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Fate of Herbicide Granstar (Tribenuron Methyl) in Wheat Field in AI-Nasiriya Governorate

Ihsan Flayyih Hasan AI-Jawhari[1*] and Khudier Jouda Yasir AI-Seadi[2]
Department of Environment and Pollution, Marshes Research Center, Thi-qar University, Iraq
dr.ihsan_2012@yahoo.com

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Abstract

Eleven filamentous fungi isolated from the application field soil with tribenuron methyl herbicide. Aspergillus niger, Aspergillus flavus were more frequency with 88.8%, 77.7% respectively, while Penicillium funiculosum, Aspergillus ostianus and Aspergillus versicolor were moderate frequency with 55.5%, 44.4%, 44.4% respectively, but the remaining 5 fungal species were isolated with a very frequency with 22%, 11%. Also the results obtained that the total numbers of fungi were increased after one day from application with granstar herbicide and continue to increase with all weeks and the higher numbers of fungi calculated in fifth week, and the results also showed that the higher degradation of granstar was determined in this week. The results showed that A.niger was more resistance among other isolated fungi with all concentrations of (TBM) in solid PDA medium and the colony diameter of this fungus was equal with control (8.5cm), also the herbicide (TBM) not appear any effect on this fungus with all concentrations. However the results showed that TBM was inhibited A.versicolor 15%, 5% in 25, 50 ppm respectively, but the colony diameter was increased to 8.5cm when compared with control and TBM was inhibited A.flavus 35% in 75ppm concentration only, but this herbicide inhibited P.funiculosum 6%, 9% in 25, 50ppm respectively. The statistical methods showed a significant difference between isolated fungi, but no significant differences between concentrations were obtained. The results showed that TBM was inhibited the mycelial dry weight of A.niger with all concentrations in liquid mineral salts medium, and the inhibition percent reached to 9%, 13%, in 25, 50, 75ppm respectively, also the results showed that the inhibition percent was 2.0%, 31% in 25, 75ppm with A.flavus. However the results showed that the dry weight of A.flavus was increased in 50ppm concentration, and the mycelial dry weight of this fungus reached to 0.761gm when compared with control (0.700gm) and in same time the results showed that the inhibition percent was reached to 14% in 25ppm with P. funiculosum. Also the results showed that the mycelial dry weight of P.funiculosum was increase to 0.519 gm, 0.704gm in 50, 75ppm, when compared with control (0.511 gm). Only among with all fungi under study the mycelial dry weight of A.versicolor was increased in all concentrations of TBM, and also the statistical methods showed no significant differences between concentrations and in the same time this fungus show the higher ability to degraded granstar to other different compounds.

Keywords: Biodegradation**,** Filamentous fungi, Soil, Tribenuron methyl, Wheat.

Introduction

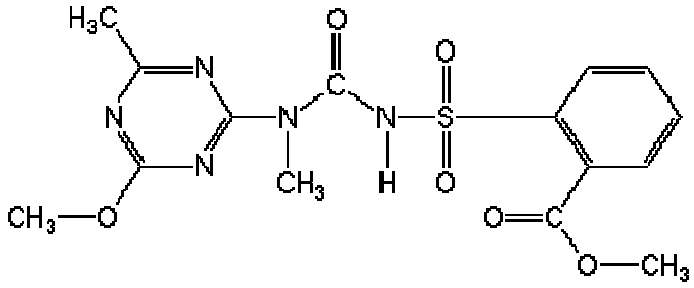
Tribenuron methyl (TBM) is a one of the sulfonylurea herbicide family and is widely used in weed control. This herbicide interred to Iraq last ten years. However sulfonylurea herbicide are to be highly efficient at allow dosage to sensitive crops[1,2]. TBM and with other metabolites was polluted soil and groundwater[3,4]. Although previous studies showed that only 25% from TBM was mineralized after 126 day in sandy soil[5].

Sulfonylurea compounds are limited in soil by many factors such as physical–chemical characteristic of soil, acidity, temperature, moisture content, as well as soil microorganisms[6,7]. Natural hydrolysis of TBM is slowly happened and can be speed up by increasing acidity and light irradiation[8].

Herbicides and their metabolites have negative effects on algae[9], invertebrates, ciliates, protozoa[10], fungi, actinomycetes and bactria[11,12]. Fungi can used to increased detoxification of polluted environment with herbicides[13] and fungi play in important role in the environment to removal and breakdown of many pollutant and in the same time herbicide propanil was cleavage to five metabolites by *Aspergillus niger*, *A.flavus*, *A.candidus*, *A.restrictus*, *Penicillium sp.*, *R.stolinifer*, *Trichoderma lignorum* and *T.harzianum* after treatment rice field[14]. However bacteria and fungi have different sensitivity to most herbicides and this sensitivity is also influenced by other factors. Microscopic fungi are more sensitive to herbicide in lower doses[15]. Many studies referred that sulphonylureic herbicides causes significant decreases in soil microbial population[16]. The purpose of the present investigation was to examine persistence of tribenuron methyl in wheat field soil, and to determine whether or not this application causes any toxic to soil, also the present investigation is the first study done around this herbicide in fields and studying effect on soil fungi in Iraqi agricultural fields.

Materials and Methods

Chemical: All chemicals used in present investigation are bring from BDH CO. and the purity was (98.99%), Tribenuron methyl known as 2[4-methoxy-6-methyl-1,3,5-triazin-2-yl methyl carbamoysulfamoyl] benzoate and the structure of this herbicide show in Scheme-1, tribenuron methyl purchased from local market under the trade name (granstar) – Italy.



Scheme-1
Structure of tribenuron methyl

Field application: The field experiment was conducted on a silt y clay loam soil (Table-1) at AI- Fadilyia area in AI-Nasiriya governorate-south of Iraq. A separted field (1 hectare) was seeded with wheat grains (*T.astevium*) in November, 2015. Granstar (tribenuron methyl) was applied field when the grass reached to 3-4 leaves stage at the recommended field dose of (20gm/hectare, dissolved in 200L water). The field was drained 2days before application and kept drained 2days after that. Soil samples were collected randomly in depth 5-20cm before 1h from application with granstar (tribenuron methyl) and put in plastic sac to mixing. The soil was then analyzed to estimate the moisture, pH, Electric conductivity, texture (Table-1) and also collected soil sample after 1h from application and after first day with this herbicide. Sampling was begun on the 7th day after application and continued at regular intervals (week after week), up to the 6th week and all samples were stored (under freezing) at (-18^{o}C) until analyzed to determined the residue of tribenuron methyl in soil.

Determination of tribenuron residue in soil: Extracted from soil sample**:** The method of analysis was adopted with minor modifications to method[17]. Soil samples (25gm) was dehydrated in oven with 50^{o}C during 30minute to reduce moisture and shaken with 50mL mixture (40mL acetonitrile:10 mL water) and after shaking 10minute with (150rpm) orbital shaker incubator. The sample was filtered by Whattman No.1 filter paper. The residue extracted secondly with mixture (40 mL acetonitrile: 10mL water) also the residue extracted thirdly with (20mL acetonitrile: 5mL water) and filtered sample was added to a chromatographic column to removal pigments. The filtered sample was evaporator in water bath until one drop and redissolved with 10mL acetonitrile and concentrated to (1mL final volume). The latter extract was then analysis by FTIR spectroscopy.

Table-1
Some physical and chemical characteristic to field soil

Studying characteristic	Degree
Sand	60 %
Silt	70 %
Clay	30 %
Texture	Silty Clay Ioam
pH	7.98
E.C	15.2 ds / m
Soil moisture	27.8 %

Isolation of fungi: Soil samples were collected (1h pre application, 1h after application, 1 day,1,2,3,4,5,6 weeks) after application and bring to the laboratory to assess the herbicidal effect on the fungal populations in the soil. 1gm soil was suspension in 9mL of sterile distilled water in test tube and mixing 5 minute, serial dilutions were made until the dilutions were made up to 10^4. 1mL from 10^4 were piptted and transferd to Petri dishes, then added Potato Dextrose Agar (PDA) amended with 250mg L^{-1} chloramphenicol to inhibitor added into sterilized (121^{o}C,15min) media to dishes by using poured plate method. The inoculated media plates were covered and allowed to dry. After 30 minute, the plates were transferred to darkness incubator. Fungal population in the PDA plates was enumerated by their colony forming unit (CFU). The CFUs were determined after 7days by counting the visible colonies. The total up of the colonies was used to calculate the $CFUg^{-1}$ dry weight of soil using the formula :

$$\text{CFU / g dry weight of soil} = \frac{\text{Colony forming unit x dilution factor}}{\text{Amount of aliquot x dry weight of soil (g)}}$$

Effect of tribenuron methyl on isolated fungi in solid medium: The effect of the herbicide on the fungal colony development was conducted on PDA medium in Petri dish (8.5 cm diameter). The treatment involved PDA mixed with the herbicide as 0.0, 25, 50, 75ppm concentration, while the control was without herbicide treatment. The herbicide-PDA medium was prepared by adding the herbicide into sterilized PDA (121^{o}C, 15min) and mixed manually before pouring into the Petri dishes. The Petri dishes were covered and allowed to dry for 30 minute in sterile condition, fungal (5mm) subculture of 7days old was transferred aseptically using sterile inoculation lope to the center of the herbicide-PDA medium, also work with control and then incubation at 25^{o}C in darkness. All treatments were done in triplicate The effect of herbicide on the fungal isolated was measured by the radial growth of fungal colony in

both control and herbicide –PDA plates for 7 days using centimeter ruler. The measurements were expressed as inhibition percentage of the colony, during used the formula of[18]:

$$\text{Growth inhibition percentage} = \frac{D_C - D_T}{D_C} \times 100$$

Where: D_C is the average diameter of fungal colony in control, and D_T is the average diameter of fungal colony with herbicide treatments.

