Myco-Degradation of Petroleum Product by Fungal Species Isolated from Petroleum Contaminated Soils

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Abstract: Microbial degradation is the major and ultimate natural mechanism that can clean up the petroleum hydrocarbon pollutants from the environment. Due to release of hydrocarbons in environment soils and water bodies are contaminated that cause extensive damage to local ecosystem. During present investigation myco-degradation in hydrocarbon contaminated soils has been assessed. Total eight species of six genera of fungi have been isolated from soil contaminated with petrol / petroleum products that facilitate the candidacy as bioremediation agents. Fungal isolates have been identified and in *vitro* examined their ability to degrade petroleum product (Petrol, Diesel and Mobil oil). All fungal isolates exhibited hydrocarbon degrading ability whereas the highest potential was observed in *Curvularia lunata;* however, myco-degradation potential of other fungal isolates were found as - *Curvularia lunata* > *Fusarium solani* > *Aspergillus niger* > *Aspergillus fumigates* > *Penicillium notatum* >*Fusarium oxysporum* > *Rhizopus nigricans* > *Mucor sp.* Assessment of growth pattern in different concentration of Petrol, Diesel and Mobil oil revealed that soil inhabiting fungi - *Curvularia lunata*, *Fusarium solani* and *Aspergillus niger* have greater ability to degrade petrol / petroleum product. Hence this strain can be used in cleaning oil polluted sites to protect the soil and environment.Ultimately findings revealed the extents to which the isolate could degrade crude oil hydrocarbon.

Index Terms- Myco-degradation, Hydrocarbon degraders, Soil fungi, Petroleum products.

I. INTRODUCTION

Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment. Hydrocarbons in environment are biodegraded primarily by bacteria and fungi. Physical, chemical and biological factors have complex effects on hydrocarbon biodegradation in soil (Bossert and Campeau 1995). Petroleum compounds consist of four fractions: saturated hydrocarbons, aromatic hydrocarbons, nitrogen-sulphur-oxygen containing compounds and asphaltenes. Normally, of the saturated hydrocarbons, the straight-chain *n*-alkanes are most susceptible to biodegradation, whereas branched alkanes are less vulnerable to microbial attack. Polycyclic aromatic hydrocarbons occur extensively as pollutants in soil and water and are important environmental contaminants because of their recalcitrance. These compounds also constitute a potential risk to human health, as many of them are carcinogens (Deziel, *et al*., 1996).

Liquid petroleum has become one of the most prevalent pollutants in industrialized and developing countries (Joshi *et al.,* 2011). Its transportation and global usage has increased the tendency to pollute the environment (Tyagi *et al.,* 2011). The source of pollution is usually accidental spills, uncontrolled landfills, leaking underground storage tanks or improper storage crude oil (Plohic *et al.,* 2002). Due to oil mobility, it may cause considerable damage not only in soil, but also in water intakes or ground water reservoirs (Jina *et al.,* 2014). The rate of oil spillage reported in the country has been rising with a corresponding increase in petroleum production (Onifade *et al*., 2007). Oil spills pose serious environmental challenges due to the possibility of air, water, and soil pollution (Trindade *et al.,* 2005). These oil spills are dangerous for health, drinking water, natural resources and disturb the economy (Gesinde *et al.,* 2008).When petroleum products are burned as fuel, they give off carbon dioxide, a greenhouse gas that is linked with the global warming.

Several workers *(*Alexander, 1994; Scragg, 2001, Chaudhary *et al*., 2009, Jyothi *et al.,* 2012, Jina and Ali, 2014*)* have performed with a variety of bacteria, fungi and yeasts, transforms potentially toxic compounds into non-toxic compounds to obtain energy and nutrients. Biodegradation is a biologically catalysed reduction process of complex chemicals.

Fungi have advantages over bacteria because of their fungal hyphae and potential hydrolytic enzymes, which can penetrate and degrade the hydrocarbons contaminated soil *(*Balaji and Ebenezer, 2008*;* Messias *et al.,* 2009*;*Hidayat *et al.,* 2012, Venkatesagowda *et al.,* 2012*).*

In the present course of investigation we have isolated and identified fungal species from soil (contaminated with petroleum & petroleum product) samples and performed the assessment of potentiality of fungal isolates for myco-degradation of the petroleum product / diesel / Mobil oil.

II. MATERIALS AND METHODS

Sampling

Contaminated soil samples were collected from different automobile garages and petrol pumps, determining the sampling sites at different location of city where spilled of diesel, kerosene, petrol, grease and motor oil had occurred over a period of 10 years. The samples were stored in refrigerator maintaining below 10^{o}C temperature for isolation & identification of fungi and further investigation.

