Poll Res. 39 (1) : 12-18 (2020)

ISSN 0257–8050

BIODEGRADATION OF CRUDE OIL BY NEMATODE TRAPPING FUNGI ISOLATED FROM IRAQ

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ABSTRACT

This is the first report around the world to determinate the capability of some species nematode trapping fungi to biodegrade of crude oil. The tested fungi have ability to biodegrade the crude oil. *A.cookedickinson*, *A.microscaphoides*, *A.thaumasia* and *C.rosea* recorded high ability to biodegrade of crude oil reached to 70-75% after 7 days incubation, whereas other tested fungi caused moderate susceptibility 30-35%. The ability of tested fungi to biodegrade of oil was slightly different after 30 days compare with their ability after 7 days. pH values decreased after 30 days of incubation of all fungal liquid media from 7.1 to 6.4 or 6.2 depending on the type of species. *A.cookedickinson* gave the highest fresh and dry weight reached to 8.83g and 0.52g respectively after 30 days of incubation, followed by *A.thaumasia* (7.565g fresh weight and 0.435g dry weight). *A.oligospora* gave the lowest fresh and dry weight of fungal mycelium to reach 3.23g and 0.185g respectively. On other hand, *A.conoides* showed inability to degrade oil compounds or growth on mineral salts medium.

KEY WORDS: Nematode trapping fungi, Biodegradation, Crude oil, pH, Arthrobotrys.

INRTODUCTION

Crude oil or Petroleum hydrocarbons (PHs) are very important energetic resources and materials for different industries in the world, including oil-derived products and the refinery-petrochemical industry and are also widespread used in many chemical processes (Brown *et al*., 2017 and Marchand *et al*., 2017). PHs are becoming a global problem for the environment. They are highly persistent in the environment, toxic and present significant health risks to human (Hentati *et al*., 2013). PHs are major environmental pollutants in oil producing countries as a result of extraction and processing of the oil. Therefore, crude oil spills from pipelines, refineries wide-scale production, transport and shipping activities cause damage to the environment (Arulazhagan *et al.*, 2010). PHs are a big group of chemicals that have caused a major concern because of their widespread distribution into the environment, bioaccumulation potential, harmful effects and biodegradation resistance (Al-Hawash *et al*., 2018). The crude oil is a complex mixture of alkanes, aromatic hydrocarbons and nitrogen-, oxygen- and sulfur-containing compounds (Cai *et al*., 2016).

The contamination changes in the physicochemical and biological characteristic of the soil because the oil may be toxic to soil microorganisms and plants. Its effects on living organisms and its fate in environment vary which depending on the crude oil components and concentration (Minai-Tehrani and Herfatmanesh, 2007).

Bioremediation is the use of naturally-occurring microorganisms or genetically-engineered microorganisms (bacteria and fungi) by man, to detoxify man-made pollutants (Odgen and Adams, 1989). There has been increasing interest by researchers in the application of organisms and nutrients to contaminated soils for effective biodegradation of oil. Naturally-occurring microbial communities that respond to the presence of contaminating hydrocarbons normally have more than one type of hydrocarbon utilizing microorganisms (Obire *et al.*, 2008; Sajna *et al.*, 2015). Various bacteria and fungi use some crude oil fractions as a sole carbon source and change them to

non-toxic compounds such as CO2 (Cerniglia, 1992). Various strains of white-rot fungi capable of degrading aromatic compounds (Barr and Aust 1994 and Behnood *et al*., 2013).

Nematophagous fungi are group of fungi that are ubiquitous in nature and have been well distributed in variety of ecological habitats and environment worldwide with the ability to infect and nematodes for the benefit of nutrients. (Muhsin and kasim, 1998 and Singh *et al*., 2014 and Zhang *et al*., 2016). Nematophagous fungi have concentrated on soil agriculture and animal husbandry or forestry (Su *et al*., 2007 and Askary, 2015) or freshwater environments (Hao *et al*., 2005). The distribution and occurrence of nematode-trapping species and groups of fungi are associated with specific soil variables in moisture, particular pH, nutrients (N, P, K), nematode density and heavy metal (Gray, 1985 and Mo *et al*., 2008 and Anfal and Kasim, 2019). Many studies indicated that number of hydrolytic enzymes produced by nematophagous fungi such as proteases, lipases, amylases, chitinase, acid phosphatase and pectinases have been detected *in vitro* (Lopez-Llorca and Duncan, 1988 and Dackman *et al*., 1989 and Kasim, 2016). The ability of these fungi in biodegradation of crude oil has not been studied previously. Therefore, the aim of this study is to determine the capability of some species of nematode-trapping fungi that isolated from Iraqi soil to utilize and degrade hydrocarbons in crude oil

