Original Research Article

Screening of Diesel Oil Degrading Species of *Aspergillus* for the Production of Different Enzymes Using Agar Plate Assay Method

Abstract

We have earlier isolated *Aspergillus luchuensis,A.violaceofuscus,A.niveus,A.terreus,A.japonicus* and*A.ustus*from naturally degraded diesel oil in the soil using nutrient rich technique. These species were cultured to produce enzyme on 10% (w/v) diesel oil broth containing 0.5 and 1.0% (w/v) jaggery at 37 and 45℃ temperature separately for 7, 14 and 21 days. It was observed the all 6 species produced luxuriant mycelial growth at day 7 on both temperatures, however, at 45℃, the liquid broth dried on 14th day of incubation and the secretory extracellular enzymes produced at 37℃ were screened for the production of amylases, gelatinases, lipases, cellulases, pectinases, proteases and manganese peroxidases using agar plate assay method. It was found that all 6 species produced all 7 enzymes at higher concentration at day 21 on diesel oil containing 1.0% jaggery. The production of enzyme increased with increasing period of incubation from day 7 to 21. *A. luchuensis* produced highest activities of proteases, *A. violacefuscus* - amylases, *A. niveus* - gelatinases, *A. terreus*and *A. ustus* - pectinases, and *A. japonicus* produced highest activities of manganese peroxidases. All these enzymes contribute in the degradation of diesel oil in nature. This study revealed that the production of varying amounts of different enzymes by all these 6 species of *Aspergillus* has significant ecological and bioremediation potential.

Keywords: Diesel oil degrading fungi, *Aspergillus*, Agar plate Assay, Hydrocabons, Enzymes

Introduction

Petroleum hydrocarbon contamination from oil and gas industry, oil spills, tank leaks, lubrication, petroleum exploration, transportation, and services, are the major issues affecting soil environmental pollution today[1]. Further, anthropogenic activities result in the annual release of 5 million tons of petroleum oil into the environment that cause oil pollution [2].In terms of environmental contamination, the extraction, processing, and transportation of petroleum oil and its products are of special concern around the globe[3]. Crude oil is commonly referred as fossil fuel and is categorized as non-renewable energy. It is made up of a viscous liquid that contains both simple and complex hydrocarbons. For the petrochemical sector, it serves as a source of several fuels and chemical feed stock [4].The diesel is a complex mixture and a typical pollutant, made up primarily of aliphatic hydrocarbons with a chemicals

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mainly composed of C9 to C23 and a few aromatic chemicals[5]. Diesel fuel physically prevents water and oxygen from moving from soil environment to seed, which delays seed emergence and lowers plant growth and seed germination rates. Used engine oil contains significant amount of toxins that affect soil enzymatic activities and survival of soil microbiome including eco-friendly earthworms and biofertilizers [6].Remediation of diesel contaminated oil environments has attracted the attention of scientific community[7, 8]. Hydrocarbon compounds make bondswith several soil components and are difficult to remove or degrade[9].Most soil enzymes are known to be produced from fungi, bacteria, actinomycetesand animal remains[10]. Oxidoreductase and hydrolases are the two major groups of enzymes that are most frequently utilized to assess the impacts of TTE and PHC pollutants[11]. While oxygen picks up electrons and is reduced, the hydrocarbon loses electrons and is oxidized. Water and carbon dioxide are the end products. Through the actions of living organisms, most organic compounds and many inorganic ones are vulnerable to enzymatic attack[12].Based on fungal enzymes and their impact on the hydrocarbon bio-degradation system, the soil fungi providea powerful and unique biodegrading mechanism, which is sustainably beneficial[13], however,there is little knowledge of the enzymatic mechanisms and the accompanying genetic routes of hydrocarbon breakdown by/in fungi, despite the vast examination of the many pathways. By secreting extracellular enzymes that convert the refractory hydrocarbons into intermediates with lesser toxicity, fungi aid in the breakdown of recalcitrant hydrocarbons. In order to break down the various hydrocarbons, they have complimentary substrates[14].Furthermore, the fungi can survive in harsh environments and are tolerant of high concentrations of refractory substances. The apical dominance of the fungi provides them mechanical power to penetrate the hard substrates[15]. A mixed population of fungal strains has reportedly been used in several recent researches to increase the bio-degradation effectiveness, particularly in situations when the oil concentration is high or the substances are bound with complex molecules [16, 17].The presence of extensive network of mycelia and their abilities to secrete high activities of extracellular enzymes, many filamentous fungi have been described as bio-remediation agents. The majority of rot fungi produce high redox potential enzymes for the oxidation of lignin, including laccases, lignin peroxidases, and manganese peroxidases (MnP). Dehydrogenase, ß-glucosidases, proteases, phosphatases, and arylsulfatasesare some of the key enzymes that have been suggested as a way to track the activity of microbial communities[18].Since lignin is a cross-linked phenolic polymer, these enzymes are typically utilized to break down lignin in nature. However, because of their low specificity, they can also break down other phenolic compounds, like those found in hydrocarbons[5]. In previous paper, we have reported six species of *Aspergillus* that were selectively present in diesel oil contaminated soils [19] and

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in this paper we are describing the extracellular enzymes that are produced by these 6 species of *Aspergillus*with an aim to bio-remediate diesel-contaminated soils.