Isolation and identification of soil fungal species

Potato dextrose agar media (PDA) and Sabouraud's agar media (Sandven and Lassen, 1999: Guinea *et al*., 2005) were used for fungal analysis of soil samples. Pour plate method and Pure culture by point inoculation method were employed for isolation of fungus by applying the soil samples on agar plates. Single colony from the primary plates was sub-cultured on the fresh Sabouraud's dextrose agar each supplemented with 300 mg l - 4 Rose-Bengal. The sub-cultured was carried out to purify the fungal isolates, for which this was further incubated at room temperature for 48 hours. Identification of fungal isolates was performed through microscopic observation of temporary stained slides.

Selection of test fungi

All isolated fungal cultures were taken in culture plates. Petrol / Diesel / Mobil oil has been placed over Potato Dextrose Agar plates (PDA) spreading with glass rod on the surface of media. Screening was done by using cork borer (4mm in diameter) by well diffusion method. The wells were saturated with fungal strains and incubated for 6 days at 26 ± 2°C. At interval of 4 & 6 days the culture plates with and without supplementation of Petrol / Diesel / Mobil oil were examined for growth.

Assessment of fungal growth in petroleum product

Qualitative determination of fungi, growing on petroleum product incorporated PDB (Potato Dextrose Broth) media, was determined by culturing of fungi. 0.1ml, 0.5ml, 1ml, 1.5ml, 2ml petrol, diesel and mobile oil was poured in 100ml individual conical flask containing PDB media and inoculated with fungi with one control each which were subjected to a period of 10 days at 26 ± 2°C and 37°C respectively, the control and radically growing colonies were examined for their growth as degrading ability

III. RESULTS AND DISCUSSION

Conferring the fungal occurrence in contaminated soil of this region, numerous samples were examined and isolated fungal strains have been identified. During present study total 8 species of 6 genera has been identified of fungal isolates on the basis of microscopic observation i.e. *Aspergillus niger, Aspergillus fumigatus, Curvularia lunata, Fusarium solani, Fusarium oxysporum, Mucor sp., Penicillium notatum & Rhizopus nigricans* as their features mentioned in Table 1 and shown Plate 1: Fig.(i – viii). The occurrence of *Aspergillus niger, Curvularia lunata, Fusarium solani* and *Rhizopus nigricans* were found more frequent.

Fungal isolates have been screened to confer their ability to degrade petroleum product (Petrol, Diesel and Mobil oil) and observed mycelia growth has been mentioned in Table 2 that pictorially presented in Fig. 1. Mycelia growth of all isolates was measured after 48 and 72 hours of inoculation in both supplemented and non-supplemented culture plates. All fungal isolates exhibited degrading ability whereas the highest potential was observed in Curvularia lunata and the ability of other fungal isolates were found as *Curvularia lunata > Fusarium solani > Aspergillus niger > Aspergillus fumigates > Penicillium notatum >Fusarium oxysporum > Rhizopus nigricans > Mucor sp.* Having better degrading potential three fungal isolate - *Curvularia lunata, Fusarium solani* and *Aspergillus niger* were selected for further growth assessment.

By taking Optical Density, the growth pattern of *Curvularia lunata, Fusarium solani* and *Aspergillus niger* in different concentration of Petrol, Diesel and Mobil oil was examined. OD was measured at 3 days interval up to 15 days to analyse the myco-degrading potential of selected fungi - *Curvularia lunata, Fusarium solani* and *Aspergillus niger* and observed data have been incorporated in Table – 3, 4 and 5 respectively and their mode of degrading potentiality has been shown in Fig. 2, 3 and 4.

The increase in biomass, and increase in OD, between 3 to 15 days in all the concentrations of Petrol, Diesel and Mobil oil, strongly suggests that *Curvularia lunata, Fusarium solani and Aspergillus niger* has got a significant potential to degrade petrol / petroleum product. Diesel was found as a most suitable carbon and energy source for the test fungi, however Mobil oil has more or less similar test and better than petrol. The fungus was able to multiply within the days of study, indicating that it was able to degrade and utilize the oil for its growth and development, hence the concomitant increase in the concentration of the broth (turbidity). The gradual increase in the absorption of the broth indicates mycelia growth, hence degradation of hydrocarbons, decline in the decrease in OD suggests a reduction in the fungal population and that the hydrocarbon has been degraded, mostly between 3 and 15 days.