MATERIALS AND METHODS

Crude oil

Crude oil was supplied by the Misan Oil Company (Misan Province, Iraq) and brought to the laboratory in a tightly closed dark bottle and kept in a cool, dark place until used.

Fungal Isolates

Nine species of nematode-trapping fungi (*Arthrobotrys conoides* (Ac), *A.cookedickinson* (Ack), *A.eudermata* (Ae), *A.microscaphoides* (Am), *A.oligospora* (Ao), *A.rutgeriens* (Ar), *A.thaumasia* (At), *Clonostachys rosea* (Cr) *Drechslerella brochopaga* (Db) (were supplied by Fungi Laboratory, Biology Department, College of Sciences, University of Misan isolated from agriculture soil of Misan province/southern Iraq) were tested to evaluate the most appropriate partners for biodegradation of crude oil.

Determination of the Fungal Ability in Biodegradation of Crude Oil

10 ml flask containing sterilizer 2 ml mineral salts medium (MSM) (containing (gI-1): (NH4)2SO4, 2; KH2PO4, 4; Na2HPO4, 6; FeSO47H2O, 0.2; CaCl2, 0.001; H3BO3, 0.000015; MnSO4, 0.00001; ZnSO4, 0.00007; CuSO4,0.00001) were prepared, 10˜l of crude oil were added per flask, then inoculated with 5mm disk cut from the axenic fungal culture (grown on corn meal agar medium) of each isolate (except control) and incubated at 25 °C for 7 days (duplicates for each fungus and control). The susceptibility of fungi to break down crude oil was determined by the amount of decomposed oil as follows: 0 indicates that crude oil remains unchanged, which means that fungus has not been able to biodegrade crude oil, + indicates little biodegradation ranging from 30-25% of the total amount of crude oil, ++ indicates high biodegradation ranging from 75-70% of the total amount of crude oil (Lemos *et al*., 2002).

Determination of the Fungal Ability in Biodegradation of Crude Oil after 30 days.

250 ml flask containing sterilizer 100 ml MSM were prepared, 0.1 ml of crude oil were added per flask (except control) then inoculated with 3 disks (5mm in diameter) cut from the axenic fungal culture of each isolate and incubated at 25 °C for 30 days (duplicates for each fungus and control). The susceptibility of nematode-trapping fungi to break down crude oil was determined as above and the amount of fungal growth of tested fungi was measured (Lemos *et al.* 2002)

RESULTS

Iraq one of the most impartment oil producing countries in world, therefore, soil and water may be contaminating as a result of extraction and processing of the oil. In nature, many microorganisms such as bacteria and fungi can be decrease these contaminations by degrade the oil and its products compounds. Nemato-phagous fungi are significantly more abundant in the Iraqi soils (Muhsin and Kasim, 1998; Anfal and Kasim, 2019). This is the first study around the world to measure the ability of nematode trapping fungi to biodegrade of crude oil and indicates that the tested fungi have ability to biodegrade the crude oil. Tested fungi showed different capability of breaking

down of oil compounds. Table 1 shows that *A.cookedickinson*, *A.microscaphoides*, *A.thaumasia* and *C.rosea* have high ability to biodegrade reached to 70-75% after 7 days, whereas *A.eudermata*, *A.oligospora*, *A.rutgeriense* and *D.brochopaga* caused moderate susceptibility 30-35%. On other hand, inability to degrade oil compounds was observed when using *A.conoides* (Figure 1 and 2).

Table 1. Ability of nematode trapping fungi to biodegrade the crude oil after 7 and 30 days.