Materials and Methods

6 species of*Aspergillus* were isolated from diesel contaminated soils as using nutrient rich technique on 2% agar medium supplemented with 10% diesel oil as described earlier[19] . These were identified as (A) *Aspergillus luchuensis*(B) *A.violaceofuscus*(C) *A.niveus*(D) *A.terreus*(E) *A.japonicus* and (F) *A.ustus*using standard reference books [20,21]. These were maintained at 4℃ in refrigerator on slants and at -20℃ in 2% (w/v) glycerol in the form of mycelium. The soil samples were collected around Shobhit Institute of Engineering & Technology, Deemed to-be-University, Meerut and from the petrol and diesel pumps situated on NH-58 in Meerut.

Production of Enzymes: For the production of different enzymes, 60 mL of broth with 0.5% and 1% (w/v) jaggery + 10% diesel oil was taken in 250 mL Erlenmeyer flasks and autoclaved at 15 Pa for 15min. When cooled, 1 mL aliquot of 7-day old fresh culture was inoculated aseptically separately for each of the 6 test species of *Aspergillus*[(A) *Aspergillus luchuensis*(B) *A.violaceofuscus*(C) *A.niveus*(D) *A.terreus*(E) *A. japonicus*and (F) *A.ustus*] in 20 flasks of which 10 were incubated at 37℃ and 10 at 45℃ temperature separately.For the production of crude enzyme preparation, three flasks for each of the test species were harvested after 7, 14 and 21 days of incubation by centrifugation at 15000xg at 4℃ for 1 h which was used as crude enzyme. Cell free enzyme was produced by filtering the crude enzyme through 0.22µM membrane filter. All 6 species of *Aspergillus* produced luxuriant growth at 37℃ and 45℃ temperature on 7-day of incubation, however, at 45℃ the growth was reduced after 7th day of incubation may be because of evaporation of water from the medium during long incubation. Hence, for the estimation of different enzymes activities, the flasks harvested at 37℃ temperature were used.

Agar Plate Assay method for Screening of Enzymatic Activity: Enzymatic activity of the extracellular secretory enzymes produced from the 6 isolated test species of *Aspergillus* was assessed using agar plate assays method[22]. The medium was prepared using 2% (w/v) agar + suitable substrate for the test enzyme, autoclaved at 15 Pa for 15 min and poured in sterile 10 cm diameter Petri dishes aseptically, wells were cut asepticallyusing sterile Cork borer (6 mm diam.) and 100µL of crude enzyme preparation from each of the enzyme prepared from each of the 6 isolated test species was added carefully and aseptically. It was found that though, the cell free extract produced radial enzyme activities but the crude enzyme extract showed higher activities and produced very little mycelial growth during agar plate assay.

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The crude enzyme preparation sowed minute cell fragments and exhibited very high enzyme activity. The plates were incubated at 37±1°C for 48-72 h for cell free enzyme preparations, while the plates were incubated only for 24 h for crude enzyme preparation. The zone of activity in the form of halo area around the well, was noticed and measured. The halo area was measured on both x and y axis and the radial enzyme activity was calculated as follows:

$$\text{Radial Enzyme Activity} = \frac{(\text{Diam on x + y axis}) - (\text{diam of well x 2})}{4}$$

where diameter of well cut by cork borer was 6 mm

Different substrates were used for estimation of different enzyme activities. 2% (w/v) starch was used as substrate for amylases activity; 1% tributyrin for lipases; 10% skim milk for proteases; 2% gelatin for gelatinases; 1% birchwood xylan for xylanases were used as specific substrates for estimation of specific enzyme activities. For cellulases activity the agar plates containing 2% carboxymetholecellulose (CMC) were used while for pectinases 5% apple pulp (after peeling) was used as substrate. For manganese peroxidasesactivity, the grounded wood chips were used as substrate. Staining was made with desired stain when the halo area of zone of enzyme activity was not clearly visible.

