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Research Article

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A Bility of Sediments Fungi in Biodegradation of Diesel Fuel

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ABSTRACT

Five filamentous fungi used in this study to show the ability of these fungi in biodegradation of diesel fuel , these fungi were Aspergillus niger, Aspergillus, flavus , Aspergillus versicolor , Penicillium funiculosum and Rhizoctonia solani .The results showed that A. niger was resistence to diesel fuel in 1% , 3% , 4% concentration in solid medium , and the colony diameter of this fungus reached to 5.8 ,6.4,6.7 cm respectively, but this fungus was sensitive toward diesel fuel in 5% with 3.7 cm colony diameter . However the results showed that diesel fuel not appear any effect on the colony of A. flavus ,but the A. versicolor was sensitive in 3%, 4%, 5% concentration and the colony of this fungus was increased to 7.2 ,7.9 cm with 1%, 2% when compared with control . Also the results showed that the colony diameter of P. funiculosum decreased in 1% , 2% , 3% , 4% concentration except that the colony diameter of this fungus was increased in 5% when compared with control . And in the same time the results showed that R. solani was sensitive with all concentrations of diesel fuel. The stasitical methods obtained different significance with fungi and also with 5% concentration when compared with control .

The results showed that all fungi under study were resistence with diesel fuel in all concentrations in mineral salts medium during determined dry weights of these fungi with compared control after seven days incubation. The stasitical methods obtained different significance with different fungi and also with concentrations of diesel fuel.

The results showed that all fungi can degraded diesel fuel to other compounds by usin FTIR Spectroscopy.

Keywords: *Environment, Ecofriendly, Microorganisms, Solid media, Iiquid media*

INTRODUCTION

The contamination of environment by crude oil and petroleum products has become a serios problem on ecosystem and human health[25]. Among petroleum products, diesel oil is a complex mixture of alkanes and aromatic compounds[11], diesel fuel produce from the distillation of crude oil has a carbon range between C_8 and C_{26} [1] with high content of polyaromatic hydrocarbons[32]. Although diesel is a commonly used fuel for vehicles and machines so as effected on ecosystem[14].

Diesel hydrocarbons can accumulate in food chains at varios levels, However causing carcinogensis of some organs, mutagenesis in the genetic material[15].

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The effect of these compounds on the natural environment depends on the surface of the area contaminanted by the petroleum products , chemical composition and depth at which pollutants occur[34] . The technology commonly used for soil remediation includes mechanical , burying, evaporation, dispersion , and washing. However, these technologies are expensive, incomplete decomposition of contaminants, time- consuming and less effective[26,8]. Biological methods can treatments and removing oil spill . Bioremediation technology is a safe, economical , more efficient[31,13] and to be promising, practical, also to complete minerlization of hydrocarbons to carbon dioxide and water[33]. The principle of bioremediation depend on using microorganisms to destroy hazardous contaminants and convert them to harmless products[8,18]. Many microorganisms such as bacteria , fungi and yeast use their enzymatic activity to utilize hydrocarbons as a sole carbon and energy[13,2]. Fungi have adventages over other microorganisms in that they produce several extracellular enzymes that can interact with some types of polycyclic aromatic hydrocarbons . However fungi play in important role to degradation during by excretion extracellular enzymes such as cellulose, Laccase and other compounds so as crude oil, insecticide and herbicides were degradation by *A. niger, F. solani, Penicillium sp.* and *Trichoderma lignorum*[2,28,21]. Fungi are also tolerant to high concentrations of recalcitrant compounds . Some recent studies have been reported to use a mixed population of fungal strains that could enhance biodegradation efficiency,especially on high concentrations of oil[18,20,5].

The aim of this study is to degrade diesel fuel by locally isolated fungi from Abu – Subat marshe under different concentrations of diesel fuel. This study is the first about fungi isolated from the region of centeral marshes in AI-Nasiriya governorate (South of Iraq).

MATERIAL AND METHODS

Chemicals

Diesel fuel was collected from local stations in AI-Nasiriya city . All chemicals used in this study are purchased from BDH Co. , and the purity of this chemicals are 99.99% .