Fungal species	Rate of biodegradation	
	After 7 days	After 30 days
Arthrobotrys conoides (Ac)	0	0
A. cookedickinson (Ack)	++	++
A. eudermata (Ae)	+	++
A. microcaphoides (Am)	++	++
A. oligospora (Ao)	+	+
A. rutgeriens (Ar)	+	+
A. thaumasia (At)	++	++
Clonostachys rosea (Cr)	++	++
Drechslerella brochopaga (Db)	+	+

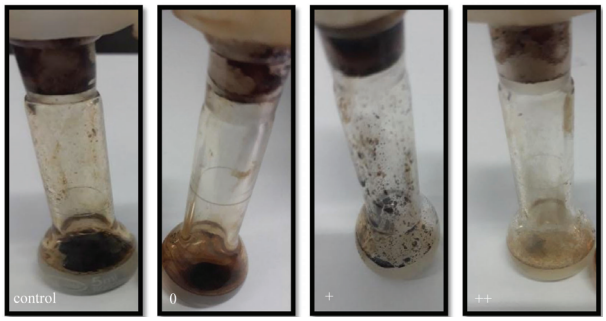

Fig. 1. Biodegradation indicator of crude oil: control MSM medium without fungi, 0 crude oil remains unchanged, + little biodegradation ranging from 30-25% of the total amount of crude oil, ++ high biodegradation ranging from 75-70% of the total amount of crude oil (Lemos *et al.*, 2001).

The ability of tested fungi was slightly different after 30 days were the susceptibility of species to biodegrade of oil was similar to that of 7 days except the *A.eudermata* which had been an ability to biodegrade reached to 70-75%.

The results showed that the tested fungi revealed a distinct growth of mycelium. Some species gave dense mass of mycelium in the liquid medium after biodegra-dation of crude oil, while others species formed a net of mycelium, at the bottom of flask under the crude oil which floats on the surface of the

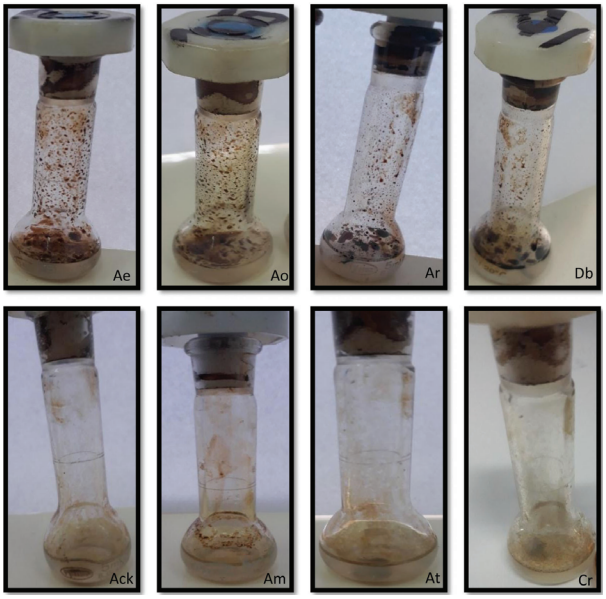

Fig. 2. Biodegradation of crude oil by nematode trapping fungi after 7 days incubation: *A.eudermata* (Ae), *A.oligospora* (Ao), *A.rutgeriens* (Ar) and *D.brochopaga* (Db) gave biodegradation ranging from 30-35% , *A . A.cookedickinson* (Ack), *A .microcaphoides* (Am), *A.thaumasia* (At) and *C.rosea* (Cr) gave biodegradation ranging from 70-75%

liquid medium. Moreover, the crude oil was transformed from a shiny to a no shiny and semi-solid layer. The biomass of fungal colonies increased over time and varied between species (Figure 3).

The results showed that pH values decreased after 30 days of incubation of all fungal liquid media and that they varied depending on the type of species. The pH values of *A.oligospora*, *A.rutgeriens* (30-35% biodegradation) and *A.thaumasia* (70-75% biodegradation) decreased to 6.4, on other hand the rest species gave 6.2 (Table 2).