Effect of tribenuron methyl on mycelial dry weight in mineral salts medium: Prepare synthetic mineral salts medium (MSM), this medium involved $FeSO_4.7H_2O$, 0.1g; $MnCI_2.4H_2O$, 0.1g; $ZnSO_4.7H_2O$, 0.1g and $MgSO_4.7H_2O$, 1.0g. All components of mineral salts to this medium were dissolved in 1L of aseptic distilied water. Mineral salts medium was divided to 50mL and added to 250mL conical flasks. MSM sterilized by autoclaved with (121^{o}C, 15min) and after decreased the temperature of medium, herbicide 25, 50,75ppm concentration was added, while the control was without herbicide treatment. Fungal (5mm) subculture of 7 days old was transferred aseptically using sterile inoculation lope to the MSM in all conical flasks and also work with control. All flasks were covered with non absorbent cotton wool and then incubation 7 days at 25^{o}C in darkness. All treatments were done in triplicate. After 7 days incubation the MSM was filtered through Whattman No.1 filter paper and then the mycelial of isolated fungi were dehydrated in oven with 65^{o}C during 30minute to measure the dry weight of fungi mycelium by sensitive balance.

Biodegradation of tribenuron methyl by isolated fungi: Prepare synthetic mineral salts medium (MSM) this medium involved $FeSO_4.7H_2O$, 0.1g; $MnCI_2.4H_2O$, 0.1g; $ZnSO_4.7H_2O$, 0.1g and $MgSO_4.7H_2O$, 1.0g. All components of mineral salts to this medium were dissolved in 1L of aseptic distilled water. Mineral salts medium was divided to 50mL and added to 250 mL conical flasks. MSM sterilized by autoclaved with (121^{o}C, 15min) and after decreased the temperature of medium, 4mL of tribenuron methyl herbicide was added to produce final concentration (75ppm) while the control was without herbicide treatment. Fungal (5mm) subculture of 7 days old was transferred aseptically using sterile inoculation lope to the MSM in all conical flasks and also work with control. All flasks were covered with non absorbent cotton and then incubation 7 days at 25^{o}C in darkness. All treatments were done in triplicate. All conical flasks were shaking manually three times to mixed cultures of fungi. After 7 days incubation the MSM was filtered through Whattman No.1 filter paper the filtered samples were separated transferred to test tube, 1mL acetonitrile with 1mL distilled water was added and then transferred to centrifuge with 10.000 rpm in minute, then after this step, the supernatant was removed and concentrated to 1mL by evaporation in water bath, then the sample was analysis by FTIR to determined the biodegradation TMB to other metabolites.

Statistical Analysis: All applications were analysis by using (ANOVAs) in program SPSS (version 10.0).

Results and Discussion

Isolation of fungi: Figure-1 showed the density of fungi in soil treatment and without treatment with granstar (TBM), the total numbers of fungi were decreased to $8x10^4$ after 1h treatment because the fungi were no adapted after short time with TBM treatment but the total numbers of fungi were increased to 15x 10^4 after 1 day from treatment, this result may be due to that the herbicide molecule was activated to fungi and also these fungi can used (TBM) as a source of carbon after degrading and analysis to metabolites. This result was similar when the numbers of fungi were increased in soil after treatment with propanil herbicide but the numbers of bacteria was decreased[14].

Figure-2 showed that the numbers of living fungi were 15 x 10^4 in first week and the numbers were increased in all weeks, except the numbers are dreased in second week. under normal conditions, herbicide microbial degradation consists of three phases after application microbial degradation begins with a lag phase when breakdown is relatively slow because the microorganisms or enzymes are adapting to the recently applied herbicide and in the final phase the degrading microbes also returns to a slower rate due to depletion of the herbicide present in the soil or an accumulation of toxic metabolites that reduce the rate of degradation[17]. Also the addition of a herbicide to the soil can change and effect on the microorganisms population and can its ability to degrade the chemical[17]. The increased carbon source from the herbicide may lead to an increased in microbe populations due to the additional energy source. However the exposure of soil fungi to herbicide application caused short term growth-inhibitory effects on soil fungal population[19], also other studies referred that some effects on soil microorganisms upon single application of herbicide[20] and even when herbicides applied higher than the recommended field rate was found to cause effects on microbial biomass[21,22]. Highly numbers were calculated in the present investigation in the third week with (49 x 10^4) Figure-3.

Table-2 showed that eleven filamentous fungi isolated from the application field soil with tribenuron methyl herbicide. *A.niger*, *A.flavus* were more frequency with 88.8%, 77.7% respectively, while *P.funiculosum*, *A.ostianus* and *A.versicolor* were moderate frequency with 55.5 %, 44.4% , 44.4 % respectively, but the remaining 5 fungal species were isolated with a very frequency with 22%, 11%. These results were similar that *Penicillium sp.* and *Fusarium sp.* were a strong resistance of molds toward (TBM)[23] and the results obtained also found that *Fusarium sp.*, *Chaetomium sp.*, *Mucor sp.*, *Humicola sp.*, *Penicillium sp.*, *Aspergillus sp.* Were frequently found in soil samples[24]. However certain types of fungi were found in soil

samples treated with increasing doses of herbicides while other fungi were reduced and still others disappear as a result of their sensitivity to xenobiotic and other species appear (*Stachybotrys sp.*, *Cladosporium sp.* and *Aspergillus sp.* and also showed that *Fusarium sp.* and *Penicillium sp.* are present in a large number in experimental variants and suggest the increased of found these fungi due to resistance to the action of tribenuron methyl[23], but in the present investigation the results were different with the results obtained that fungal colony was increased inhibition with increased treatment rate of many herbicides[19].

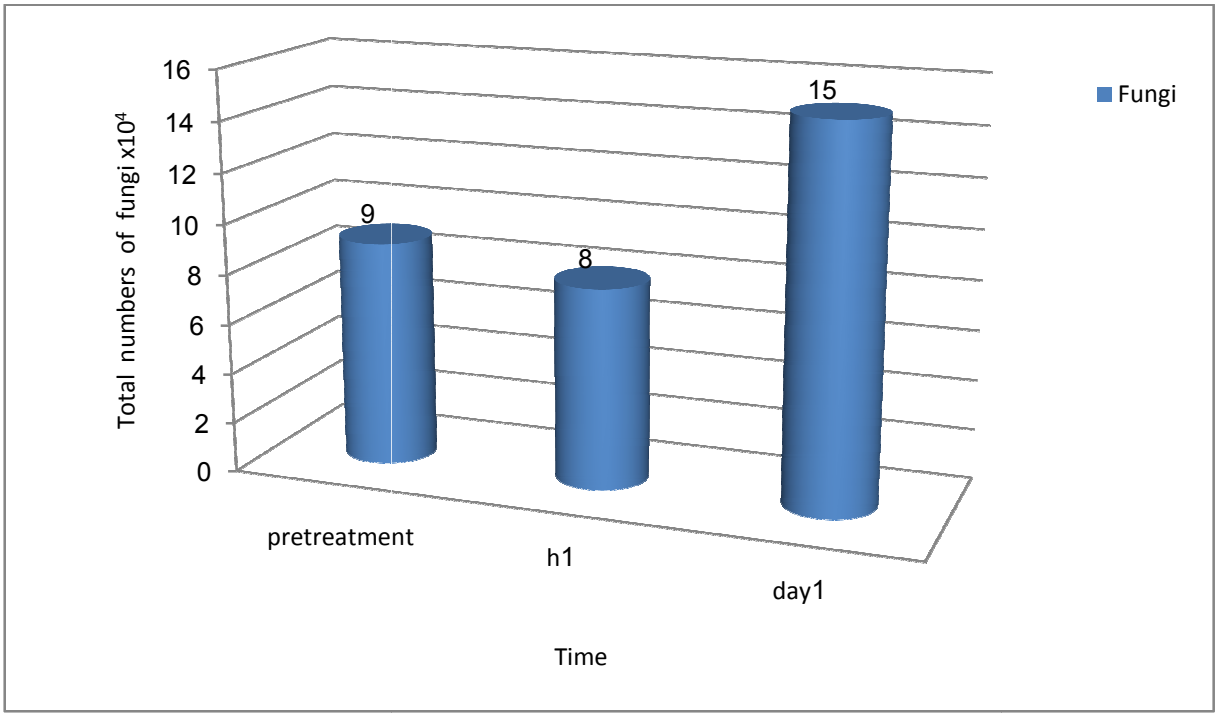


Figure-1
Density of fungi in soil treatment and without treatment with granstar (TBM)

