliter of the pesticide was injected via a microsyringe .It became possible to estimate

the amount of the remaining pesticide and compare it with the standard treatment after get chromatogram.

The effect of the pesticide on the tested fungi

The liquid culture synthesis medium was prepared as described in Ouhib *et al.* [14] from (0.1g) $ZnSo_4$.$7H_2O$, (0.1g)$MnCl_2O$. H_2O ,(0.1g) $FeSO_4$,. $7H_2O$ and (0.1g) $MgSO_4$. $7H_2O$. These material were solved in one liter of distilled water and the pH was modified to 6.8 ,after sterilizing the medium powered in glass flasks its size was 50 ml . Increasing concentrations of round up (0.00, ……..0.09,0.1) were then added and after , mixing well , the flasks were inoculated by discs of tested fungus (5ml diameter) and incubated under 27c° for seven days . the growth was observed very closely daily and finally the colonies were filtrated by Buchner funnel and the remaining part was dried by putting it in oven under 40° for overnight . At last , the dry colonies were weighed by a sensitize balance and result put down .

The Effect of the living mass amount of the fungi and temperature on the degradation of round up.

This effect was investigated by repeating the same steps above by using different amounts of fungal inoculums and incubation under different Temperature [6,12].

Results and Discussion

The degradation of the pesticide Round up by effect of two tested fungi:-

-*Fusarium* Fungus

The table(1) shows the role of the fungus in degradation the round up pesticide . These is a significant effect in all the concentrations used but the most effective one was 40 ppm which gave rise to a continuously increasing percentage of degradation reaching to 19.25% on the ninth day , the concentration 50 ppm comes next the degradation personates reaching 15.61 % on the ninth day .

Many studies showed that round up can degrade by the effect of soil microorganisms quicker that by water since the former produces larger amount of oxidizing enzymes such as peroxidase the result of the degradation is CO2 and Glyoxilate ,Aminomethylphosphonic acid (AMPA) the last compound keeps an degenerating into methylamine then Ammonia ,Formaldehyde and CO2 . AS for Glyoxilate , it degrade into Aminocids Carbohydrates , organize acids and CO2[(11)].

Table (1) the degradation of Round up by effect of *Fusarium* in different times.

Concentration ppm	3 days		6 days		9 days		Degradation %
	with	without	with	without	with	without	
30	29.1	29.6	28.5	29.3	26.3	28.1	12.31
40	37.5	39.7	35	37.8	32.3	36.8	19.25
50	44.3	50	43.9	49.5	42.2	48.5	15.61
60	59.2	60	58.5	60	57.1	59.7	4.83

- *Altenaria* fungus

The Table(2) shows the role of *Altenaria* in degrading round up .This fungus also accelerates the degradation of this pesticide but it is less effective than *Fusarium* . The most effective concentration was 30 ppm giving 13.33% percentage followed by concentration 40% which gave 3.5% . While other concentrations gave no percentage of degradation of the pesticide depends on a number of factors such as the nature of the pesticide , environmental conditions such as temperature , moisture ,pH and the microorganisms ability in producing the required enzyme.

High concentration of the pesticide can give bad negative result on environment because the living creatures cannot exploit it at source of hydrogen or nitrogen whish makes it toxic to them[(4)] .

Table (2) the degenerating of the Round up by effect of *Altenaria* in different time.

Concentration ppm	3 days		6 days		9 days		Degradation %
	with	without	with	without	with	without	
30	27.1	27.1	26.6	29.1	26	28.1	13.33
40	39.5	39.5	39.2	40	38.6	36.8	3.5
50	50	50	50	50	50	48.5	0
60	60	60	60	60	60	59.7	0

The effect of the living mass (mg) on the adequacy of the two fungi , *Fusarium* and *Altenaria* in degradation round up .

The figure (2) shows a positive relation ship between the amount of the living mass used and the adequacy of the fungi in degenerating the herbicide reaching to 74% with 1600 mg of *Fusarium* while reaching to 53% with the same amount of *Altenaria* . The increase in the mass used results in increasing the enzymes content hence accelerating degradation.