Fresh and dry weight of fungal colonies were differed between tested fungi. *A.cookedickinson* recorded the highest fresh and dry weight reached

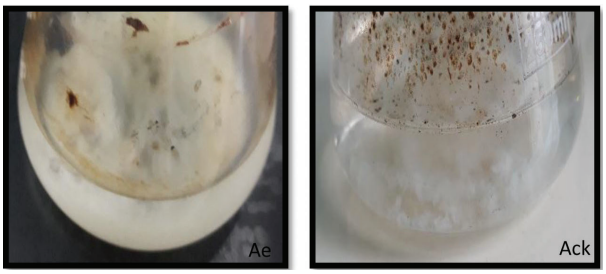

Fig. 3. Growth of mycelium, *A. eudermata* (Ae) showed dense mass of mycelium, *A.cookedickinson* (Ack) showed net of mycelium

to 8.83g and 0.52g respectively after 30 days of incubation, followed by *A.thaumasia* (7.565g fresh weight and 0.435g dry weight) and *C.rosea* 7.225g and 0.41g respectively.

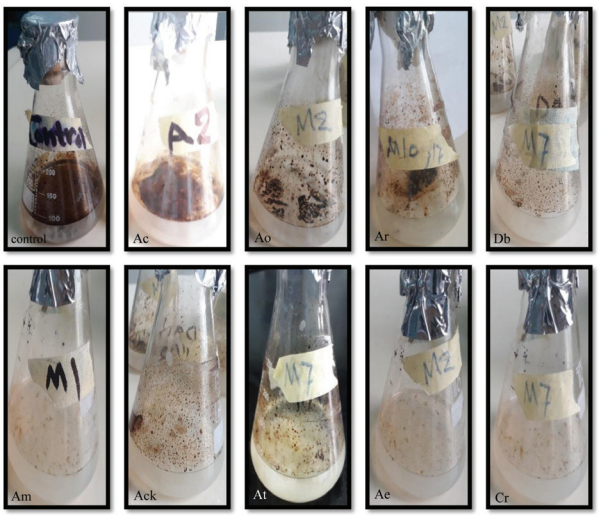

Fig. 3. Biodegradation of crude oil by nematode trapping fungi after 30 days incubation: *A.conoides* (Ac) no biodegradation potential, *A.oligospora* (Ao), *A.rutgeriens* (Ar) and *D.brochopaga* (Db) gave biodegradation ranging from 30-35%, *A. cookedickinson* (Ack), *A .microcaphoides* (Am), *A. thaumasia* (At), *A .eudermata* (Ae) and *C.rosea* (Cr) gave biodegradation ranging from 70-75%.

while *A. oligospora* gave the lowest fresh and dry weight of fungal mycelium to reach 3.23g and 0.185g respectively and *A. rutgeriens* 3.535g and 0.26g respectively, on the other hand, the rest tested fungi revealed different values of fungal growth (Figure 4, Table 2).

DISCUSSION

The results indicate that many of the nematode trapping fungi isolated from agriculture soil of Misan province were capable of degrading crude oil. Furthermore, there are no published reports dealing with ability of these fungi to degrade of crude oil. Sunlight and microorganisms (fungi and bacteria) have ability to degrade of petroleum that spills to soil even in relatively large quantities, so the petroleum did not persist for long periods in the most soils (Atlas, 1981 and Alrumman *et al.,* 2015). The existence of microbial populations in environment that respond to the presence of contaminating hydrocarbons normally have more than one kind of hydrocarbon utilizing microorganisms (Obire *et al*., 2008). Recently, many studies showed that fungi were better degraders than traditional bioremediation techniques including bacteria (Batelle, 2000). Moreover Many reports indicated that the most fungi capable of biodegrading the petroleum oil, though at different rates (Adekunle and Adebambo, 2007, Elshafie *et al.,* 2007 and Al-Jawhari, 2014 and Reyes-Ce´sar, *et al*., 2013).