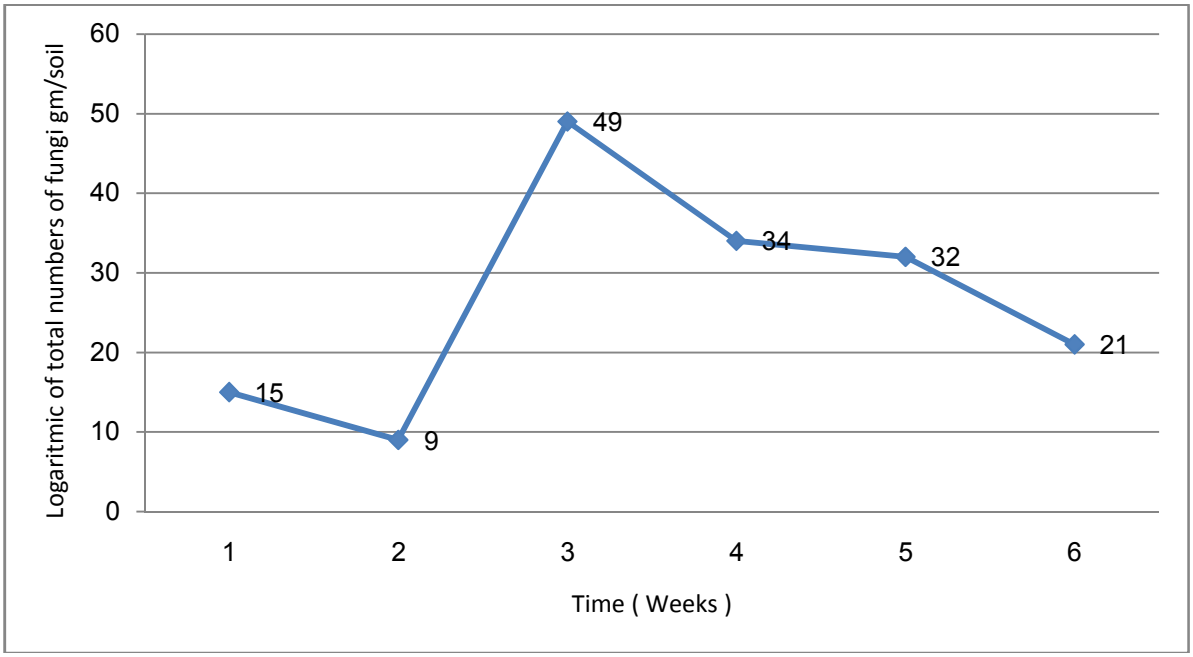


Figure-2
Logaritmic of total numbers of fungi in field soil after treatment with granstar

Table-2
Appearance percent to fungi species in all soil samples treatment with granstar

Fung	Samples number to species appear	Appearance %
Aspergillus niger	8	88.8
A.flavus	7	77.7
A.ostianus	4	44.4
A.versicolor	4	44.4
A.sydowii	1	11.1
Penicillium funiculosum	5	55.5
P.notatum	1	11.1
Rhizoctonia solani	2	22.2
Trichoderma harzianum	2	22.2
Trichoderma opacium	1	11.1
Stephyllium sp.	2	22.2

The results showed that Deuteromycetes was more dominant genera with 78.37% included eight species belong to 3 genus (Table-3) although the total calculated to genus *Aspergillus* was 12.3 x 10^5, *Trichocladium* was 4.0 x 10^5, then *Trichederma* and *Stemphlium* with 3.3 x 10^5, 3.0 x 10^5 respectively, but the total calculated of *Pencillium* and *Rhizoctonia* reached to 2.6 x 10^5, 2.0 x 10^5 respectively (Table-4). As well as (Table-4) appear the frequency of all genus in all soil samples, this table showed that the frequency of *Aspergillus* was higher than other fungi (45%) and *Trichocladium* was the second genus with frequency (14.8%) and in the same time this table showed that *Rhizoctonia* was the lower genus frequency (7.4%).

However (Table-3) showed that Ascomycetes was appear with 16.21 and including 2 species belong to one genus, while Basidomycetes was appear with 2.70% and including one species belong one genus (Table-2). The differences in species numbers isolated from field soil on PDA medium due to the faster growth of Deuteromycota when compare with Ascomycetes and Basidomycetes. The results were similar with the results obtained that Deuteromycetes were more dominant than Ascomycetes and Zygomycetes[25].

Effect of tribenuron methyl on isolated fungi in solid medium: The growth ability of the isolated fungi was done under 0.0,25,50,75 ppm concentrations of granstar (tribenuron methyl) and was expressed as diameter of the colony (Figure-3).

Table-3
Frequancy of fungi genera by using pour plate

Fungi genera	Genus number	Frequency %
Deuteromycetes	4	78.37*
Ascomycetes	1	16.21
Basidomycetes	1	2.70

* Frequency of fungi genera = Total number of fungi genera / Total number to all fungi genera x 100

Table-4
Total number of fungi genus in all samples with frequency to genus in field soil treatment with granstar

Genus	Total numbers of genus in all samples	Frequency %
Aspergillus	12.3 x 10^5	45
Penicillium	2.6 x 10^5	9.6
Rhizoctonia	2.0 x10^5	7.4
Trichoderma	3.3 x 10^5	12.2
Trichocladium	4.0 x 10^5	14.8
Stemphylium	3.0 x 10^5	11.1

$$\text{Frequency} = \frac{\text{Total calculated of genus in all samples}}{\text{Total calculated of all genus in all samples}} \times 100$$

The results showed that *A.niger* was more resistance aong other isolated fungi with all concentrations of (TBM) in solid PDA medium and the colony diameter of this fungus reached to 8.5cm, also the herbicide (TBM) not appear any effect on this fungus with all concentrations. However the results showed that TBM was inhibited *A.versicolor* 15%, 5% in 25, 50 ppm respectively but the colony diameter was increased to 8.5 cm when compared with control and inhibited *A.flavus* 35% in 75 ppm concentration only but this herbicide inhibited *P.funiculosum* 6%, 9% in 25, 50ppm respectively but the colony diameter was increased to 8.5 in 75ppm when compared with control. The statistical methods showed significant differences between isolated fungi and also no significant differences between concentrations were obtained. The inhibitory effect of herbicide on growth of the fungus through soil treatment was lower than with the direct exposure in vitro[26]. This indicated that herbicides in soil may undergo certain natural processes (biological, chemical and physical) such as temperature, pH , sun light, wind, rain, moisture, large numbers of microorganisms which could reduce its toxicity to the fungal population also these microorganisms can used herbicides as a

sole of carbon and energy. The results in present investigation were similar with the results obtained that Glyphosate was less toxic and inhibited the radial growth of *Penicillium sp.* and *Aspergillus sp.* by 18-58%, but *Mucor sp.* was more susceptible with 63–80% inhibition and Metusulfuron methyl caused minimal inhibition (< 20%) of fungal development[19]. These results due to the differing abilities of fungal mycelia to absorb herbicides for their utilization[27]. However *Aspergillus sp.* and *Penicillium* were also reported as active degrader of herbicides[28]. Also propanil was activated the growth of fungi in solid media with the concentration 0.01, 0.04, 0.3 ppm. *sp* [14].

Effect of tribenuron methyl on mycelial dry weight in mineral salts medium: Figure-4 showed that TBM was inhibited mycelial dry weight of *A.niger* with all concentrations in liquid mineral salts medium and the inhibition percent reached to 9%, 13%, in 25, 50, 75ppm. Also Figure-5 showed that the inhibition percent was 2.0%, 31% in 25, 75ppm with *A.flavus*, this result was similar with the results obtained that the direct exposure of the fungi in vitro to the herbicides paraquat and glufosinate – ammonium could be very toxic, whereas glyhosate was moderately toxic and metsulfuron- methyl had caused the lowest inhibitory effects to the fungi[19]. The results in present investigation showed that the dry weight of *A.flavus* was increased in 50 ppm concentration and the mycelial dry weight of this fungus reached to 0.761gm when compared with control (0.700 gm) and in same time the results showed that the inhibition percent was reached to 14% in 25ppm only with *P.funiculosum*. This result refer that TBM was moderate inhibition effect on this fungus also this result was similar with results obtained that glyphosate was moderate growth-inhibition effects on fungal species likely to be due to its degradation by microorganisms as a source of phosphoorus by direct cleavage of the C-P bound producing sarcosine or by intermediate aminomethyl phosphonic acid[29]. Also the results in present investigation was similar with the results obtained that metsulfuron – methyl was the lowest inhibitory effects to fungal species in low doses and its ability to be degraded by soil fungi[30,31], in present investigation the results showed that the mycelial dry weight of *P.funiculosum* increase to 0.519 gm, 0.704 gm in 50 , 75 ppm, when compared with control (0.511 gm). Only among with all fungi under study the mycelial dry weight of *A.versicolor* was increased in all concentrations of TBM and also the stasitical methods showed no significant differences betwen concentrations. These results may be refer that to the ability of this fungus to used TBM to nutrition substances in medium with no added any carbon, nitrogen and energy source and then TBM was activated to these fungi due to the direct contact betwen herbicide and fungi in different concentrations of herbicides. The high concentrations fromthiourea was highly activated to *Fusarium oxysporum* than low concentrations[32]. Also these results were similar with results obtained that all fungi studied were exhibit different activated to exposure with propanil herbicide in liquid mineral salts medium[14], also these difference due to the differing abilities of fungal mycelia to absorb herbicides for their utilization[27] and in the same time the inhibitory effect of herbicide on growth of the fungus through soil treatment was lower than with the direct exposure in vitro[26]. This indicated that herbicides in soil may undergo certain natural processes (biological, chemical and physical), such as temperature, pH, sun light, wind, rain, moisture, large numbers of microorganisms which could reduce its toxicity to the fungal population, also these microorganisms can used herbicides as a sole of carbon and energy.