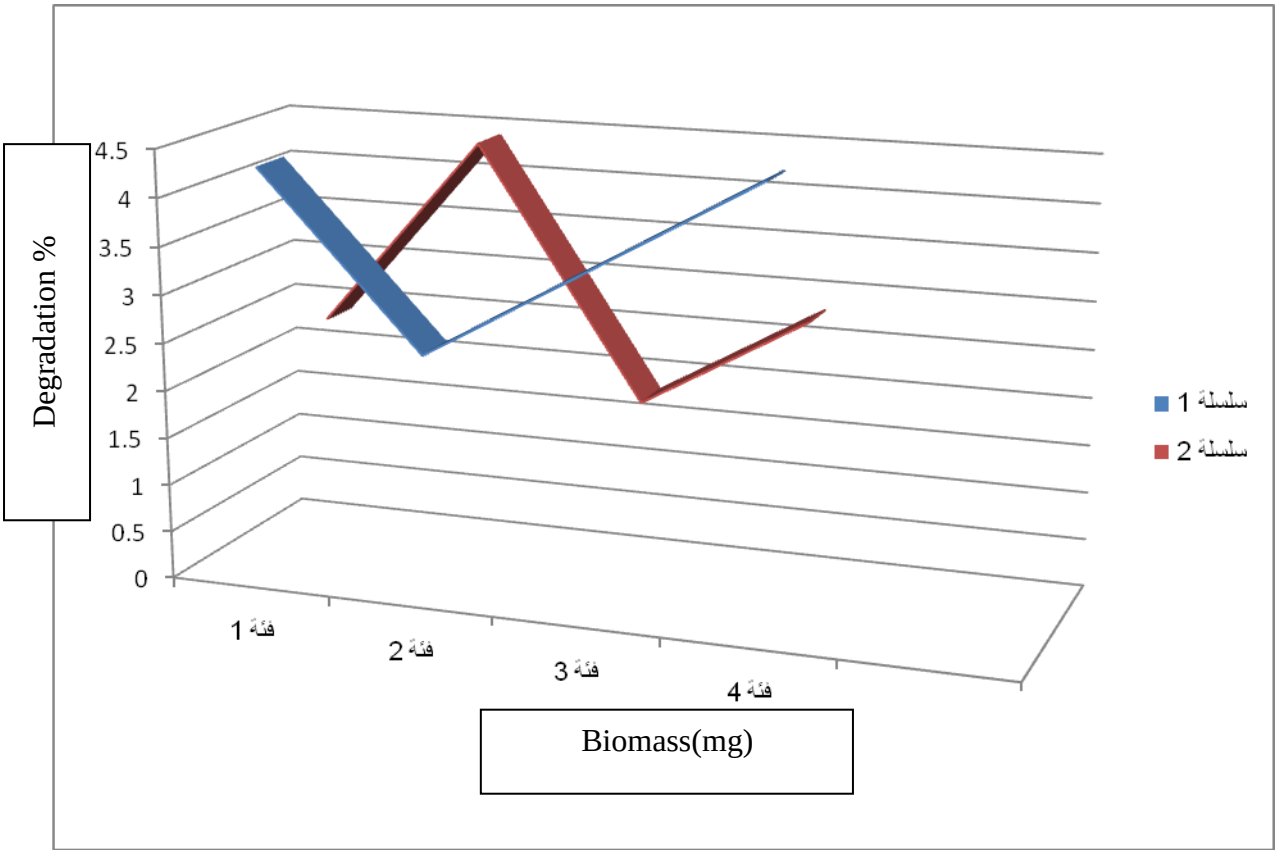

Figure (2) The effect of the biomass (mg) on the adequacy of the two fungi , *Fusarium* and *Altenaria* in degradation round up .

The effect of Temperature on the adequacy of the tow fungi *Fusarium* and *Altenaria* in degenerating Round up .

Difference in Temperature influence the ability of the tow fungi in degradation round up . The percentages of degenerating Increase with the Increase in Temperature reaching the optimum state at 25°c for both fungi (77% in Fusarium treatment and 52% in Altenaria treatment) . The increase after this became opposite , the ability of the fungus decrease with the Increase of temperature . this agrees with the result in

Abd-Naser and Sani(2008) [5] Who indicate that the Increases in temperature more 40° cause the total damage of the fungal enzymes.