Organisms and culture conditions

A. niger, A. flavus, A.versicolor, P. funiculosum and *R. solani* were obtained from Marshes Researches Center, Thi-qar University, Environment Laboratory, Iraq. These fungi isolated by Dr. AI- Jawhari from the upper surface of a sediments in Abu – Subat marshe in AI-Nasiriya governorate (South of Iraq). Stock cultures were maintained on the potato dextrose agar slants , subcultured periodically and stored at 4°C . Mineral salts medium containing (g I^{-1}): K_2HPO_4, 1.71; KH_2PO_4 , 1.32 ; $NaNO_3$,0.42 ; $MgSO_4 . 7H_2O$, 0.42 ; $Cacl_2$, 0.02 was used for the induction experiments. All media were autoclaved at 120°c for 20 min. Diesel fuel at 1%, 2%, 3%, 4%, 5% was used as carbon source and energy for the biodegradation.

Determination of the fungal growth ability under Diesel fuel pollution in solid medium

The growth assay was used to find the resistant fungal species to diesel fuel contamination. The assay were conducted by comparing the growth rates of fungal strains, as colony diameter, on the diesel fuel contaminated and control petri dishes. Test dishes were prepared by adding diesel fuel to warm PDA solution. In order to have 0.0 , 1%, 2%, 3% , 4%, 5% concentration of diesel fuel in all plates, the solution was thoroughly mixed manaually, right before it was added to the plates. Pure PDA was used in control plates. All dishes were incubated with 5 mm plugs of fungal mycelia taken from agar inoculums plate. The dishes were incubated at 25°C in an incubator. Fungal mycelia extension on the plates (colony diameter) was measured using with measuring tape after 7 days and compared with control plates.

Biomass determination on diesel fuel hydrocarbons in mineral salts medium

Fungal biomass was determined by filtering the culture broth through Whattman No.1 filter paper . Determination of dry weight of mycelia of fungal strains by harvested after 7 days incubation in flasks containing liquid mineral salts medium amended with diesel fuel and compared with other flasks without containing diesel fuel (control) on filter paper by filtration and dried in the oven with 65 °C in 30 min. pH was determined with pH meter. The difference between gain in treatment and control was considered to due biodegradation activity of fungi.

Biodegradation of Diese fuel

Determination of residual diesel fuel was carried out by using quantatitative analysis by FTIR Spectroscopy with some modification of method[29]. Residual diesel fuel was extracted with hexane. For sample preparation, 5 mI from mineral salt medium treatments with different fungi strains was transferred in to 20 mI glasses vial and 5 mI of hexane was added to it. The glasses vial was shaken vigorously for about 2 minutes with periodic venting to release vapor pressure . The organic layer was allowed to separate for 10 minutes and was recovered into the another glasses vial . The aqueous layer was re-extracted twice with 2 mI of hexane , as well as the aqueous layer was re-extracted third with 2 mI of hexane. The combined extract was dried by passing through the funnel containing the anhydrous sodium sulfate . The dried extract was concentrated with evaporation on hot plate.

FTIR Spectroscopy Schimadza, Japan, spectrum one equipment in the mid – IR region (500-4000 cm^{-1}) at 16 scan speed was used for analysis using the method of Fuad *et al.*[9] with minor modification .

Stasitical Analysis

The present study conducted an ANOVA (analysis of variance) which was performed on all the treatments and done using the SPSS (version 10.0) package to determine whether or not, a significance differences.

RESULTS AND DISCUSSION

Determination of the fungal growth ability under Diesel fuel pollution in solid medium