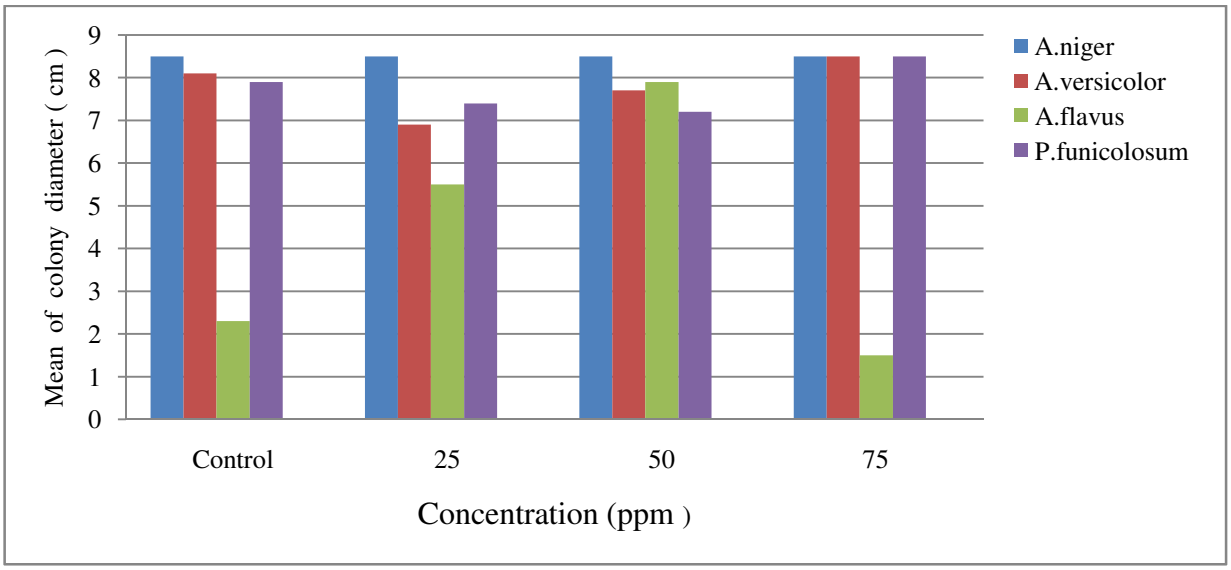


Figure-3
Effect of granstar on fungi *Aspergillus niger*, *Aspergillus vrsicolor*, *Aspergillus flavus*, *Penicillium funicolosum* in solid medium after 7 days incubation

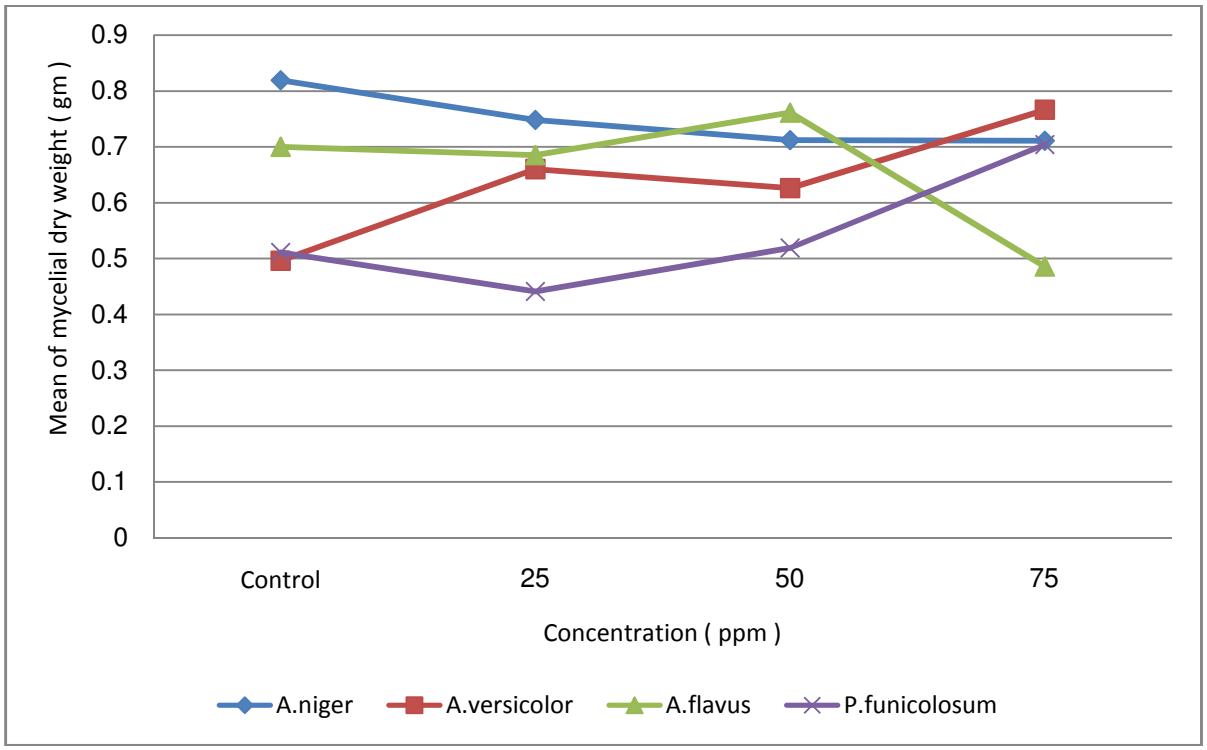


Figure-4
Effect of granstar on mycelial dry weight of fungi *Aspergillus niger*, *Aspergillus vrsicolor*, *Aspergillus flavus*, *Penicillium funicolosum*, in mineral salts medium after 7days incubation

Biodegradation of tribenuron methyl by isolated fungi: Figure-5 showed disappear a large number of peaks in region between 500- 1500, but also appear a new two peaks in region 2000 – 2500, this peaks refer nitrile group when compared with control (Figure-6) in field soil treatment with granstar (TBM) after 1h, this result may be due to that this herbicide was activated fungi in soil and these fungi were attack and utilize herbicide to a source of carbon and energy.

This results was similar with the results obtained that fungi can used to increased detoxification of polluted environment with herbicides and also fungi play in important role in the environment to removal and breakdown of many pollutant[13] and the present study also similar with the results obtained that the herbicide propanil was cleavage to five metabolites by *Aspergillus niger*, *A.flavus*, *A.candidus*, *A.restrictus*, *Penicillium sp.*, *R.stolinifer*, *Trichoderma lignorum* and *T.harzianum* after treatment rice field[14].

However bacteria and fungi have different sensitivity to most herbicides and this sensitivity is also influenced by other factors. Figure-7 showed also disappear a large number of peaks in region 500 – 1500 and appear a new two peaks in region 2000 – 2500 and appear a new one peak in region 3500, this peak refer to amine group was found when compared with standard granstar (Figure-6), this result also was correlation with increased the density of soil fungi after one week from application soil field with herbicide granstar. Also (Figure-8) showed disappear a large number of peaks in region 500 – 1500 and appear a new two peak in region 2000–25500 after second week from application field soil when compared with control (Figure-6), this result due to may be that were adaptation of soil fungi and these fungi were excreted extracellular enzymes and consumed granstar herbicide to a source of carbon and energy.

Figure-9 showed that disappear a large number of peaks in region between 500-1500, but also appear a new four peaks in region 2500 – 3000, in third week after application in field soil, this result refer to that produce carboxylic acid and this result was a correlation with larger increasing of total numbers of fungi in soil after third week from application, Figure-3 showed that the total numbers of fungi reached to 49 x10^5, these fungi can degraded herbicide and utilize this herbicide to a source of carbon and energy and also transformed this herbicide to other compounds. The increased of fungi numbers refer to appear a new peaks when compared with standard granstar.

This results was similar with the results obtained that the herbicide propanil was cleavage to five metabolites by *Aspergillus niger*, *A.flavus*, *A.candidus*, *A.restrictus*, *Penicillium sp.*, *R.stolinifer*, *Trichoderma lignorum* and *T.harzianum* after treatment rice field[14], but the results in present study were different with the results that herbicides and their metabolites have negative effects on fungi and bacteria[11,12].

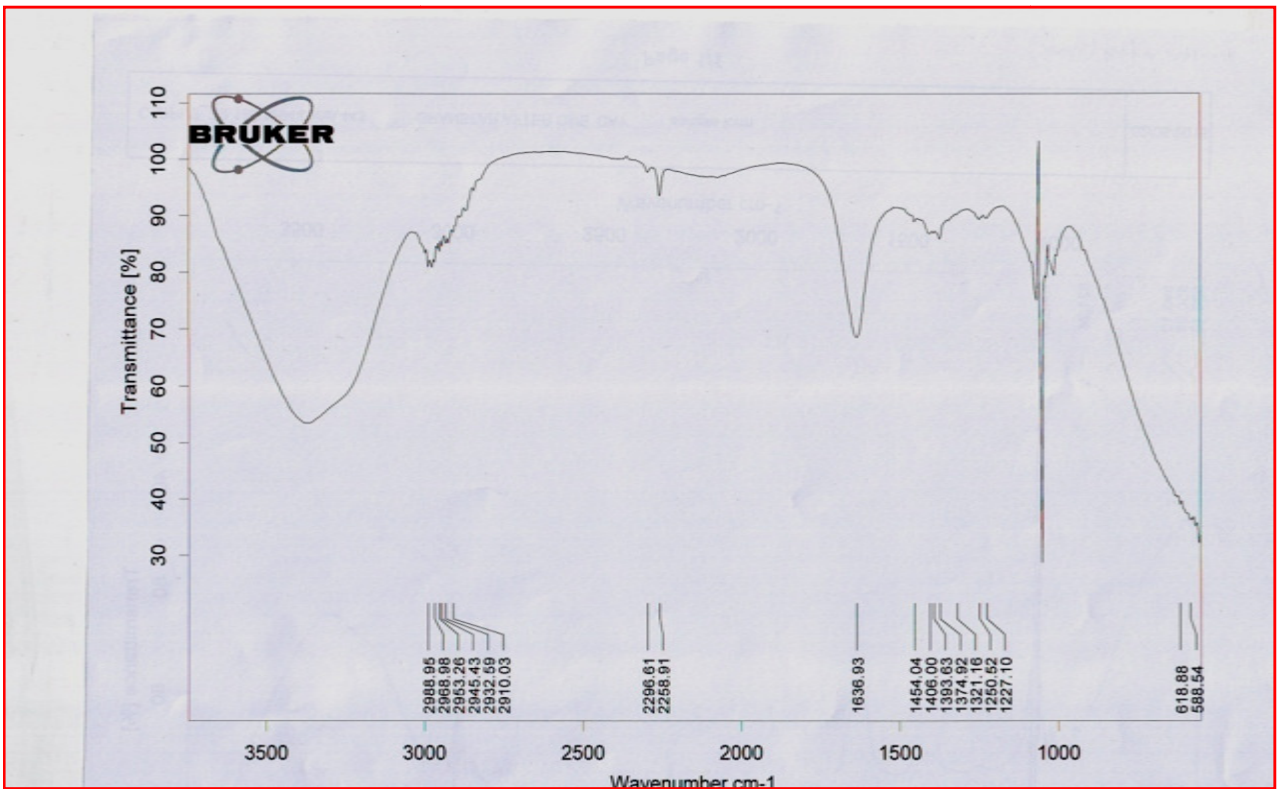


Figure-5
Biodegradation of granstar (TBM) in field soil after 1hour from application