The gradual increase in the absorption of the broth indicates mycelia growth, hence degradation of hydrocarbons, decline in OD suggests a reduction in the fungal population and that the hydrocarbon has been degraded, as similar findings have been reported by several workers. Sakineh *et al.* (2013) found that *Penicillium sp* degrade the petrol and petroleum product in 2% of concentration. Ihsan *et al.* (2014) found the growth ability of *Fusarium solani* degrading the petrol and petroleum product carried out in 2% concentration. Judith *et al.* (2002) had also found *Penicillium* sps. and *Aspergillus fumigatus* to degrade petrol and petroleum product (Mobil oil) in 2% concentration.

IV. CONCLUSION

Soil is the natural material which consists of both organic and inorganic compounds that support in the survival of microbe, flora and fauna on earth. But the man-made pollution creates an alteration of the nutrients present in the soil and use of microbial colonies has been affected. Alliteration makes the soil to release of more CO_2 from them it leads to the cause of environmental pollution such as climate change and global warming etc. The biological method of degradation is useful to degrade the toxic pollutant to nontoxic chemical. In present investigation all the strains isolated from the soil were capable of consuming Petrol and Petroleum product as a sole carbon source. To prevent development of hazardous waste the process of bioremediation has been followed. Our present study

follows the isolation of hydrocarbon degrading bacteria from the contaminated soil with petrol, diesel and Mobil oil. The finding reveals that soil microbes have great potential for their oil degrading capacity.

The biological method of degradation is useful to degrade the toxic pollutant to non-toxic chemical. Hence this strain can be used in cleaning oil polluted sites to protect the soil and environment.

Table 1: Characteristics features of fungal isolates from soil samples of different study area and sampling sites.

ISOLATES	Media (Preferred)	Colony Features	Microscopic Features
Aspergillus fumigatus	Potato Dextrose Agar (PDA)	Typical blue - green surface pigmentation	Typical columnar, uniserrate conidial heads
Aspergillus niger	Potato Dextrose Agar (PDA)	Colonies white to dark to pale yellow in early stage while became black at maturity	Jet black conidia, reverse usually grey, spherical conidial head, rough with maturity.
Curvularia lunata	Potato Dextrose Agar (PDA)	Dark – brown colonies, velvety in appearance,	Mycelium branched, septate, conidia are inequilaterally.
Fusarium solani	Sabouraud's Media (SB)	Light yellow, moist appearance, red with cottony and orange brown mycelium	Hyphae septet, small conidia in chain, sickle shaped long conidia were also observed.
Fusarium oxysporum	Sabouraud's Media (SB)	Red with cottony and orange brown mycelium, white to pink colony	Hyphae septet, small conidia in chain, sickle shaped long conidia were also observed.
Mucor sp.	Potato Dextrose Agar (PDA)	Filamentous colonies, fluffy appearance, white in colour	Globular sporangia round black, elevated column like columella. Non-/partially septate hyphae.
Penicillium notatum	Potato Dextrose Agar (PDA	Velvety or woolly colonies initially white and become green	Conidiophores green in colour, repeatedly branched, brushy head.
Rhizopus nigricans	Potato Dextrose Agar (PDA	Filamentous colonies, fluffy appearance, white in colour	Globular sporangia round black, wi elevated column like columella. Non- /partially septate hyphae.

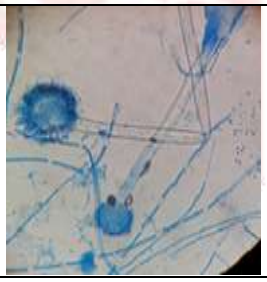

Fig. i *Aspergillus fumigatus*

Fig. ii *Aspergillus niger*

Fig. iii *Curvularia lunata*

Fig. iv *Fusarium solani*

Fig. v *Fusarium oxysporum*

Fig. vi *Mucor sp.*

Fig. vii *Penicillium notatum*

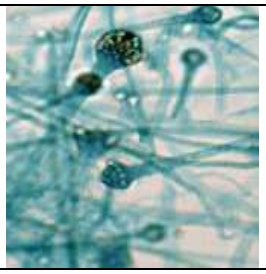

Fig. viii *Rhizopus nigricans*

Plate 1: Fig. i –vii, sowing fungal isolates as observed under microscope

Table 2: Mycelial growth (mm.) after 48 & 72 hour incubation in PDA supplemented with / without petrol, Diesel & mobile oil.