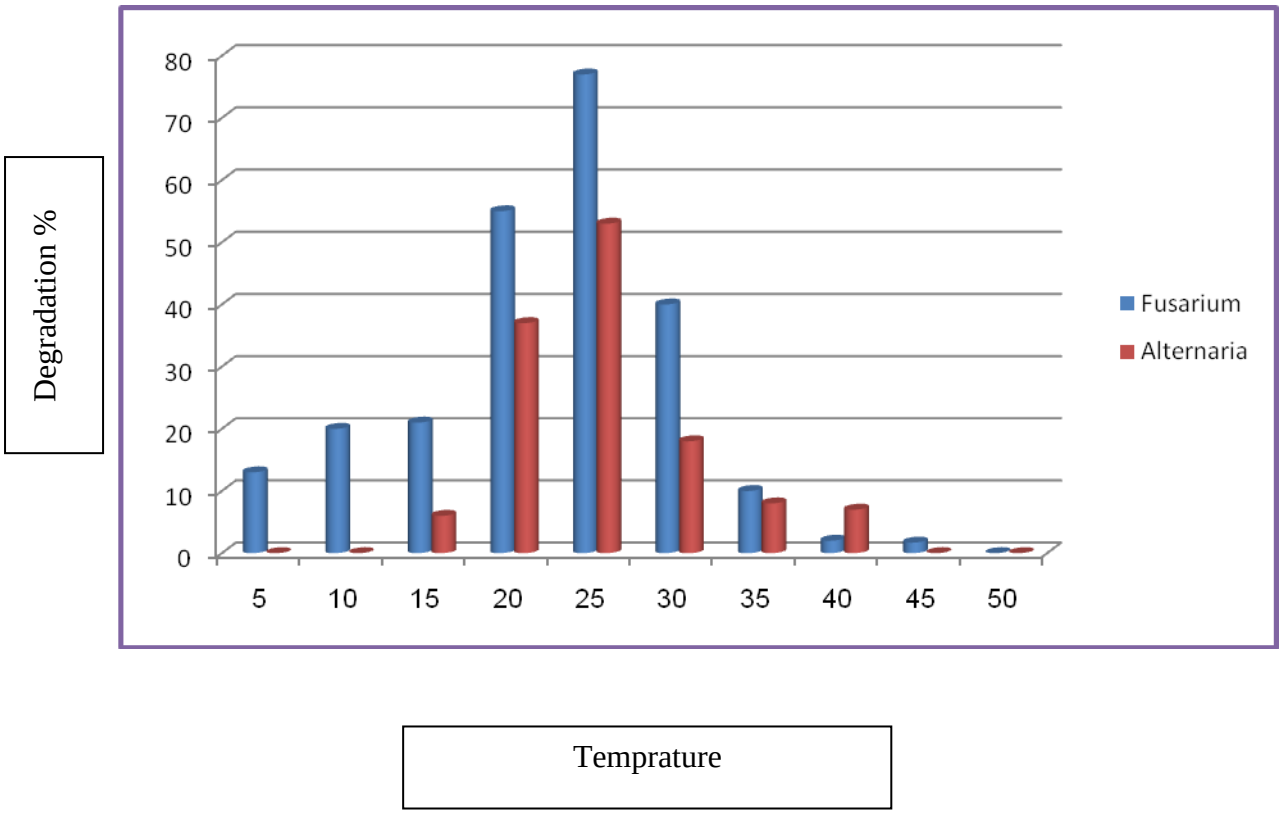

Figure (3) The effect of Temperature on the adequacy the two fungi *Altenaria* And *Fusarium* .

The effect of pH on the adequacy of the two fungi *Altenaria* And *Fusarium*

Extremist values of pH had no effect as compared with moderate values which enhanced the growth of the fungi. The optimal amount of *Fusarium* was attained with 7 pH value which gave a percentage of degradation 58%.

As for *Altenaria*, it showed 33% percentage with 7 pH value. This agrees with previous results that the optimum values of pH enhances the activity of the enzyme hence causing the increase in the place of the degradation of the pesticide[9,7] .