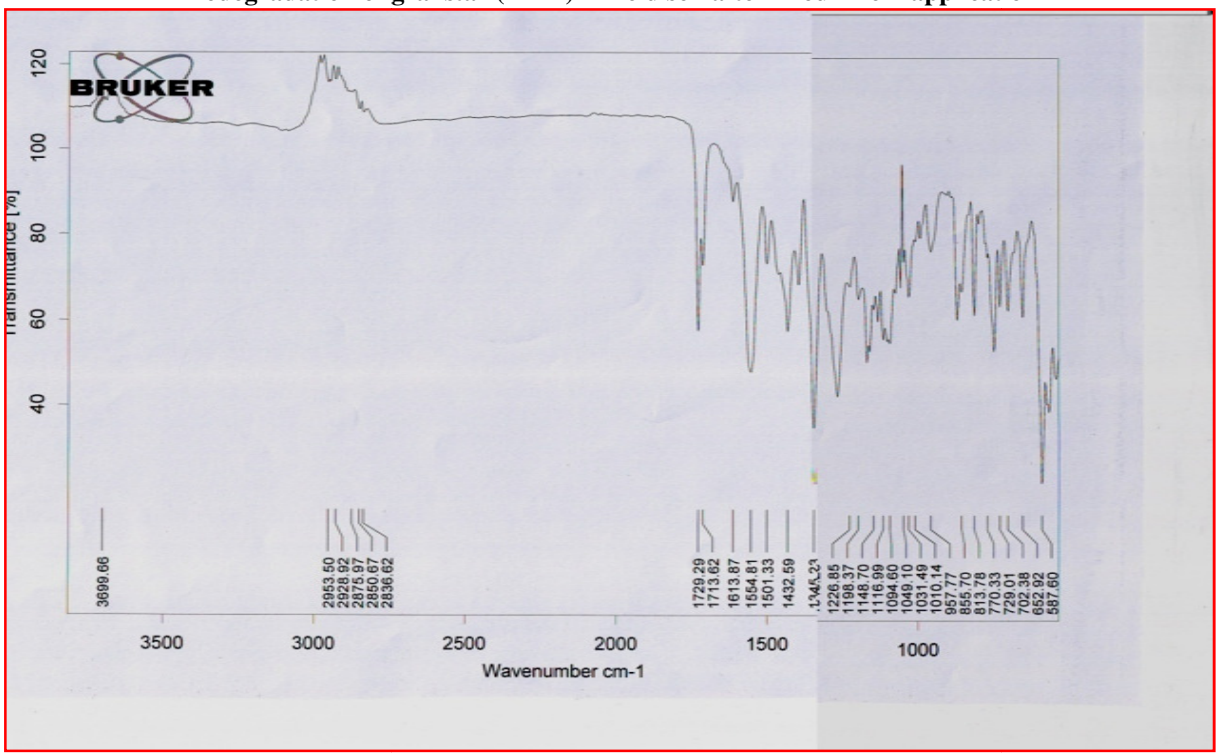


Figure-6
Granstar (TBM) – Standard

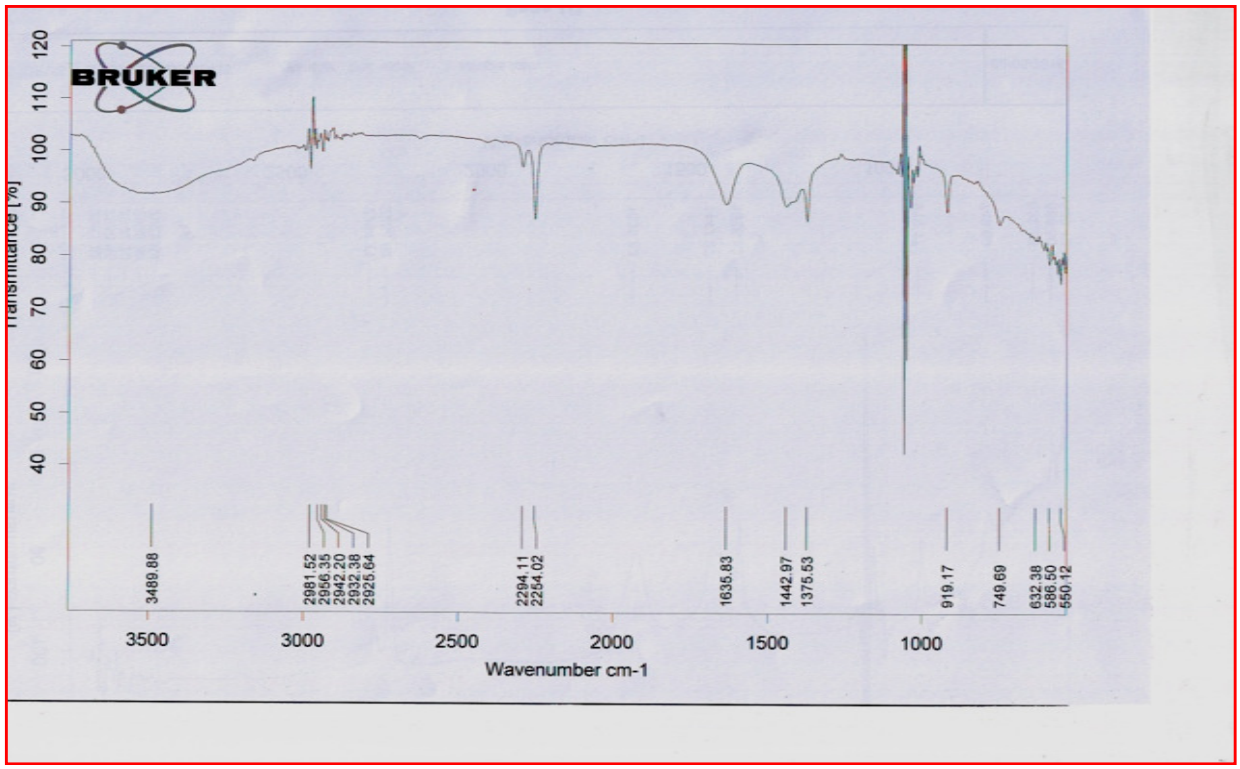


Figure-7
Biodegradation of granstar (TBM) in field soil after first week from application

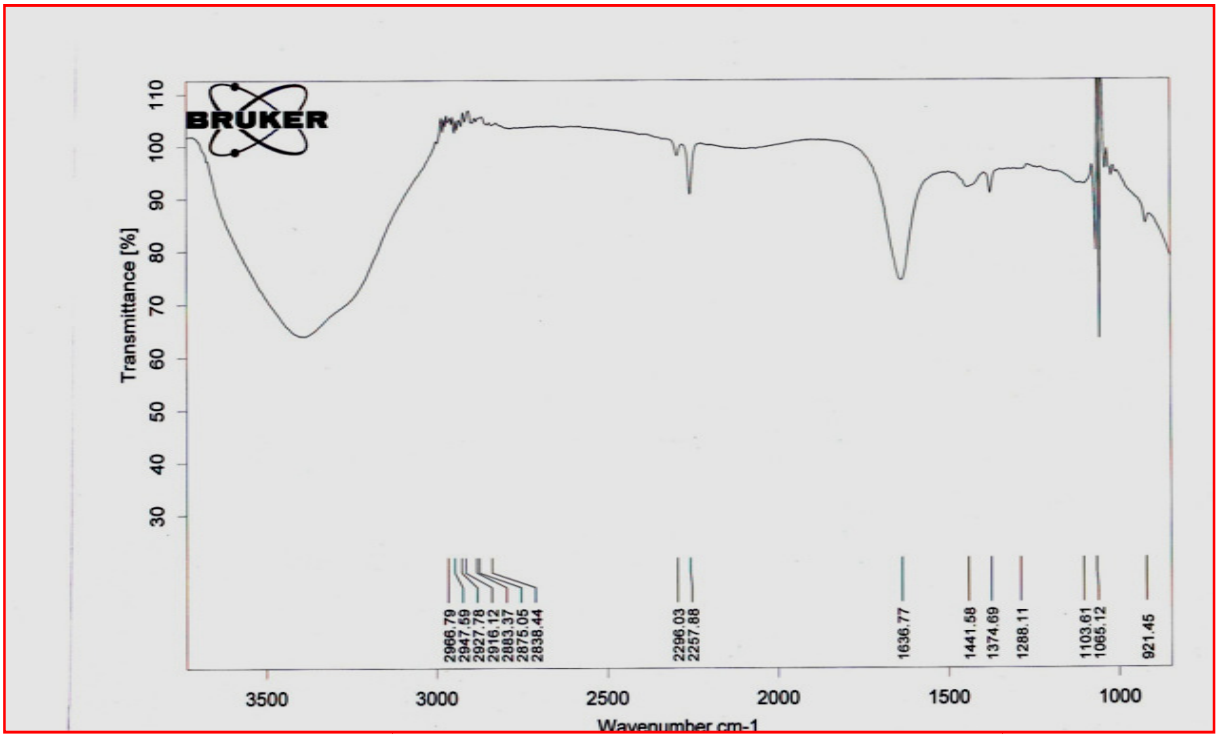


Figure-8
Biodegradation of granstar (TBM) in field soil after second week from application