Agematu *et al*. (1993) showed that the secondary metabolism of several fungi contains laccases (the enzymes are secreted out in the medium extracellularly). Many of soil as well as some freshwater ascomycetes species capable of producing laccase (Scherer and Fischer, 1998, Abdel-Raheem and Shearer, 2002 and Junghanns *et al.,* 2005). Moreover, fungi are one of the best oil-degrading organisms and are capable of using crude oil as their sole source of carbon and energy (Viswanath *et al.,*2008, Shraddha *et al*., 2011 and Upadhyay *et al.*, 2011). The results showed that the fungi isolated had been increased growth rates in the media containing petroleum compared to only when minimal salt broth was used, which gave on growth. Mohsenzadeh *et al.* (2012) showed that the growth and variation of many fungi cannot

Table 2. Fresh and dry weight of biomass (g) and decrease in pH of tested nematode trapping fungi after 30 days of crude oil biodegrading

Fungal species	Wet weight (g)	Dry weight (g)	Decrease in pH
Arthrobotrys conoides (Ac)	0	0	7.1
A. cookedichison (Ack)	8.83	0.52	6.2
A. eudermata (Ae)	5.51	0.365	6.2
A. microcaphoides (Am)	5.28	0.345	6.2
A. oligospora (Ao)	3.23	0.185	6.4
A. rutgeriens (Ar)	3.535	0.26	6.4
A. thaumasia (At)	7.565	0.435	6.4
Clonostachys rosea (Cr)	7.225	0.41	6.2
Drechslerella brochopaga(Db)	3.43	0.215	6.2

inhibited by petroleum pollution, moreover, the growth may be increased when fungal species used oil compounds as nutrients. Many fungi can use the hydrocarbons as substrates for growth by probably releasing extra cellular enzymes and acids which are capable of breaking down the hydrocarbon molecules to releases carbon (IV) oxide, water and energy used to synthesize cellular components and therefore create cellular biomass (Keeler, 1991 and Al-Nasrawi, 2012). Rudd *et al.* (1996) showed that the biodegradation of about 25% of the crude oil leads to increase mycelial mat weights.

The rate of biodegradation is influenced by many factors such as the kind of microorganisms, environmental and physical factors such as nutrients, moisture, soil type, pH, temperature, oxygen water holding capacity and nutrient limitations. (Aharoni *et al*., 2017 and Avishai *et al*., 2017). The pH can be highly changing and must be taken into consideration when developing biodegradation methods. The environmental pH affects processes such as cell membrane transport and catalytic reaction balance well as enzyme activities (Bonomo *et al.,* 2001 and Al-Hawash *et al.,* 2018). pH is a controlling in determining biodegradetion in soil and the pH range of 5.0 to 7.8 favored the degradation of oily sludge in the soil (Dibble and Bartha, 1979 and Leahy and Colwell, 1990). In this study the pH of liquid cultures in flasks reduced after the incubation from 7.1 to 6.4 or 6.2 after 30 days. Many workers have provided that the pH value reduced after the incubation (Oboh *et al.*, 2006 and Al-Jawhari, 2015). Organic acids and other metabolic products produce by microbial degradation of hydrocarbon consequently probably produced account for the reduction in pH levels (Nwachukwu and Ugoji, 1995 and Oboh *et al.*, 2006).

Zhang *et al.* (2011) indicated that nematode-trapping fungi are found in all regions of the world, from the tropics to Antarctica. They are commonly found in soils and other sites such as decaying wood, compost, decaying leaf litter, mosses and decaying leaf litter. Nematode-trapping fungi have ability to produce many secretes extracellular enzymes such as collagenases, chitinases and proteases (Tunlid *et al.*, 1994 and Morton *et al.*, 2004), which may be degraded crude oil compounds

GC-MS analysis reveal that nematode trapping fungi have ability to produce some active compounds (Raheem and Kasim, unpublished data) such as 1,3,50 triazine, 2-amino-4,6-bis (which is used in oil treatment and desulfurization), Oleyl alcohol, heptafluorobutyrate, phthalic acide, Dodecanoicacid, Piperazine, 1- (1-methyl-4-piperidyl) -, Docosanoic acid, Eicosanoic acid, 9-hexadecenyl ester, (z), Undecanoic acid, 10-bromo (which are involved in the production of detergents), we therefore think that these fungi have the potential to degrade crude oil because its ability to produce many chemical compounds and degradative enzymes.

REFERENCES

Abdel-Raheem, A. and Shearer, C.A. 2002. Extracellular enzyme production by freshwater ascomycetes. *Fungal Diversity*. 11 : 1-19.