Fungal isolates	Without supplemented (mm.)		Supplemented with Petrol (mm.)		Supplemented with Diesel (mm.)		Supplemented with Mobil oil (mm.)	
	48 hrs.	72hrs.	48 hrs.	72hrs.	48 hrs.	72hrs.	48 hrs.	72hrs.
Aspergillus fumigatus	64.5±3.0	79.7±1.9	32.0 ±1.5	42.5 ±2.2	34.0 ±2.5	46.5 ±3.2	31.7 ±2.0	44.3 ±2.8
Aspergillus niger	68.4±2.2	78.3 ±3.2	51.7 ±2.8	58.6 ±3.4	54.7 ±2.8	63.6 ±3.6	53.7 ±3.1	64.0 ±4.1
Curvularia lunata	78.1±1.7	91.5±2.3	53.5 ±2.5	61.7 ±3.2	57.5 ±2.5	65.7 ±2.2	54.6 ±2.9	66.7 ±4.0
Fusarium solani	70.8±1.6	85.3 ±4.0	51.9 ±1.5	66.5 ±2.4	53.9 ±1.5	69.5 ±2.4	62.4 ±1.9	70.5 ±2.9
Fusarium oxysporum	64.5±3.0	76±1.9	38.0 ±2.5	49.5 ±2.6	44.0 ±3.5	56.5 ±2.2	41.7 ±2.0	54.3 ±2.4
Mucor sp.	55.3 ±2.9	67.0±2.5	34.7 ±1.9	46.6 ±2.1	35.9 ±3.4	42.5 ±3.2	31.9 ±1.9	45.5 ±2.2
Penicillium notatum	65.5±3.2	74±1.6	38.0 ±3.5	47.5 ±2.4	46.0 ±2.5	55.5 ±2.8	41.5 ±2.8	55.6 ±3.4
Rhizopus nigricans	57.3 ±2.5	72.0±3.5	37.7 ±1.9	48.6 ±2.4	37.9 ±2.5	47.5 ±2.4	34.9 ±3.1	48.5 ±2.6

Table 3: Effect of different concentration of Petrol, Diesel and Mobile oil on growth of Fungal isolates– *Aspergillus niger*

Petroleum product	Conc.	Growth of *Aspergillus niger* (Optical Density at 610 nm)					
		Initial OD	3 days	6 days	9 days	12 days	15 days
Petrol	0.5%	0.2 ±0.10	0.41±0.16	0.70 ± 0.14	1.02 ±0.13	1.18± 0.18	1.15 ±0.18
	1%	0.2 ± 0.12	0.38± 0.18	0.62± 0.18	0.92± 0.18	1.08± 0.22	1.10 ±0.26
	1.5%	0.2 ± 0.14	0.32± 0.28	0.55 ± 0.16	0.81 ± 0.15	1.00 ±0.14	1.02± 0.21
	2%	0.2 ± 0.16	0.26 ±0.22	0.46 ±0.24	0.70 ± 0.18	0.92 ±0.18	0.98 ±0.26
Diesel	0.5%	0.2 ±0.10	0.46±0.16	0.72±0.28	0.94±0.22	1.08±0.24	1.16 ±0.22
	1%	0.2±0.12	0.41±0.16	0.61± 0.12	0.81± 0.18	0.96±0.15	1.05± 0.18
	1.5%	0.2±0.14	0.35± 0.18	0.42± 0.22	0.67± 0.26	0.85± 0.18	0.91± 0.23
	2%	0.2± 0.14	0.31± 0.24	0.34± 0.16	0.52± 0.18	0.63±0.24	0.72± 0.24
Mobile Oil	0.5%	0.2 ±0.12	0.39±0.16	0.69±0.24	0.89±0.22	1.02±0.22	1.05 ±0.30
	1%	0.2±0.14	0.36±0.13	0.54± 0.22	0.73± 0.18	0.91±0.22	1.00± 0.12
	1.5%	0.2±0.16	0.32± 0.22	0.40± 0.12	0.61± 0.16	0.79± 0.25	0.88± 0.19
	2%	0.2± 0.16	0.28± 0.16	0.31± 0.16	0.48± 0.14	0.62±0.26	0.70± 0.24
Control (Without supplemented)		0.2 ±0.14	0.42±0.25	0.78±0.31	1.15±0.26	1.32±0.35	1.25± 0.31

Table4: Effect of different concentration of Petrol, Diesel and Mobile oil on growth of Fungal isolates–*Curvularia lunata*