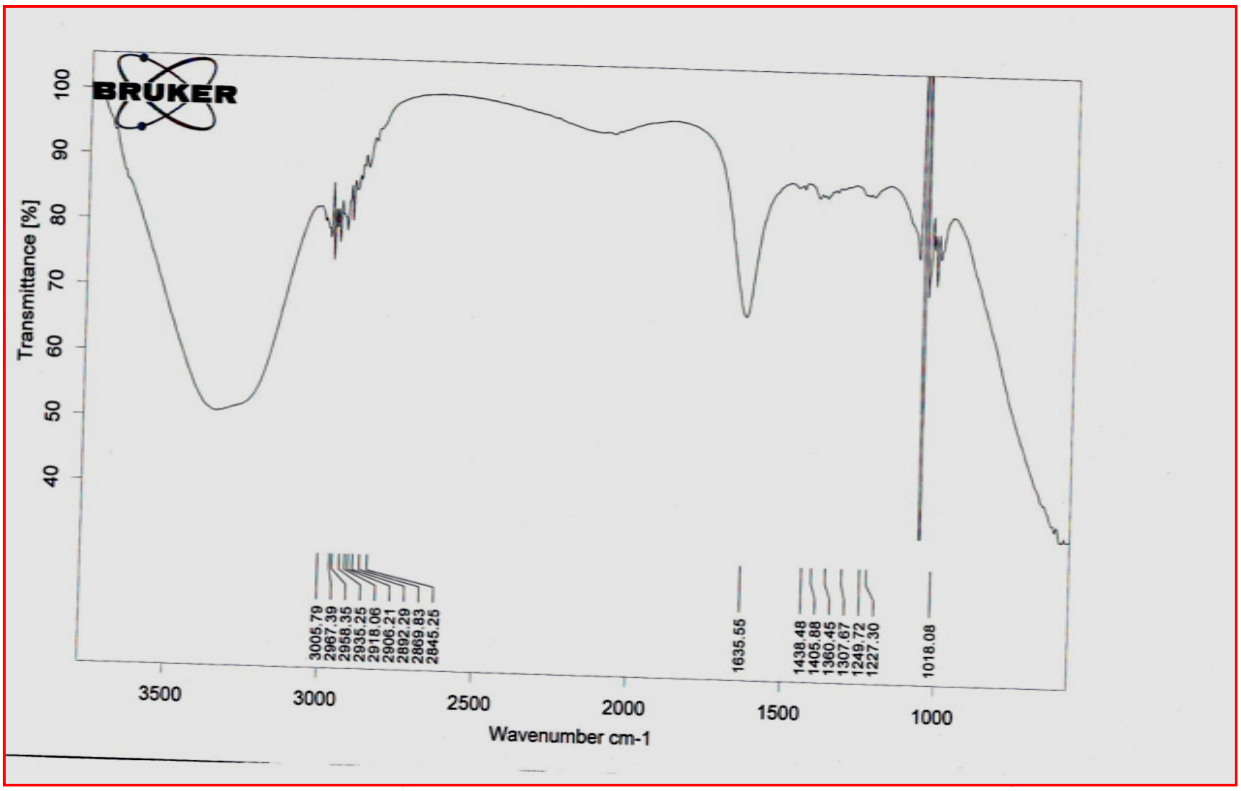


Figure-9
Biodegradation of granstar (TBM) in field soil after third week from application

Figure-10 showed that disappear a large number of peaks in region between 500- 1500, but also appear a new two peaks in region 2000 – 2500 and also appear a new four peaks in region 2500 – 3000 and one new peak was appear in region 3000 – 3500 this result was a correlation with increasing of total numbers of fungi in soil after four week from application. The results in present investigation was similar with the results obtained that metsulfuron – methyl was the lowest inhibitory effects to fungal species in low doses and its ability to be degraded by soil fungi[30,31] and also in the same time the inhibitory effect of herbicide on growth of the fungus through soil treatment was lower than with the direct exposure in vitro[26]. This indicated that herbicides in soil may undergo certain natural processes (biological, chemical and physical) such as temperature, pH, sun light, wind, rain, moisture, large numbers of microorganisms which could reduce its toxicity to the fungal population also these microorganisms can used herbicides as a sole of carbon and energy.

Figure-11 show biodegradation of granstar in field soil after five week from application, this figure show that disappear a large number of peaks in region between 500-1500 but also appear a new two peaks in region 2000–2500 and also appear a new six peaks in region 2500–3000 and one new peak was appear in region 3000–3500 also appear a new two peaks in region 3000 – 3500, this peaks refer to amine was found. Figure-11 show also that fifth week was the optimum period to degradation and transformation of granstar herbicide because produce many different metabolites when compared with standard granstar and the results showed that correlation was found with the increased of fungi numbers. The results in present investigation were similar with the results obtained that the herbicide propanil was cleavage to five metabolites by *Aspergillus niger*, *A.flavus*, *A.candidus*, *A.restrictus*, *Penicillium sp.*, *R.stolinifer*, *Trichoderma lignorum* and *T.harzianum* after treatment rice field[14].

Figure-12 showed that disappear a large number of peaks in region between 500- 1500 but also appear a new two peaks in region 2000 – 2500 and also appear a new four peaks in region 2500 – 3000 and one new peak was appear in region 3500, this result was a correlation with increasing of total numbers of fungi in soil after six week from application. This result refers the ability of soil fungi in biodegradation of granstar herbicide and utilize this herbicide as a sole of carbon and energy.

Figure-13, 14, 15, 16 showed that disappear a large number of peaks in region between 500-1500 and also appear a new peaks in region 2500 – 3000, these results refer that the a bility of fungi *Aspergillus niger*, *A.flavus*, *A.versicolor* and *P.funiculosum* to biodedegradation of granstar in mineral salts medium after 7 days incubation.

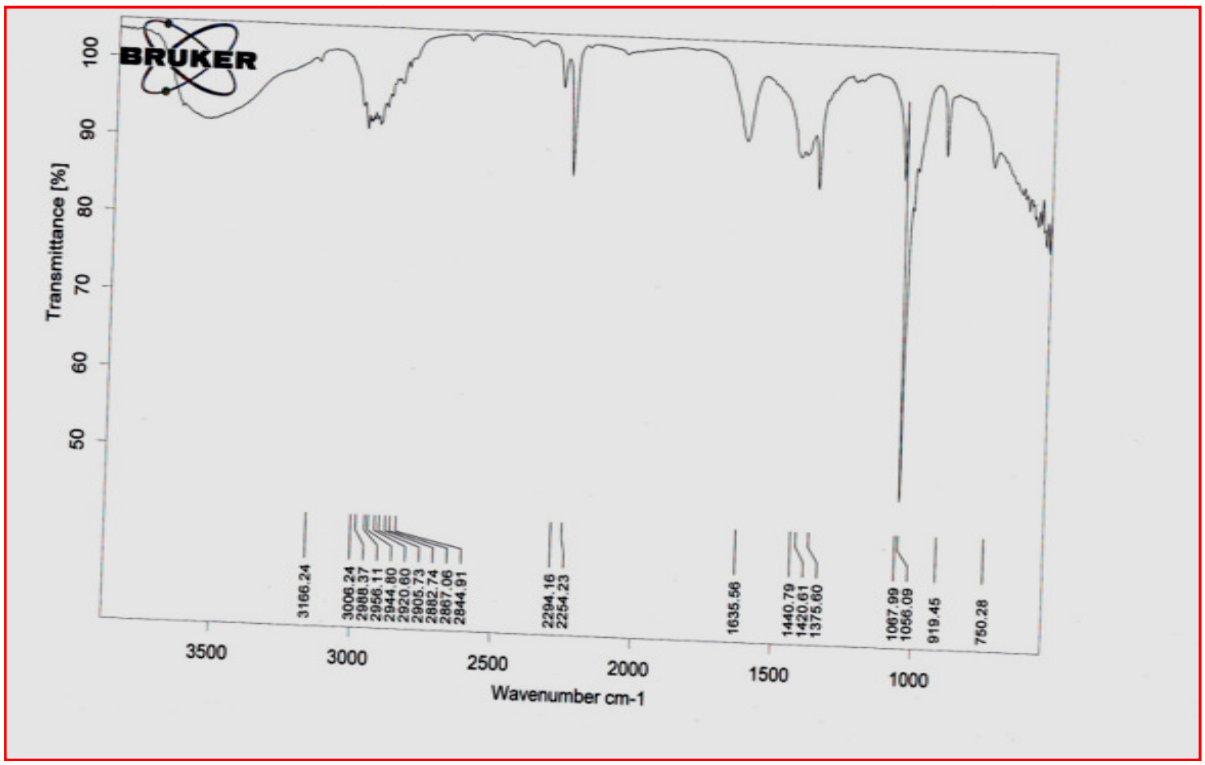


Figure-10
Biodegradation of granstar (TBM) in field soil after four week from application