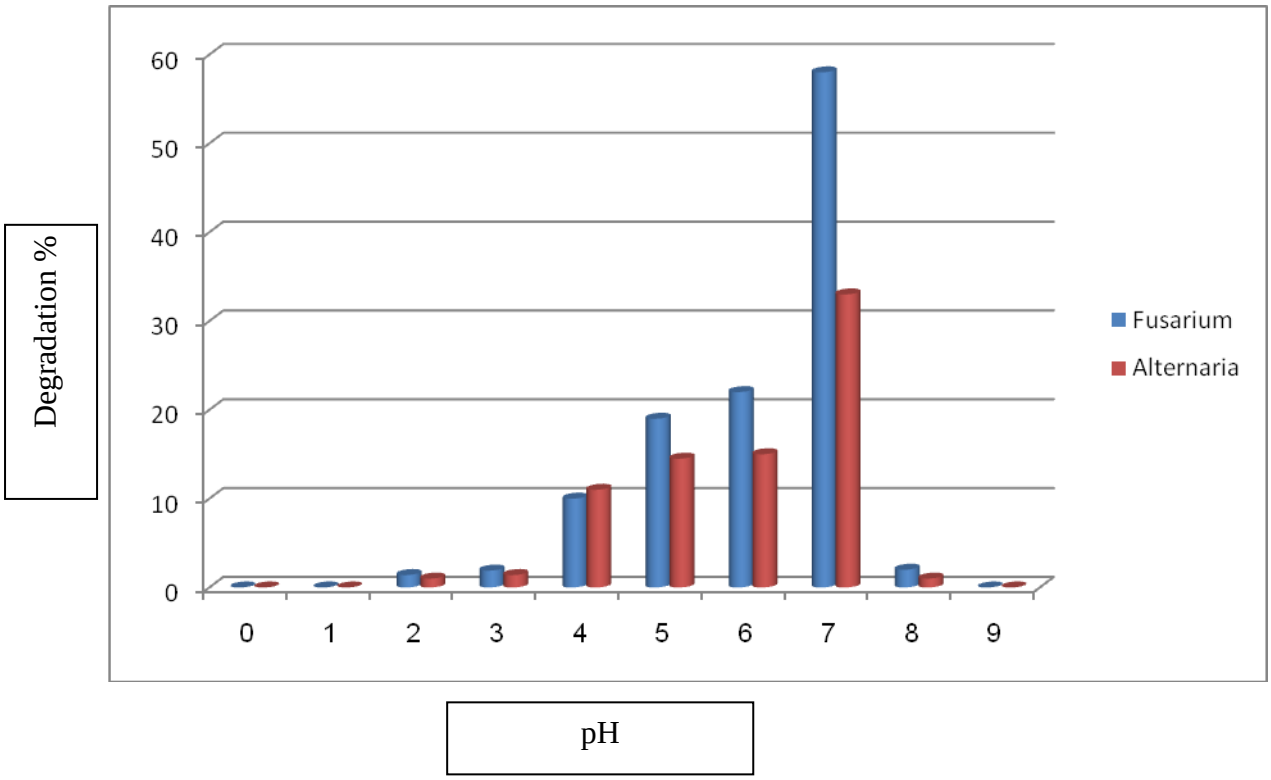

Figure (4) The effect of pH value on the adequacy the two fungi *Altenaria* And *Fusarium* .

The effect of Round up on the dry weight of Altenaria And Fusarium

Low concentration of the round up caused a significant increase in the dry weight of the two fungi . The concentration 0.04 % gave the height dry weight for *Fusarium* (37 mg) , while the control treatment

indicated (38 mg) ,then the dry weight went on decreasing with the increase in Round up concentration reaching Zero with the concentration 0.07% .

AS for *Alternaria* , it gave the best dry weight with concentration 0.55 % which gave (38.6 mg) white the control treatment indicated 20 mg .

The above result showed that there are several levels of round up that can be exploited by the fungus as nutritional source , but the increase in Round up concentration renders the fungus unable to do so , on the contrast , it starts to prohibit the growth of the fungus[3,!4].