Adekunle, A.A. and Adebambo, O.A. 2007. Petroleum Hydrocarbon Utilization by Fungi Isolated from *Detariumsenegalense* (J. F Gmelin) Seeds. *Journal of American Science*. 3 : 69-76.

Al-Hawash, Adnan B., Dragh, Maytham A., Shue Li, Alhujaily, Ahmad,Hayder, A. Abbood, Xiaoyu Zhang and Fuying Ma. 2018. Principles of microbial degradation of petroleum hydrocarbons in the environment. *Egyptian Journal of Aquatic Research* 44 : 71-76.

Al-Jawhari, I. F. H. 2015. Ability of some fungi isolated from a sediment of Suq-Al Shuyukh marshes on biodegradation of crude oil *Int. J. Curr. Microbiol. App. Sci*. 4 (1) : 19-32.

Al-Nasrawi, H. 2012. Biodegradation of Crude Oil by Fungi Isolated from Gulf of Mexico. *J Bioremed Biodegrad* 3:147. doi:10.4172/2155- 6199.1000147

Agematu, H.,Tsuchida, Kominato, Enzymatic dimerization of penicillin. *Journal of Antibiotics*. 46(1) : 141-148.

Aharoni, I., Siebner, H. and Dahan, O. 2017. Application of vadose-zone monitoring system for real-time characterization of leachate percolation in and under a municipal landfill. *Waste Manage*. 67 : 203-213.

Alrumman, S. A. Dominic, B. Standing and Graeme, I. Paton. 2015. Effects of hydrocarbon contamination on soil microbial community and enzyme activity. *Journal of King Saud University - Science*. 27: 31-41.

Arulazhagan, P., Vasudevan, N. and Yeom, I. 2010. Biodegradation of polycyclic aromatic hydrocarbon by a halotolerant bacterial consortium isolated from marine environment. *Int. J. Envlron. Sci. Te*. 7 (4) : 639-652.

Askary, T. H. 2015. Nematophagous Fungi as Biocontrol Agents of Phytonematodes. *Biocontrol Agents of Phytonematodes* (eds T.H. Askary and P.R.P. Martinelli). Pp.: 81-125.

Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: an environ-mental perspective. *Microbiology Review*. 45 (1): 108-209.

Avishai, L., Siebner, H., Dahan, O. and Ronen, Z. 2017. Using the natural biodegradation potential of shallow soils for In-situ remediation of deep vadose zone and groundwater. *J. Hazard. Mater.* 324 : 398–405.

Barr, D.P. and Aust, D. 1994. Mechanisms white rot fungi use to degradepollutants. *Environ. Sci. Technol.* 28: 78 - 87.

Batelle C.D. 2000. Mushrooms: Higher Macrofungi to clean up the environment. Environmental Issues, Fall

Behnood, M., Nasernejad, B., Nikazar, M. 2013. Biodegradation of crude oil from saline waste water using white rot fungus *Phanerochaetechryso sporium*. *Journal of Industrial and Engineering Chemistry*. 20 (4) : 1879-1885.

Brown, D.M., Bonte, M., Gill, R., Dawick, J. and Boogaard, P.J. 2017. Heavy hydrocarbon fate and transport in the environment. *Quarterly J. Engin. Geo. Hydrogeo*. 50 : 333–346.

Bonomo, R., Cennamo, G., Purrello, R., Santoro, A., Zappala, R. 2001. Comparison of three fungal laccases from Rigidoporuslignosus and Pleurotusostreatus: correlation between conformation changes and catalytic activity. *J. Inorg. Biochem.* 83 (1) : 67-75.

Cai B, Ma J, Yan G, Dai X, Li M and Guo S. 2016. Comparison of phytoremediation, bioaugmentation and natural attenuation for remediating saline soil contaminated. *Eng. J.* 6(112) : 170–177.

Ceruiglia, C.E. 1992. Bio-degradation of polycyclic aromatic hydrocarbons. *Biodegradation.* 3 : 351-368.

Dackman, C., Chet, I. and Nordbring-Hertz, H.1989. Fungal Parasitism of the cyst nematode *Heteroderaschachtii* infection and enzymatic activity. *FEMS. Microbial Ecol.* 62 : 201-208.