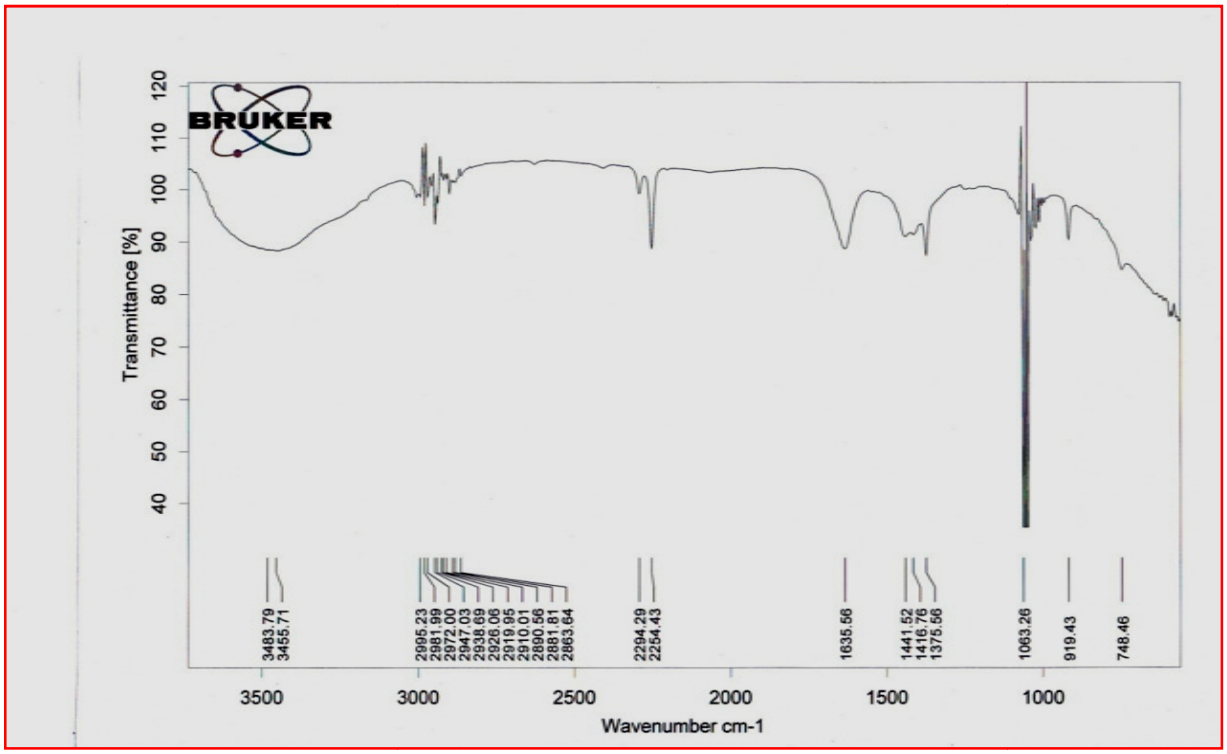


Figure-11
Biodegradation of granstar (TBM) in field soil after five week from application.

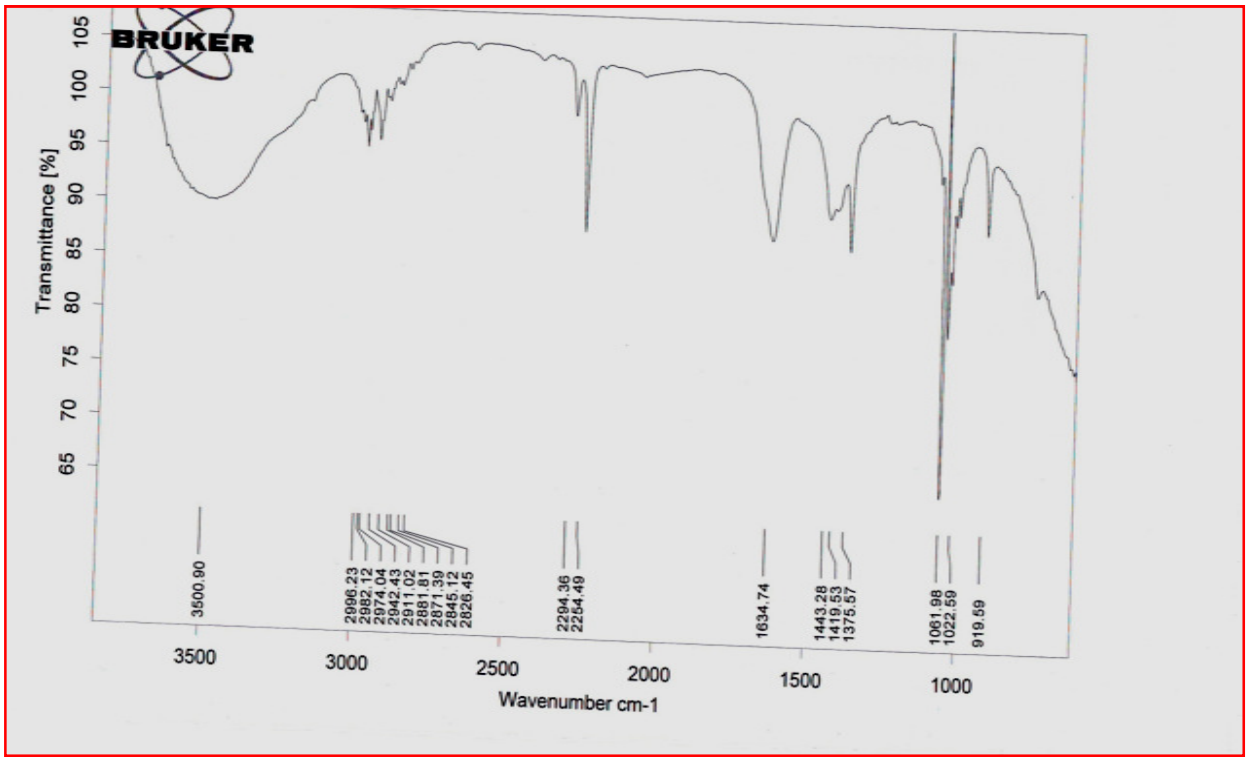


Figure-12
Biodegradation of granstar (TBM) in field soil after six week from application