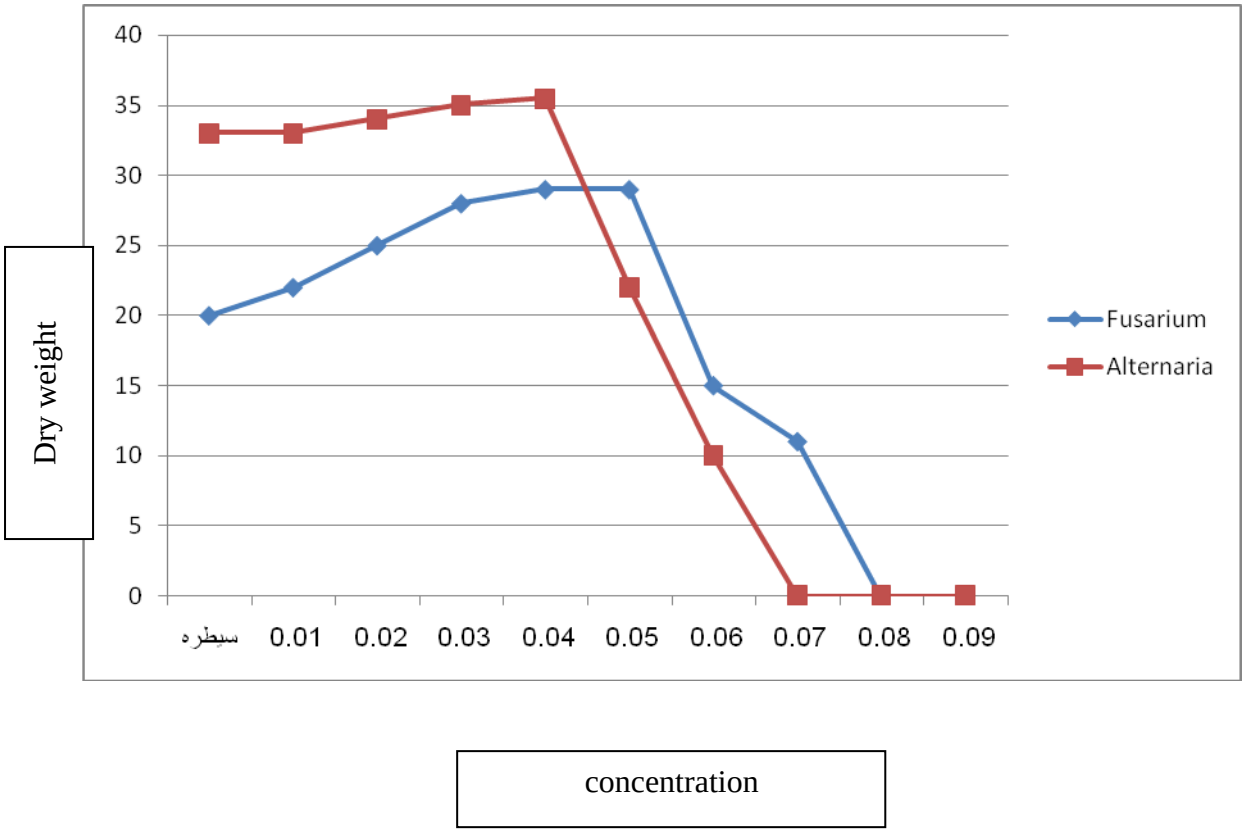

Figure (4) effect of concentrations of round up on dry weight of *Fusarium and Alternaria*.

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دور الفطرين *Fusarium* و *Alternaria* في التحلل البيولوجي للمبيد راوند اب

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الملخص

هدفت الدراسة الحالية الى التحري عن الدور الدي تلعبه فطريات التربة في التحلل البايولوجي للمبيد راوند اب (Round up) من خلال عزل الفطرين *Fusarium* و *Alternaria* من التربة ومن ثم التحري عن قدرة هدين الفطرين في تحليل المبيد اعلاه وقد تضمنت الدراسة إضافة تراكيز من المبيد هي (60,50,40,30,0) جزء بالمليون الى عينات من التربة ملوثة باللقاح الفطري ومن ثم حضن هده العينات في لمدد زمنية مختلفة (9,6,3,0) يوم بعدها تم استخلاص المبيد وتقدير المتبقي منه بطريقة الكروماتوكرافي الغازي (Gas chromatography) كدلك تم دراسة تاثير بعض العوامل في قدرة الفطرين اعلاه على تحلل المبيد الفطري منها درجة الحرارة والاس الهيدروجيني وكمية اللقاح الفطري كما تم التحري عن تاثير اظافة تراكيز من المبيد الى الوسط الغدائي السائل المنمى عليه الفطرين قيد الدراسة وقد

بينت النتائج بان هنالك قدرة معنوية للفطرين في تحلل المبيد راوند اب خصوصا عند التركيز 40 جزء بالمليون للفطر *Fusarium* اد كانت نسبة التحلل بمقدار 19.25 % وعند التركيز 30 جزء بالمليون للفطر *Alternaria* اد كانت النسبة 13.33 % وكانت درجة الحرارة الامثل للتحلل لكلا الفطرين هي عند 25 م ° والاس الهيدروجيني الامثل هو عند 7 اما كمية اللقاح الفطري فكانت دات علاقة طردية مع قدرة الفطرين في التحلل البيولوجي للمبيد ،وقد كان تاثير اضافة المبيد للوسط السائل المنمى عليه الفطرين متباينا اد ان الفطرين يستطيعان تحمل التراكيز الواطئة من المبيد مستفيدان منه كمصدر غدائي لكن التراكيز العالية تسبب تثبيط نمو الفطرين قيد الدراسة.