Petroleum product	Conc.	Growth of *Curvularia lunata* (Optical Density at 610 nm)					
		Initial OD	3 days	6 days	9 days	12 days	15 days
Petrol	0.5%	0.2 ±0.10	0.39±0.16	0.71 ± 0.19	1.05 ±0.13	1.2± 0.12	1.19 ±0.21
	1%	0.2 ± 0.12	0.35± 0.11	0.65± 0.10	0.98± 0.18	1.15± 0.23	1.13 ±0.26
	1.5%	0.2 ± 0.11	0.3± 0.08	0.56 ± 0.12	0.85 ± 0.13	1.05 ±0.17	1.02± 0.21
	2%	0.2 ± 0.16	0.28 ±0.23	0.44 ±0.27	0.71 ± 0.12	0.97 ±0.30	0.98 ±0.28
Diesel	0.5%	0.2 ±0.10	0.41±0.15	0.75±0.18	0.98±0.22	1.18±0.32	1.16 ±0.30
	1%	0.2±0.12	0.39±0.16	0.63± 0.22	0.84± 0.18	0.98±0.15	1.05± 0.12
	1.5%	0.2±0.14	0.33± 0.18	0.44± 0.24	0.71± 0.16	0.89± 0.16	0.92± 0.19
	2%	0.2± 0.15	0.28± 0.28	0.37± 0.18	0.58± 0.18	0.65±0.18	0.75± 0.24
Mobile Oil	0.5%	0.2 ±0.10	0.39±0.19	0.72±0.21	0.95±0.25	1.12±0.24	1.11 ±0.30
	1%	0.2±0.11	0.37±0.13	0.58± 0.17	0.79± 0.18	0.98±0.25	1.05± 0.12
	1.5%	0.2±0.14	0.3± 0.12	0.42± 0.17	0.65± 0.07	0.81± 0.15	0.90± 0.19
	2%	0.2± 0.16	0.26± 0.18	0.34± 0.10	0.52± 0.13	0.65±0.21	0.72± 0.24
Control (Without supplemented)		0.2 ±0.14	0.41±0.25	0.79±0.31	1.1±0.26	1.22±0.35	1.22± 0.31

Table 5: Effect of different concentration of Petrol and Mobile oil on growth of Fungal isolates– *Fusarium solani*

Petroleum product	Conc.	Growth of *Fusarium solani* (Optical Density at 610 nm)					
		Initial OD	3 days	6 days	9 days	12 days	15 days
Petrol	0.5%	0.2 ±0.10	0.54± 0.16	0.81± 0.19	1.01± 0.13	1.09± 0.12	0.99± 0.21
	1%	0.2 ± 0.12	0.47± 0.11	0.71± 0.10	0.94± 0.18	0.98± 0.23	0.91± 0.26
	1.5%	0.2 ± 0.11	0.41± 0.08	0.64± 0.12	0.82± 0.13	0.91± 0.17	0.86± 0.21
	2%	0.2 ± 0.16	0.35 ±0.23	0.55± 0.27	0.72± 0.12	0.81± 0.30	0.75± 0.28
Diesel	0.5%	0.2 ±0.10	0.57± 0.26	0.85± 0.16	1.01± 0.13	1.15± 0.12	1.04± 0.21
	1%	0.2 ± 0.12	0.49± 0.15	0.76± 0.18	0.98± 0.18	0.99± 0.23	0.96± 0.26
	1.5%	0.2 ± 0.12	0.44± 0.18	0.68± 0.15	0.87± 0.13	0.94± 0.17	0.88± 0.21
	2%	0.2 ± 0.14	0.37 ±0.23	0.56± 0.17	0.75± 0.12	0.85± 0.30	0.76± 0.28
Mobile Oil	0.5%	0.2 ± 0.10	0.38± 0.19	0.6± 0.21	0.78± 0.25	0.88± 0.38	0.87± 0.30
	1%	0.2 ±0.11	0.34±0.13	0.55 ±0.17	0.73 ±0.18	0.79 ±0.25	0.77 ±0.12
	1.5%	0.2±0.13	0.31± 0.12	0.52± 0.17	0.68 ±0.22	0.74 ±0.25	0.71± 0.33
	2%	0.2± 0.12	0.27 ±0.10	0.45 ±0.15	0.62 ±0.21	0.7 ± 0.25	0.69 ±0.31
Control (Without Supplemented)		0.2 ± 0.10	0.58± 0.21	0.9 ± 0.34	1.1± 0.29	1.19± 0.37	1.09 ±0.32