The growth ability of the isolated fungal strains was carried out under 0.0,1%, 2%, 3%, 4%, 5% concentrations of diesel fuel and was expressed as diameter of the colony (Fig1,Fig 2). The results showed that *A.niger* was resistence to diesel fuel in 1% , 3% , 4% concentration in solid medium, and the colony diameter of this fungus reached to 5.8 , 6.4, 6.7 cm respectively, but this fungus was sensitive toward diesel fuel in 5% with 3.7 cm colony diameter . However the results showed that diesel fuel not appear any effect on the colony of *A.flavus* ,but the *A.versicolor* was sensitive in 3%, 4%, 5% concentration and the colony of this fungus was increased to 7.2,7.9 cm with 1%, 2% when compared with control . Mohsenzadeh *et al.*[22] refer that the fungal species used oil compounds as nutrients and crude oil pollution cause to increase fungal growth. The results in the present study were similar to the findings of [3] which showed that the fungus *A.niger* and *R.stolinifer* are resistant to kerosene pollution. Among the studied fungus, *R.stolinifer* showed the highest resistance to all concentrations of kerosene in solid media (with 8.5 cm diameter of colony after 7 days growth), and *A. niger* also resistant. The colony diameters were determined after 7 days in the 0.0 , 5% , 10% , 15%, 20% concentration of kerosene polluted PDA media. The results in present study showed that the colony diameter of *P. funiculosum* decreased in 1%, 2%, 3%, 4% concentration except that the colony diameter of this fungus was increased in 5% when compared with control. And in the same time the results showed that *R. solani* was sensitive with all concentrations of diesel fuel. The stasitical methods obtained different significance with fungi and also with 5% concentration when compared with control. These results confirm that the biodegradation process depend on the type of hydrocarbon, the genus, species, and may be the strain of the fungus, as well as on nutritional and fermentation conditions[16]. Also [4] refer that *A. niger* showed the highest resistence to 2% crude oil pollution (with 8.5 cm diameter of colony after 7 days growth) and three fungal strains including *F.solani* (5.9 cm), *A. fumigatus* (4.5 cm), *P. funiculosum* (3.6 cm) . In the same time[17] recorded that *A. niger* showed the largest colony diameter on medium with 20 % kerosene amongst *A. terreus*, *Rhizopus sp.* and *Penicillium* sp.

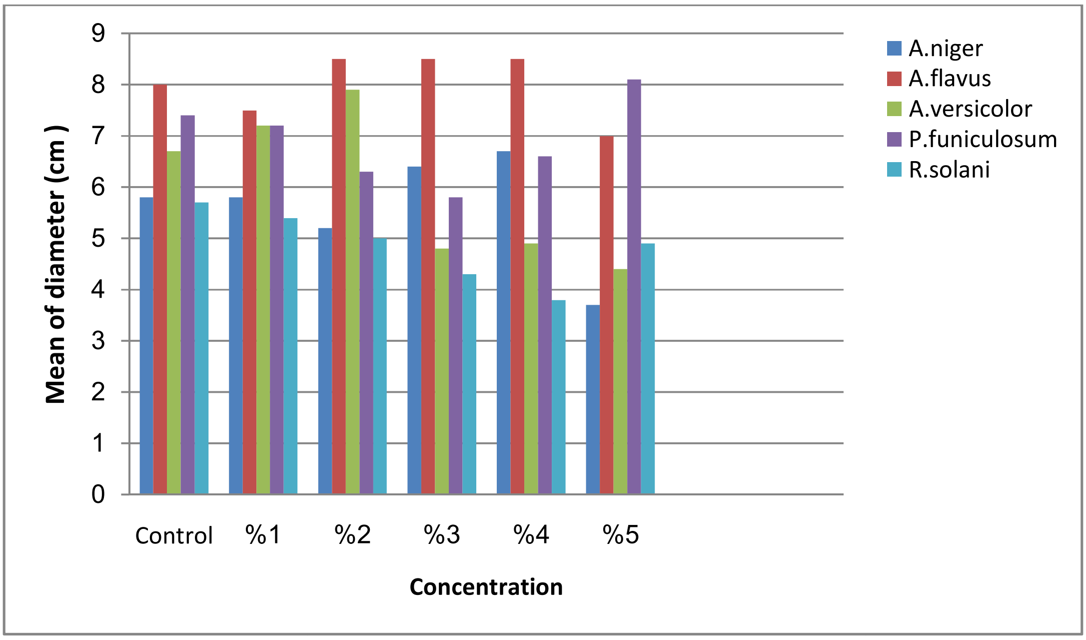
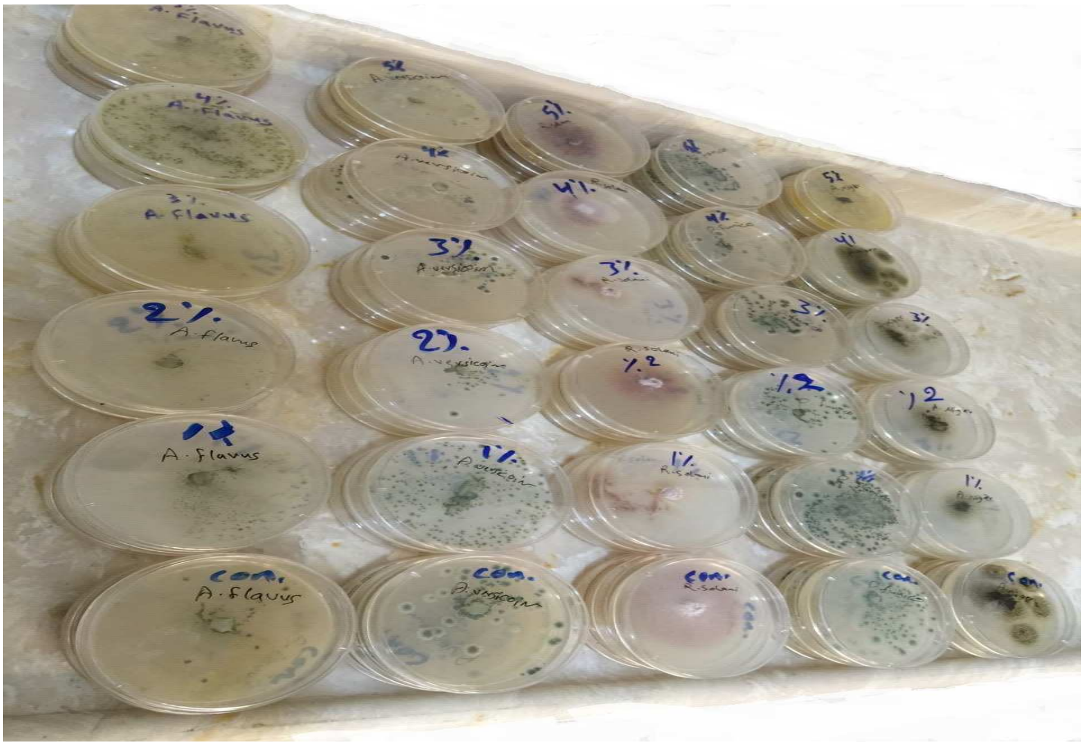