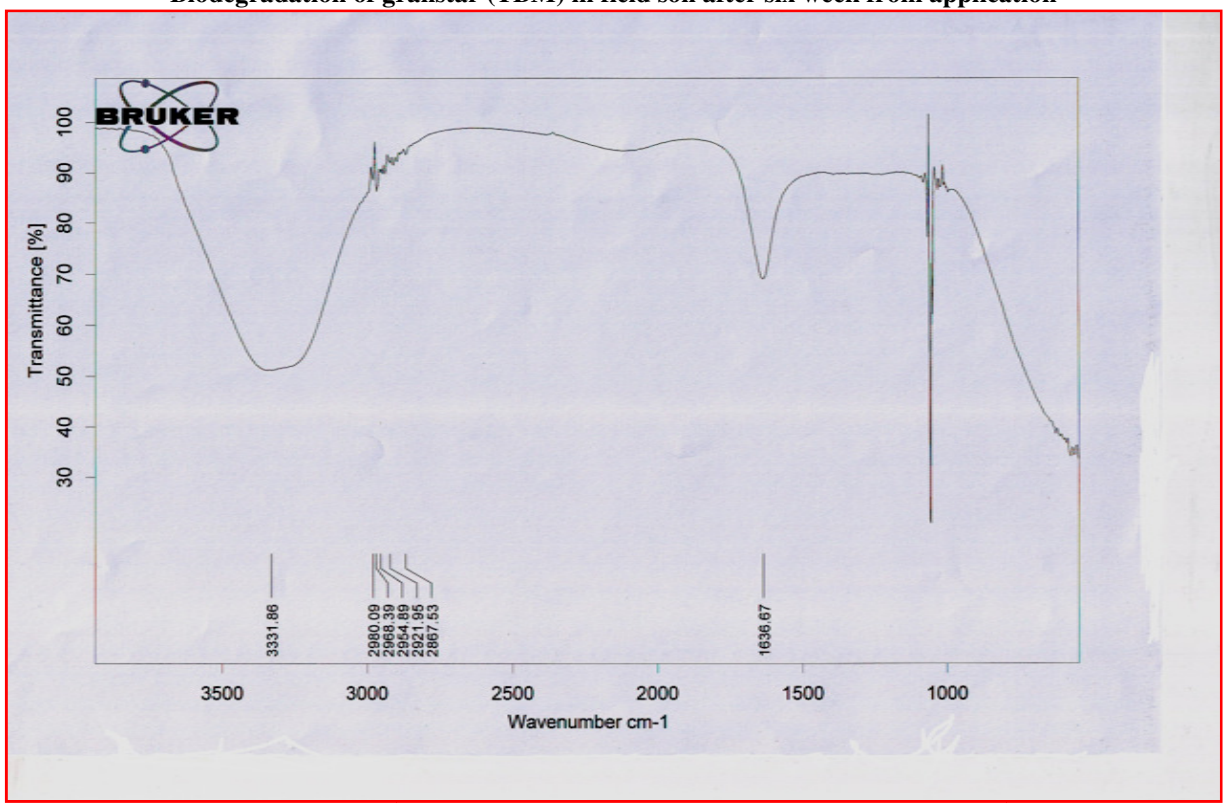


Figure-13
Biodegradation of granstar (TBM) by *A.niger* after 7 days incubation

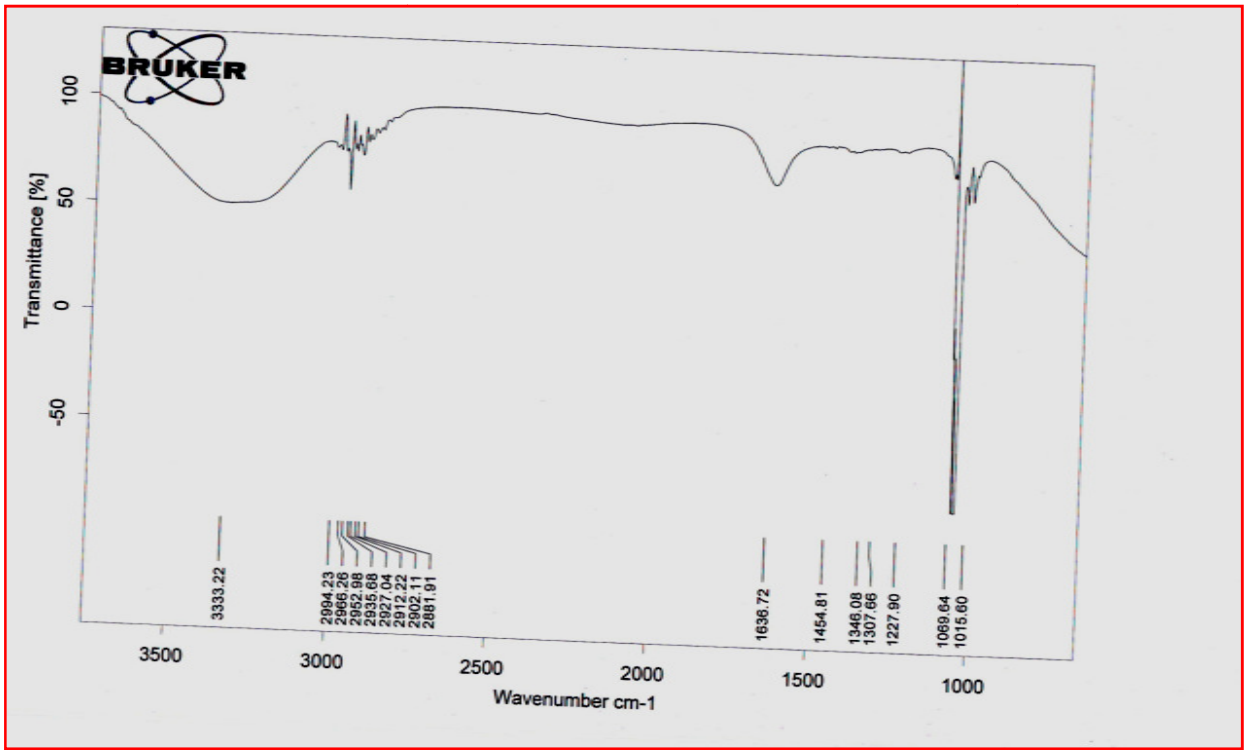


Figure-14
Biodegradation of granstar (TBM) by *A.flavus* after 7 days incubation

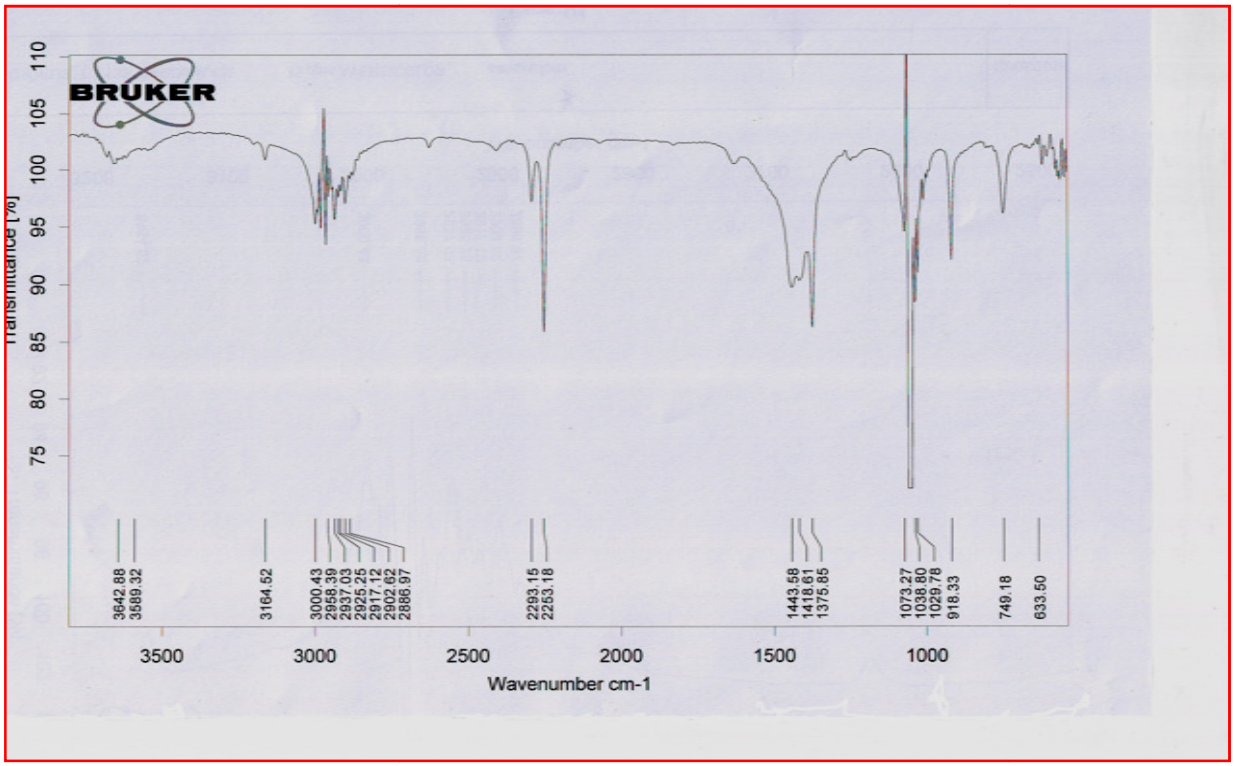


Figure-15
Biodegradation of granstar (TBM) by *A.versicolor* after 7 days incubation

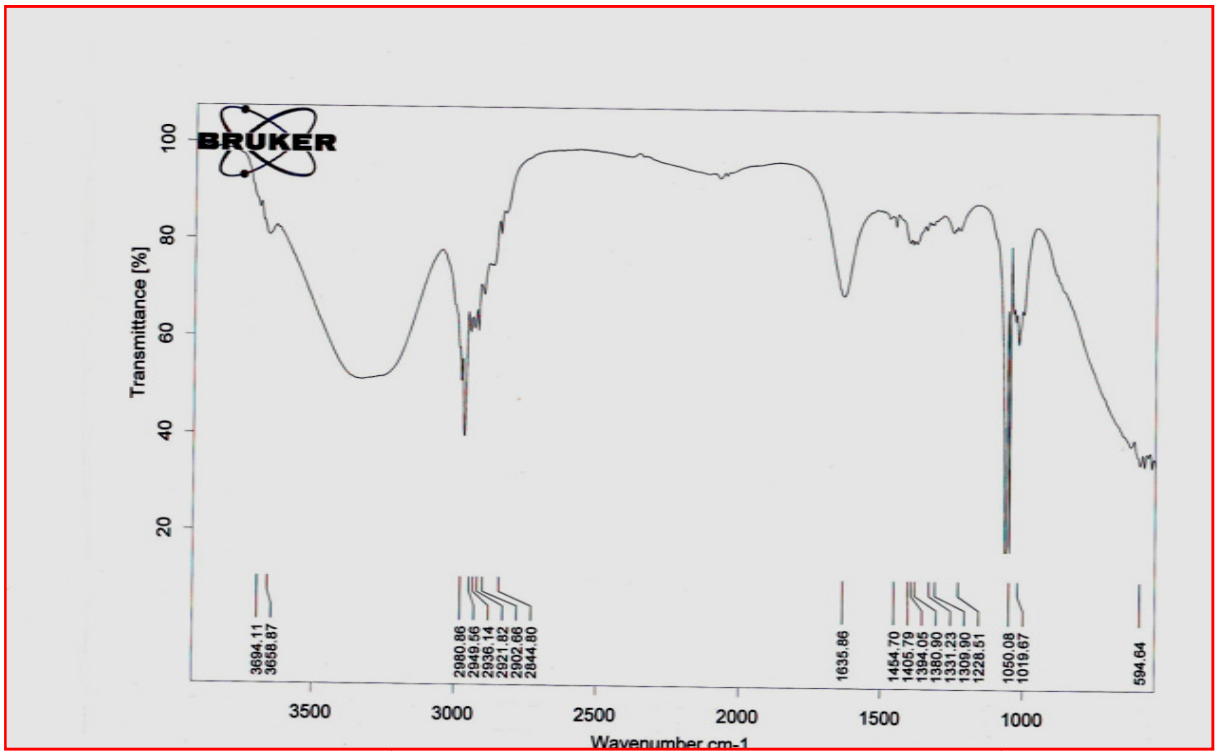


Figure-16
Biodegradation of granstar (TBM) by *P.funiculosum* after 7 days incubation

Figure-16 shows that *A.versicolor* was more efficiency to biodegradation of granstar to different metabolites than other fungi. Also these results were similar with the results obtained that all fungi studied were exhibit different activated to exposure with propanil herbicide in liquid mineral salts medium[14], also these difference due to the differing abilities of fungal mycelia to absorb herbicides for their utilization[27].

The results in present investigation showed that correlation was found between biodegradation of granstar and increased the dry weight of *A.versicolor* in all concentrations. Also the results in present investigation were similar with results obtained that the herbicide propanil was transformed to dichloroaniline by *Aspergillus niger*, *Penicillium sp.*, *R.stolinifer* and *Trichoderma lignorum* after 7 days incubation[14].

Conclusion

The results showed that all fungi isolated in this study were well adapted to degrade and utilize granstar (TBM) and convert this compound to other metabolites. Also the results showed that *A.versicolor* was more efficient to biodegradation herbicide and the five week was the optimum period to biodegradation of granstar to different metabolite. In the same time the results showed that granstar herbicide have moderate effect in some concentrations and also no effect in another concentration on fungi in mineral salts medium.

Rehabilitation of contaminated soil and water by the culture fungi (*A.niger*, *A.flavus*, *A.versicolor, P. funiculosum*) were promising as it can reduce the soil pollution with granstar to acceptable levels for reuse of land and water within a short period. The data obtained in the present investigation was advanced our knowledge of herbicide and behavior of fungi in polluted soils in different location and how these fungi to breakdown or biodegradation herbicide in environment as well as can used these organisms to removal pollution now and also in future.

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