Dibble, J. and Bartha, R. 1979. Effect of environmental parameters on the biodegradation of oil sludge. *Appl. Environ. Microbiol.* 37 (4) : 729–739.

Elshafie, A., AlKindi, A. Y., Al-Busaidi, S., Bakheit, C. and Albahry, S.N. 2007. Biodegradation of crude oil and n-alkanes by fungi isolated from Oman. *Marine Pollution Bulletin.* 54 : 1692–1696.

Gianfreda, L., Xu, F. and Bollag, J. M. 1999. Laccases: a useful group of oxidoreductive enzymes. *Bioremediation Journal*. 3(1) : 1-25.

Gray, N.F. 1985. Ecology of nematophagous fungi: effect of soil moisture, orgaic matter, pH and nematode density on distribution. *Soil Biol. Biochem.* 17 : 449 - 507.

Hao, Y., Mo, M., Su, H. and Zhang, K. Q. 2005. Ecology of aquatic nematode trapping hyphomycetes in southwestern China. *Aquatic Microbial Ecology*. 40: 175–181.

Hentati, O., Lachhab, R., Ayadi, M. and Ksibi, M. 2013. Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays. *Environ. Monit. Assess.* 185: 2989-2998.

Junghanns, C., Moeder, M., Krauss, G., Martin, C. and D. Schlosser, 2005. Degradation of the xenoestrogennonylphenol by aquatic fungi and their laccases. *Microbiology.* 151(1) : 45–57.

Kasim, Ali A. 2016. Influence of bioactive metabolites extracted from three species of nematode-trapping fungi on the growth of *Escherichiacoli* and *Staphylococcusaureus*. *World Journal of Biology and Biological Sciences*. 4(1) : 09-14, October 2016

Keeler, R. 1991. 'Bioremediation', healing the environment naturally. *R & D Magazine*. (2) : 34-40.

Lafta, Anfal A. and Kasim, A. A. 2019. Effect of Nematode-trapping fungi, *Trichoderma harzianum* and *Pseudomonas fluorescens* in controlling *Meloidogyne* spp. *Plant Archives.* 19 (1) : 1163-1168.

Leahy, J.G. and Colwell, R.R. 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* 54 (3) : 305–315.

Lemos, J.L.S., Rizzo, A.C., Millioli, V.S., Soriano, A.U., Sarquis, M.I.M. and Santos, R. 2002. Petroleum degradation by fýlamentous fungi, *9nt Annual International Petroleum Environmental Conference, October 22-25,* Albuquerque, NM.

Lopez-Llorca, L. V. and Duncan, G. H. 1988. A study of fungal endoparasitism of the cereal cyst nematode (*Heteroderaavenae*) by scanning electron microscopy. *Canadian Journal of Microbiology*. 34 : 613–619.

Marchand, C., St-Arnaud, M., Hogland, W., Bell, T.H. and Hijri, M. 2017. Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. *Int. Biodet. Biodeg.* 116 : 48-57.

Muhsin, T.M. and Kasim, Ali, A. 1998. Nematophagous fungi from soils of Iraq. *Acta. Mycol.* 33 (1) : 161-167.

Minai-Tehrani, D. and Herfatmanesh, A. 2007. Biodegradation of Aliphatic and Aromatic Fractions of Heavy Crude Oil-Contaminated Soil: A Pilot Study. *Bioremediat. J.* 11(2): 71-76.

Mo, M. H., Chen, W. M., Yang, H. R. and Zhang, K. Q. 2008. Diversity and metal tolerance of nematode-trapping fungi in Pb-polluted soils. *Journal of Microbiology (Seoul, Korea).* 46 : 16-22.

Mohsenzadeh, F., Rad, A.C. and Akbari, M. 2012. Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils. Irani. *J. Environ. Healt. Sci. Engin.* 9 : 26-34.

Morton, C. O., Hirsch, P. R. and Kerry, B. R. 2004. Infection of plant-parasitic nematodes by nematophagous fungi-A review of the application of molecular biology to understand infection processes and to improve biological control. *Nematology.* 6: 161–170.

Nwachukwu, S.U. and Ugoji, E.O. 1995. Impact of crude petroleum spills on microbial communities of tropical soils. *Int. J. Environ. Sci.* 21: 169 175.