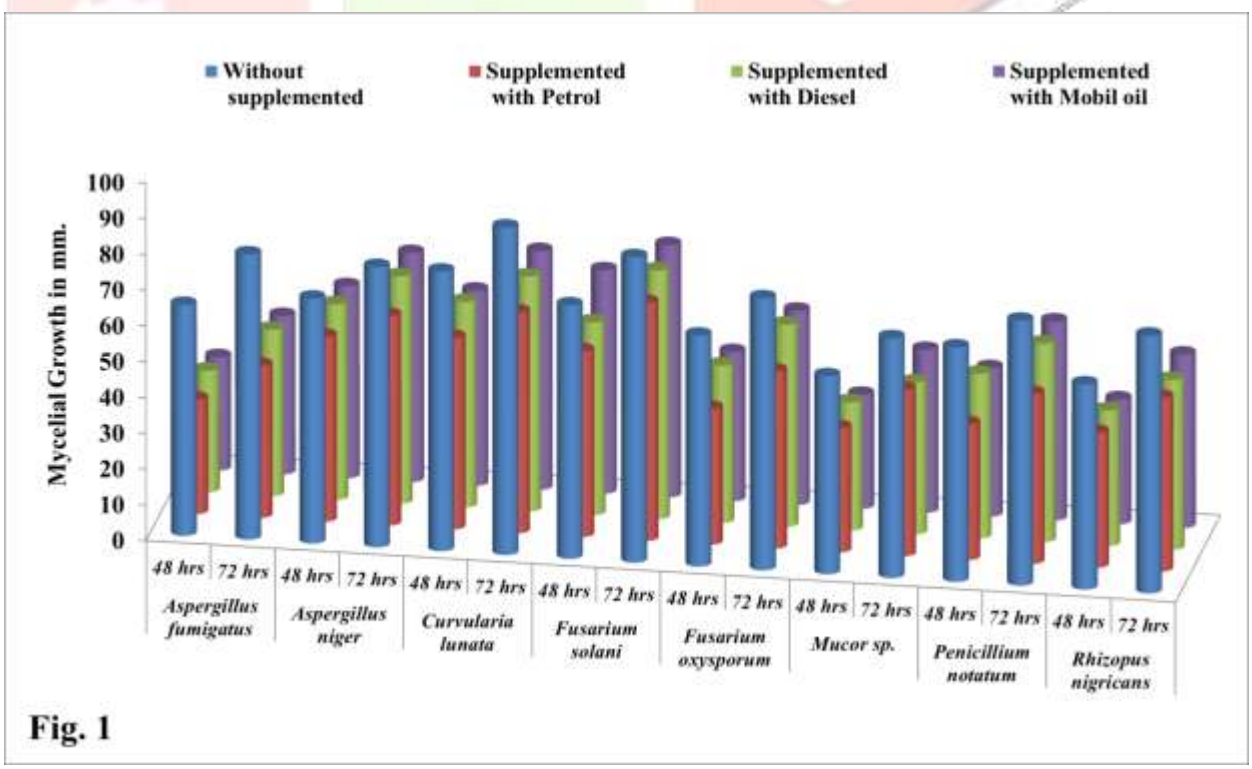

Fig. 1

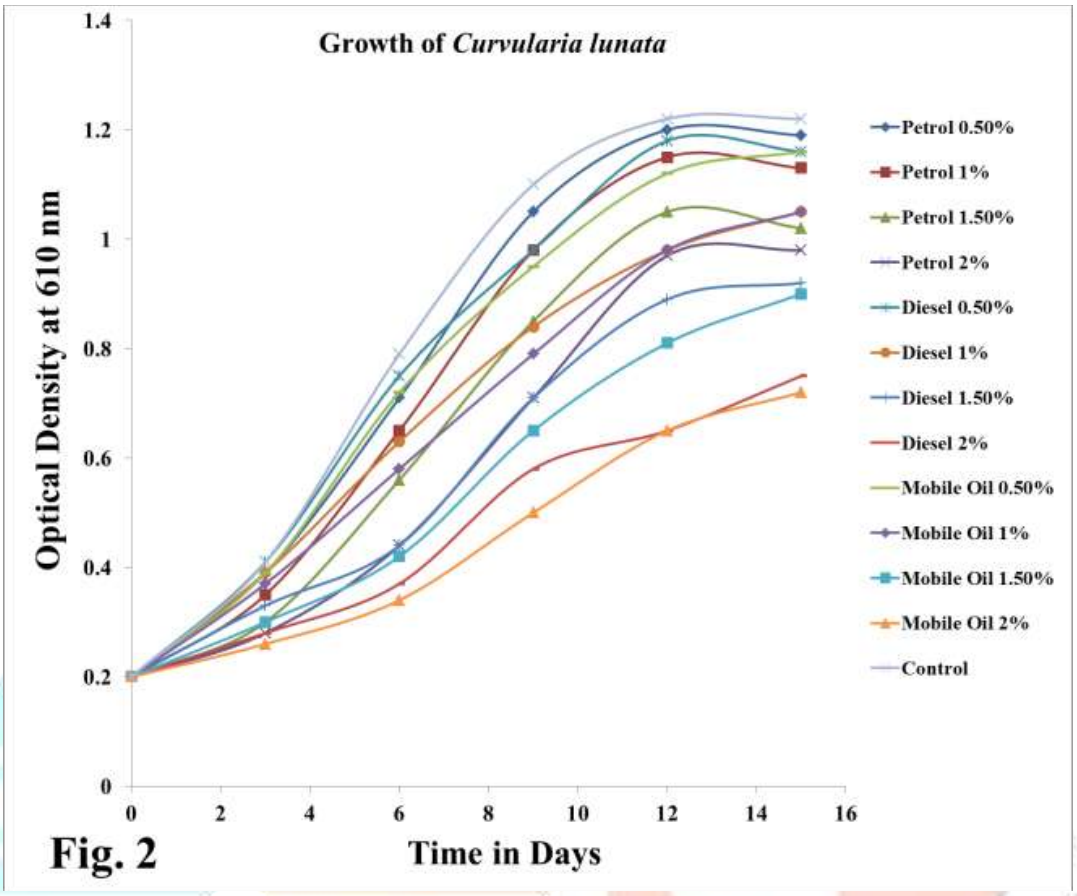
Growth of *Curvularia lunata*
Optical Density at 610 nm
Time in Days
Petrol 0.50%
Petrol 1%
Petrol 1.50%
Petrol 2%
Diesel 0.50%
Diesel 1%
Diesel 1.50%
Diesel 2%
Mobile Oil 0.50%
Mobile Oil 1%
Mobile Oil 1.50%