Fig. 1: Effect of Diesel fuel on colony diameter of fungal strains in solid media



A B C D E

Fig. 2: Growth determination of the fungi in different concentrations of diesel fuel in Potato Dextrose Agar (PDA)

A : *Aspergillus flavus* B: *Penicillium funiculosum* C : *Rhizoctonia solani*

D : *Aspergillus versicolor* E: *Aspergillus niger*

Biomass determination on diesel fuel hydrocarbons in mineral salts medium

Fig.3 showed that all fungi under study were resistence with diesel fuel in all concentrations when determined dry weights of these fungi compared with control after seven days incubation. Fig. 3 obtained that *A.niger* was the highest dry weight among than other fungi under study , the dry weight of this fungus reached to 2.84 gm in 5% diesel fuel when compared with control but the results also showed that the lowest dry weigh was calculated with *A.versicolor* (0.509 gm). The stasitical methods obtained different significance with all fungi and also with concentrations of diesel fuel. This result was similar to the findings of [3] which showed that all fungi studied are resistant to kerosene polluted mineral salts media with 10% concentration but the dry weight of these fungi were decreased with 20% concentration. Among the studied fungi, *A.niger* showed the highest resistance to 10% kerosene pollution (with 0.530 gm dry weight of mycelia after 7 days growth), and the dry weight of *R.stolinifer* reached to 0.522 gm. The same results were obtained by Hashem[12] in their study on the changes of mycelium dry weight of *A.niger* , *A.flavus* , *Curvularia lunata* , *Rhizopus sp.* and *Trichoderma sp.* on media containing different concentrations of crude oil (0.5 , 1.0 , 2.0 ml), the results showed in this study that *Trichoderma sp.* exhibited an increasing mycelium dry weight with increase in crude oil concentrations while *A.niger* dry weight reached to 2.68 mg in 2% concentration , but the lowest dry weight was calculated with *Rhizopus sp.* They had been showen that fungi were as active or more active than bacteria in the biodegradation of hydrocarbons . In this indicated that these fungi had adapted to degrade the petroleum hydrocarbons. Atlas *et al.*[7] reported that in the most environment systems which may become contaminated with petroleum hydrocarbons. There were indigenous oil degrading microorganisms capable of seeding the oil spilled and initiated microbial attack. Obuekwe *et al.*[24] in their study about the effect of oil spill on the composition of microbes in a soil , they found that the soil was dominated by a diversity of oil degrading fungi including *Penicllium sp.*, *Rhizopus sp.* *Thamanidium sp.*, *Cunninghamella* and *Candida sp.* When petroleum hydrocarbons polluted various habitats the indigenous microflora was two fold. The hydrocarbons may inhibited or caused by a death of certain microorganisms. On the other hand, there will also be increasing in numbers of certain microorganisms especially those capable of degrading the hydrocarbons[19]. In the same time[9] record that *Eupenicillium hirayamae* gained the maximum weight of (43.4 %) followed by *Cladosporium sphaerospermum* (40 %), whereas minimum weight gain (28 %) was recorded in *Alternaria alternate.*