Obire, O., Anyanwu, E.C. and Okigbo, R.N. 2008. Saprophytic and crude oil degrading fungi from cow dung and poultry droppings as bioremediating agents. *Journal of Agricultural Technology.* 4(2) : 81-89.

Oboh, O.B., Ilori, M.O., Akinyemi, J.O. and Adebusoye, S.A. 2006. Hydrocarbon. degrading potential of bacteria isolated from a Nigerian Bitumen (Trasand) deposit. *Nature Sci.* 4(3) : 1 57.

Odgen, R. and Adams, D.A. 1989. Recombinant DNA Technology: Applications. In:Carolina Tips, Vol. 52, Carolina Biological Supply Company, Burlington, NorthCarolina. 18-19.

Ogbo, E. M. and Okhuoya, J. A. 2008. Biodegradation of aliphatic, aromatic, resinic and asphaltic fractions of crude oil contaminated soils by *Pleurotus* tuber-regium Fr. Singer - a white rot fungus. *African Journal of Biotechnology.* 7 (23) : 4291-4297.

Reyes-Ce´sar, A., A ´ ngel E. Absalo´n, Francisco J. Ferna´ndez, Juan Manuel Gonza´lez, Diana V. Corte´s-Espinosa. 2014. Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. *World J Microbiol Biotechnol.* 30 : 999-1009.

Rudd, L. E., Perṛy, J. J., Houk, V. S., Williams, R. W. and Claxton, L. D. 1996. Changes in mutagenicity during crude oil degradation by fungi. *Biodegradation.* 7 : 335-343.

Sajna, K.V., Sukumaran, R.K., Gottumukkala, L.D. and Pandey, A. 2015. Crude oil biodegradation aided by biosurfactants from *Pseudozyma* sp. NII 08165 or its culture broth. *Bioresour. Technol.* 191 : 133-139.

Scherer, M. and Fischer, R. 1998. Purification and characterization of laccase II of *Aspergillus nidulans. Archives of Microbiology.* 170(2) : 78-84.

Shraddha, R., Shekher, S., Sehgal, M., Kamthania, and Kumar, A. 2011. Laccase: microbial sources, production, purification, and potential biotechnological applications. *Enzy. Rese*. 11 page.

Singh, R.K., Pandey, S.K. and Chattopadhyay, A. 2014. Biodiversity and Periodical/Seasonal Distribution of Nematode Trapping Fungi from Different Habitats. *Journal of Pure and Applied Microbiology*. 9 (1) : 767-776.

Su, H., Hao, Y. E., Mo, M. and Zhang, K.Q. 2007. The ecology of nematode-trapping hyphomycetes in cattle dung from three plateau pastures. *Veterinary Parasitology.* 144 : 293-298.

Tunlid, A., Rosén, S., Ek, B. and Rask, L. 1994. Purification and haracterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora. Microbiology (Reading, England),* 140 : 1687-1695.

Upadhyay, P., Shrivastava, R. and Agrawal, P. K. 2016. Bioprospecting and biotechnological applications of fungal laccase. *3 Biotech.* 6 : 15 DOI 10.1007/s13205-015-0316-3.

Varjani, S.J. and Upasani, V.N. 2016. Carbon spectrum utilization by an indigenous strain of *Pseudomonas aeruginosa* NCIM 5514: Production, characterization and surface active properties of biosurfactant. *Bioresour. Technol.* 221 : 510-516.

Viswanath, B., Subhosh Chandra, M., Pallavi, H. and Rajasekhar Reddy, B. 2008. Screening and assessment of laccase producing fungi isolated from different environmental samples. *African Journal of Biotechnology*. 7 (8) : 1129-1133.

Zhang, W., Xiaoli Cheng, Xingzhong Liu, and Meichun Xiang. 2016. Genome Studies on Nematophagous and Entomogenous Fungi in China. *Journal of Fungi* 2 (9): 1-14. https://doi.org/10.3390/jof2010009.

Zhang, Y., Yu, Z. F., Xu, J. and Zhang, K. Q. 2011. Divergence and dispersal of the nematode trapping fungus *Arthrobotrys oligospora* from China. *Environmental Microbiology Reports.* 3 : 763-773.