Mobile Oil 2%
Control

Fig. 2

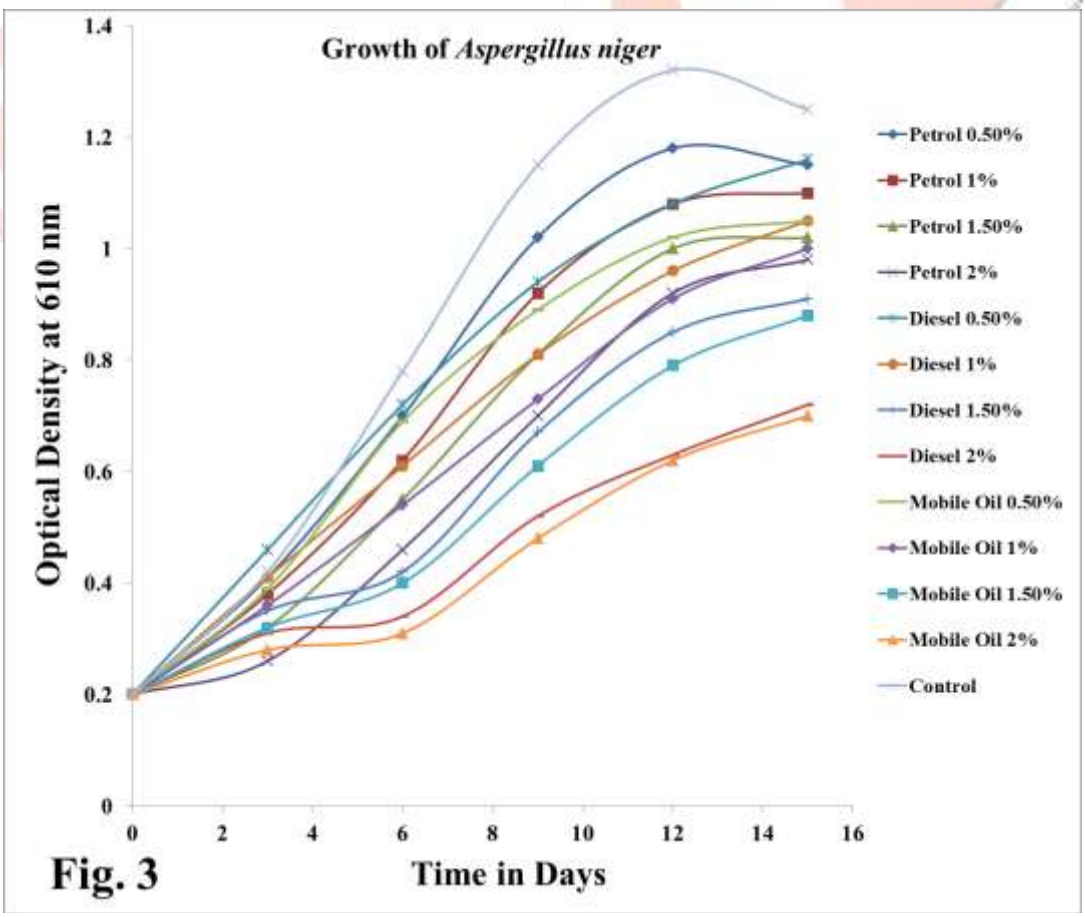
Growth of *Aspergillus niger*
Optical Density at 610 nm
Time in Days
Petrol 0.50%
Petrol 1%
Petrol 1.50%
Petrol 2%
Diesel 0.50%
Diesel 1%
Diesel 1.50%
Diesel 2%
Mobile Oil 0.50%
Mobile Oil 1%
Mobile Oil 1.50%
Mobile Oil 2%
Control