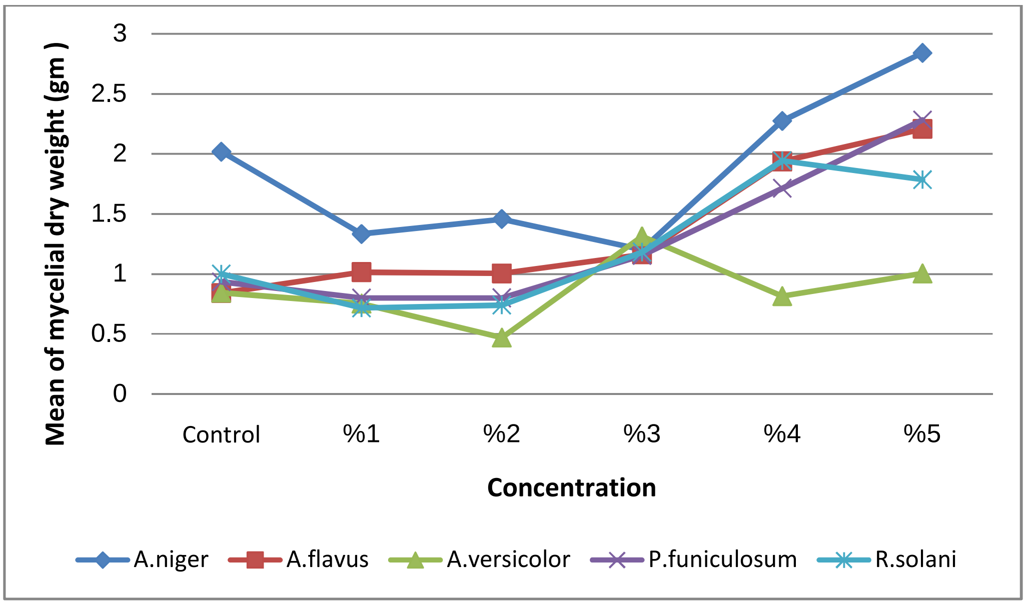


Fig. 3: Effect of Diesel fuel on mycelial dry weight to fungal strains in liquid media

Biodegradation of Diese fuel

The results showed that the axenic cultures of fungi degraded diesel fuel in mineral salts medium. Fig 5,6,7,8,9 showed disappereance of large number of band when compared with control (uninculated) Fig .4 Fig FTIR of non – degraded (uninculated) revealed three prominent peaks represented hydrocarbons due to the > CH_2 symmetric (2853, 2922, 2955 cm^{-1}). A-CH_3 symmetric and asymmetric bend for an aliphatic hydrocarbon chain and for either a linear aliphatic hydrocarbon chain or a methyl benzene derivative was observed at 1459, 1377 cm^{-1}. A ring vibration at 1040 and 1588 cm^{-1} represented alkyl cycloalkanes and aromatic hydrocarbons, and peaks 615 , 740 , 798 , 873 cm^{-1} represented mono-, tri- and tetra-substituted benzene derivatives (Fig.4). Diesel fuel extracted after incubation for 7 days showed bands at3330 , 3335 , 3339 , 3343, 3346 , 3089 cm^{-1} indicated alcohol and four sharp bands between 533 - 1639 cm^{-1} ,indicated the formation of alkyens, alkenes and carboxylic acids .

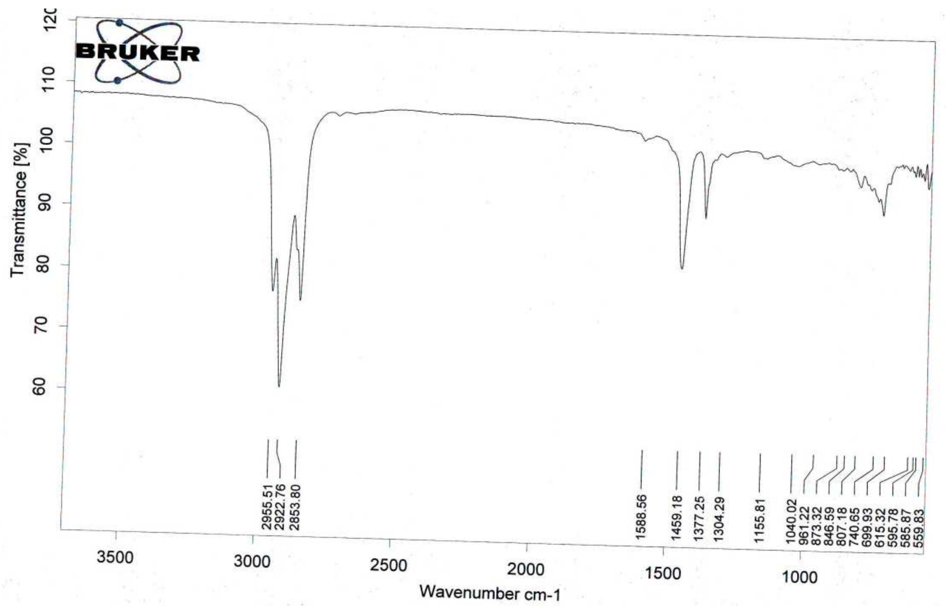


Fig. 4: Diesel fuel (Standard) – uninculated

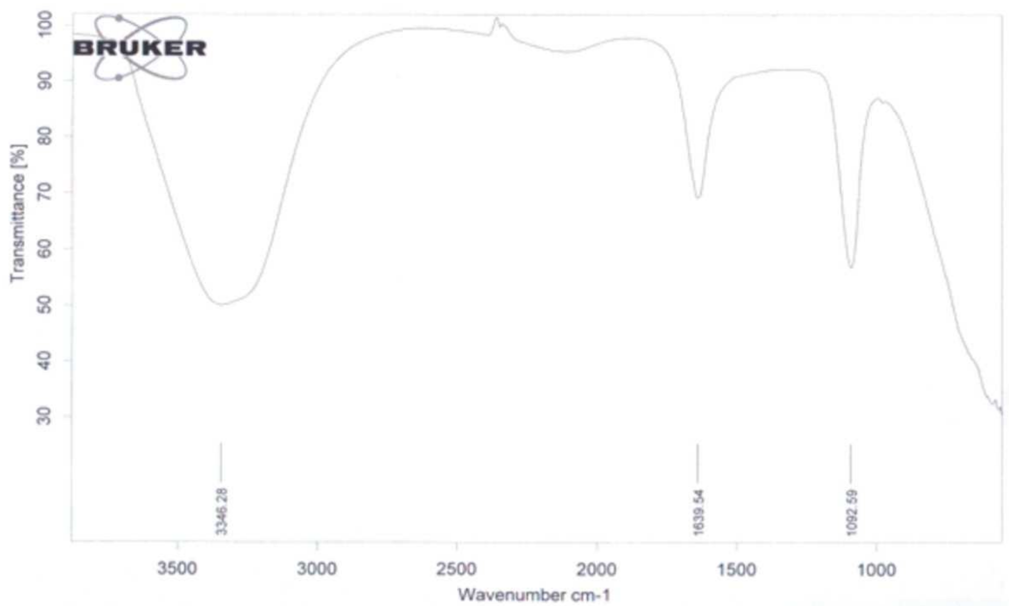


Fig. 5: Biodegradation of diesel fuel by *A.niger* after 7 day incubation

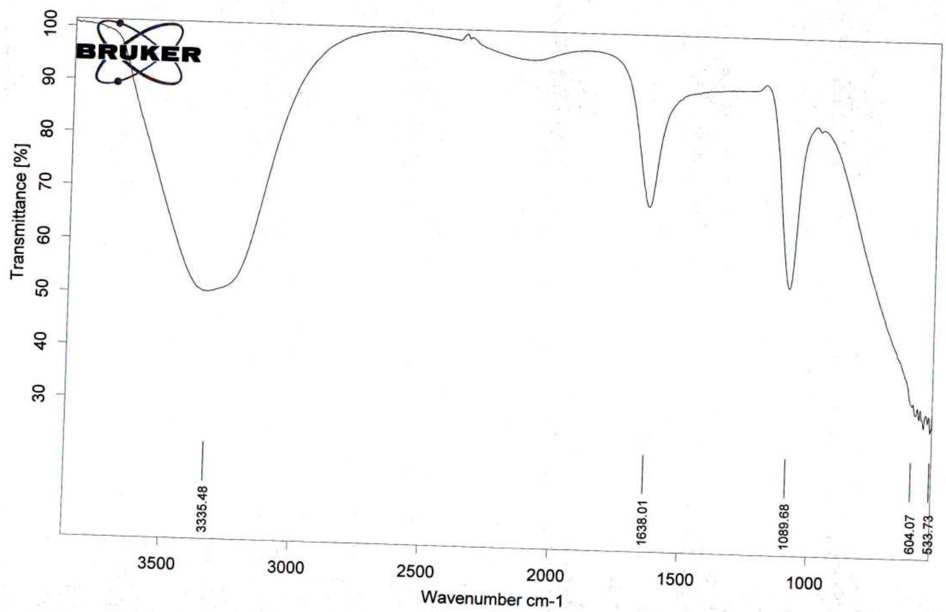


Fig. 6: Biodegradation of diesel fuel by *A.flavus* after 7 day incubation

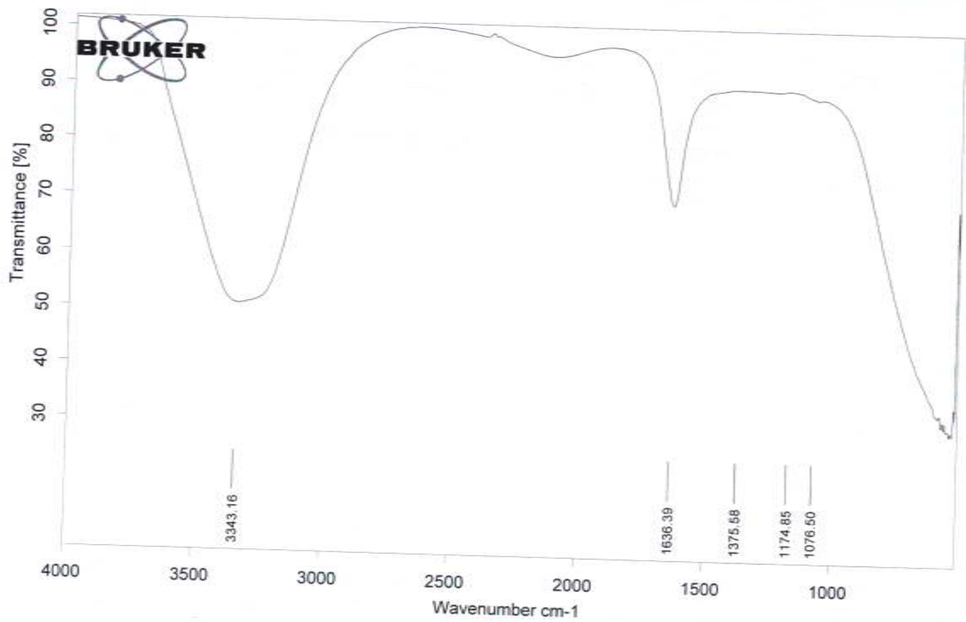


Fig. 7: Biodegradation of diesel fuel by *A.versicolor* after 7 day incubation

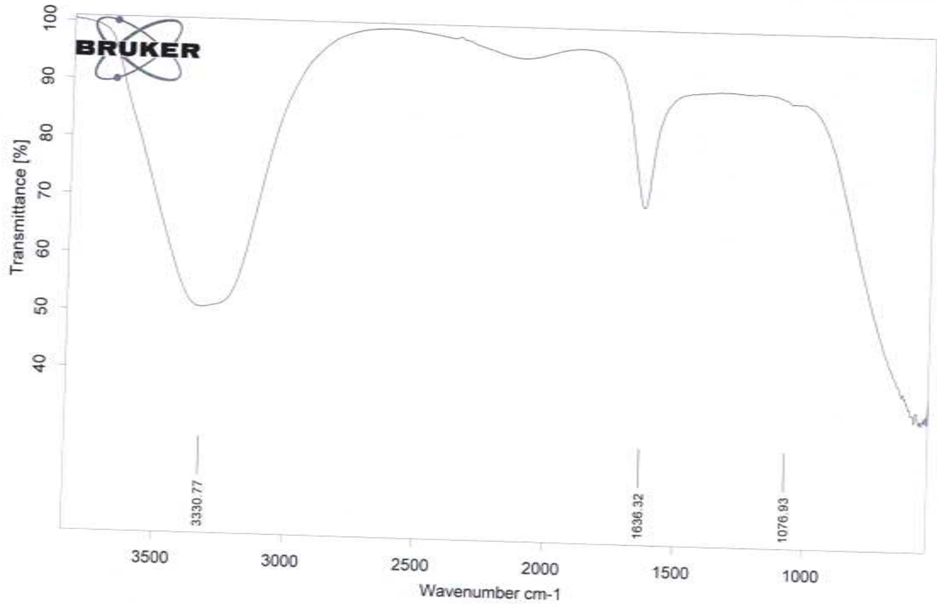


Fig. 8: Biodegradation of diesel fuel by *P.funiculosum* after 7 day incubation

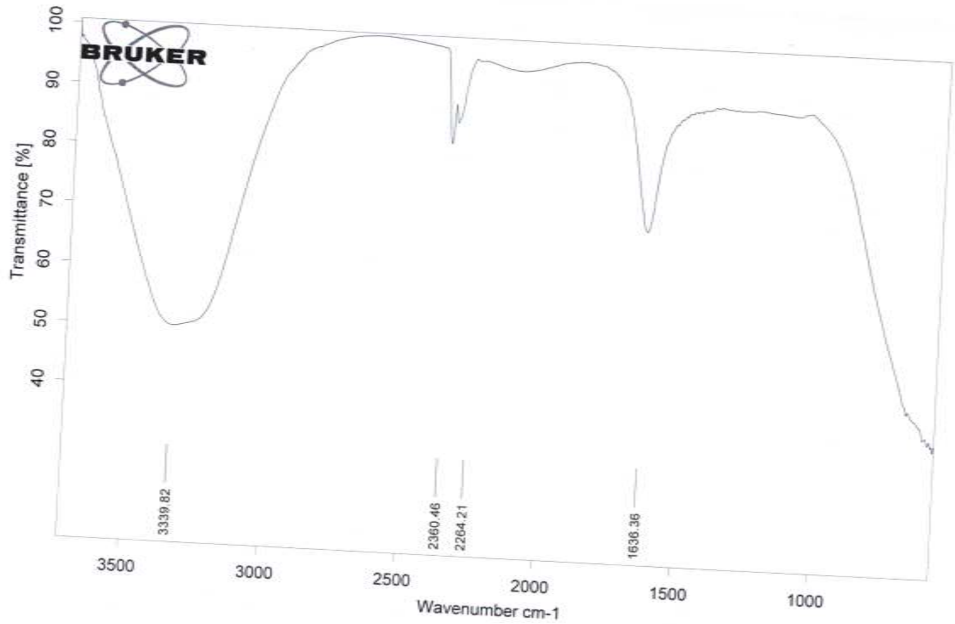


Fig. 9: Biodegradation of diesel fuel by *R.solani* after 7 day incubation

This result was similar to the findins of [30,10,4] which showed that *Aspergillus versicolor* and *Aspergillus niger* exhibited biodegradation of hydrocarbons higher than 98%. This means that the fermentation conditions stimulated productivity and or activity of diesel fuel hydrolyzing enzymes[9].

The results obtained in present study refer to produced carboxylic acids in mineral salts medium after incubation 7 days with different fungi , these acids were decreased pH in medium . The results was similar to findings of [3] which showed that the pH values was changed on kerosene during utilization by the fungal isolates from 0h to 28th days of incubation and obtained non significant in the changes in pH values. *A. niger* had the lowest pH of 4.6 after 28 days of incubation ,but the *R.stolinifer* had the highest pH value of 6.3 after 28 days incubation. The reduction in pH of the culture fluids in flasks within 28 days incubation period confirmed chemical changes of the hydrocarbon substrates which must have been precipitated by microbial enzymes[6]. However microbial degradation of hydrocarbons often leads to production of organic acids and other metabolic products[23] .

CONCLUSION

The results showed that all fungi were well adapted to degrade and utilize the diesel fuel and convert this compound to other metabolites .

Rehabilitation of oil contaminated soil and water by the culture fungi (*A.niger, A. flavus, A.versicolor, P. funiculosum* and *R. solani*) were promising as it can reduce the oil pollution 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 petroleum hydrocarbons and behavior of fungi in polluted soils in different location , and how these fungi to breakdown or biodegradation petroleum hydrocarbons in environment ,as well as can used these organisms to removel pollution now and also in future.

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