Fig. 3

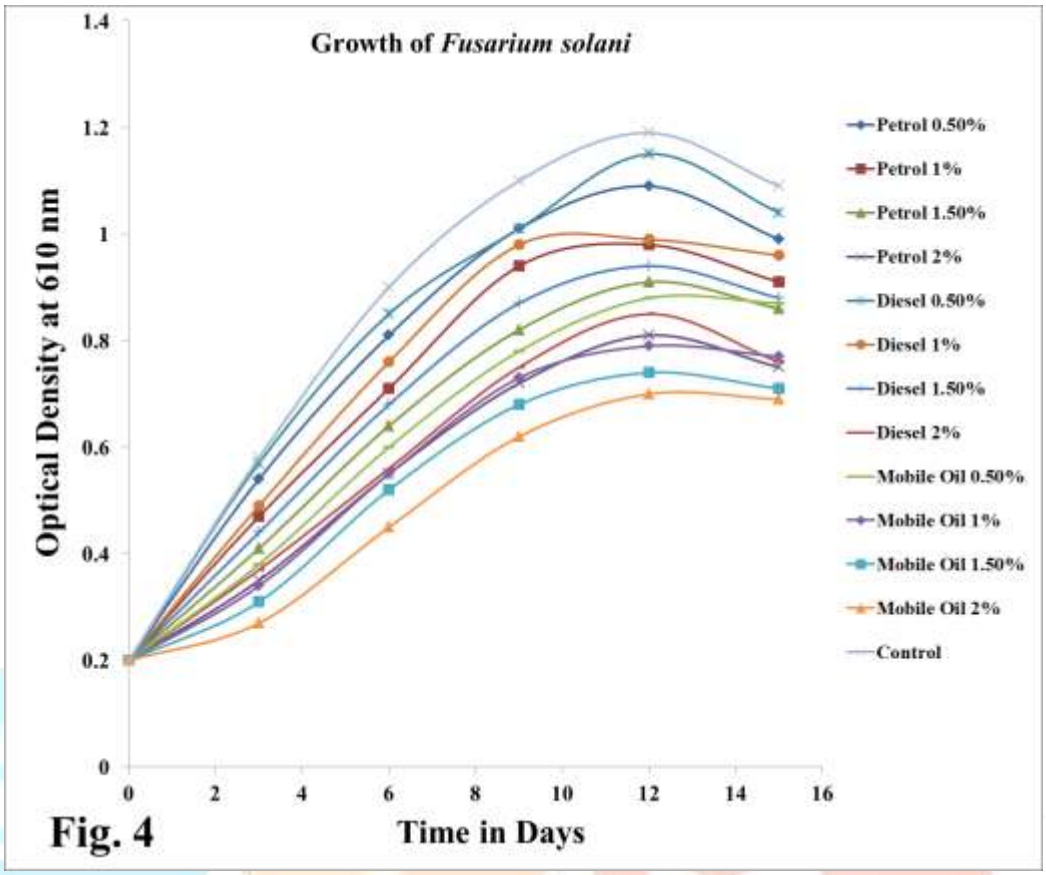

Fig. 4

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