Results and Discussion:-

The enzyme by was produced after incubation of test *Aspergillus* species at 37 and 45°C temperatures separately for 7, 14 and 21 days. It was observed that all 6 species produced luxuriant growth on incubation for 7 days at both 37 and 45°C temperatures but at later temperature the flask dried after 7th day of incubation, mainly because of the evaporation of water content from the medium. Hence, the enzyme preparations produced at 37A°C temperatures were used during the present investigation. It was further observed that the growth of all 6 test species was higher at 10% diesel oil broth supplemented with 1% (w/v) jaggery in comparison to 0.5% jaggery (Fig 1, 2). For screening of enzyme activities, agar plate assay method was used as it is fast for qualitative evaluation of extracellular enzyme activities of the microbes. The specific substrate acts as raw material for the specific enzyme to act upon resulting in the formation of halo areas around the enzyme inoculated. The halo areas may be measured and the enzyme activities may be expressed directly proportional to the radius of the halo area produced by the enzyme secreted by the microbe. Whole mycelial discs have also been used by different enzymologists to evaluate the enzyme activities of the test organism, however, during this present investigation we used cell free enzyme (after filtering the crude enzyme through 0.22μM membrane filter) and the crude enzyme both for testing the enzyme activities. The cell free enzymes produced high activities on incubation for 48-72 h

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(Tables 1 - 6) for all 6 species and for all 7 test enzymes, however, the crude enzyme exhibited greater enzyme activities but also produced little mycelial growth (Fig. 3, 4).*Aspergillus luchuensis* exhibited highest activities of all 7 test enzymes at diesel oil broth supplemented with 1% jaggery and incubated for 21 days (Table 1, Fig 4). Proteases were produced in highest amount by *A. luchuensis* followed by amylases, gelatinases, lipases, cellulases, pectinases and manganese peroxidases (Table 1). On comparison of enzyme activities of *A. luchuensis* with that of *A. violacefuscus* (Table 1 and 2), it was found that the later fungus is enzymatically more active than the former and produced highest activities of amylases followed proteases, cellulases, gelatinases, manganese peroxidases, lipases and pectinases (Table 2). *Aspergillus niveus*produced highest activities of gelatinases followed by cellulases, lipases, manganese peroxidases, pectinases, amylases and proteases (Table 3). It also showed the lipases were produced almost double at diesel oil broth containing 1% jaggery while all other enzymes produced 30-50% higher activities at oil broth supplemented with 1% jaggery. *Aspergillus terreus* exhibited highest activities of pectinases followed by proteases, amylases, manganese peroxidases, cellulases, lipases and gelatinases (Table 4). Very interestingly, *Aspergillus japonicus* produced very high activities of manganese peroxidases in oil broth containing 0.5 and 1% jaggery both on 21-day of incubation, a prime enzyme that is mainly responsible for breaking the complex bonds, followed by gelatinases, proteases, amylases, pectinases, cellulases and lipases (Table 5). *Aspergillus ustus* revealed very high activities of pectinases on 21-day of incubation at oil broth supplemented with 1% jaggery followed by amylases, proteases, manganese peroxidases, gelatinases, cellulases and lipases (Table 6). On comparison of all 6 species of diesel oil degrading *Aspergillus*, it was found that *A. luchuensis* produced highest activities of proteases, *A. violacefuscus* - amylases, *A. niveus* - gelatinases, *A. terreus*and *A. ustus* - pectinases, and *A. japonicus* produced highest activities of manganese peroxidases. All 6 species of *Aspergillus*, though, produced all 7 oil degrading enzymes in various activities but yet each species evolved its metabolism in such a way that it produced the high activities of different oil degrading enzymes (Tables 1 to 6). Supplementation of 1% jaggery with 10% diesel oil was found better for production of high activities of all 7 enzymes. The amylolytic potential of *Aspergillus* spp.(eurotiomycetes) may help them to degrade starch like polymers which is produced when the hydrocarbons are degraded. Cellulolytic activity was exhibited by all 6 species of *Aspergillus* and was measured as cellulasesactivities using carboxymethyle cellulose as substrate. Maximumcellulases activity was revealed by *Aspergillus violacefuscus*(Tables 1 to 6)which is hydrolyse the complex polymer of cellulose. Maximum pectinases activity was observed in *Aspergillus terreus*and *A. ustus* and the enzymedegradesthe hydrocarbons bonds of the diesel oil. The proteasesare hydrolytic enzymes and produce hydrochloric acid amino acids and these were also

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produced by all 6 species of *Aspergillus* isolated from naturally degrading diesel oil, however, *A. luchuensis* produced the highest activities. The lipases breakdownthe oil and produce free fatty acids, all 6 species produced varying amounts of lipases.

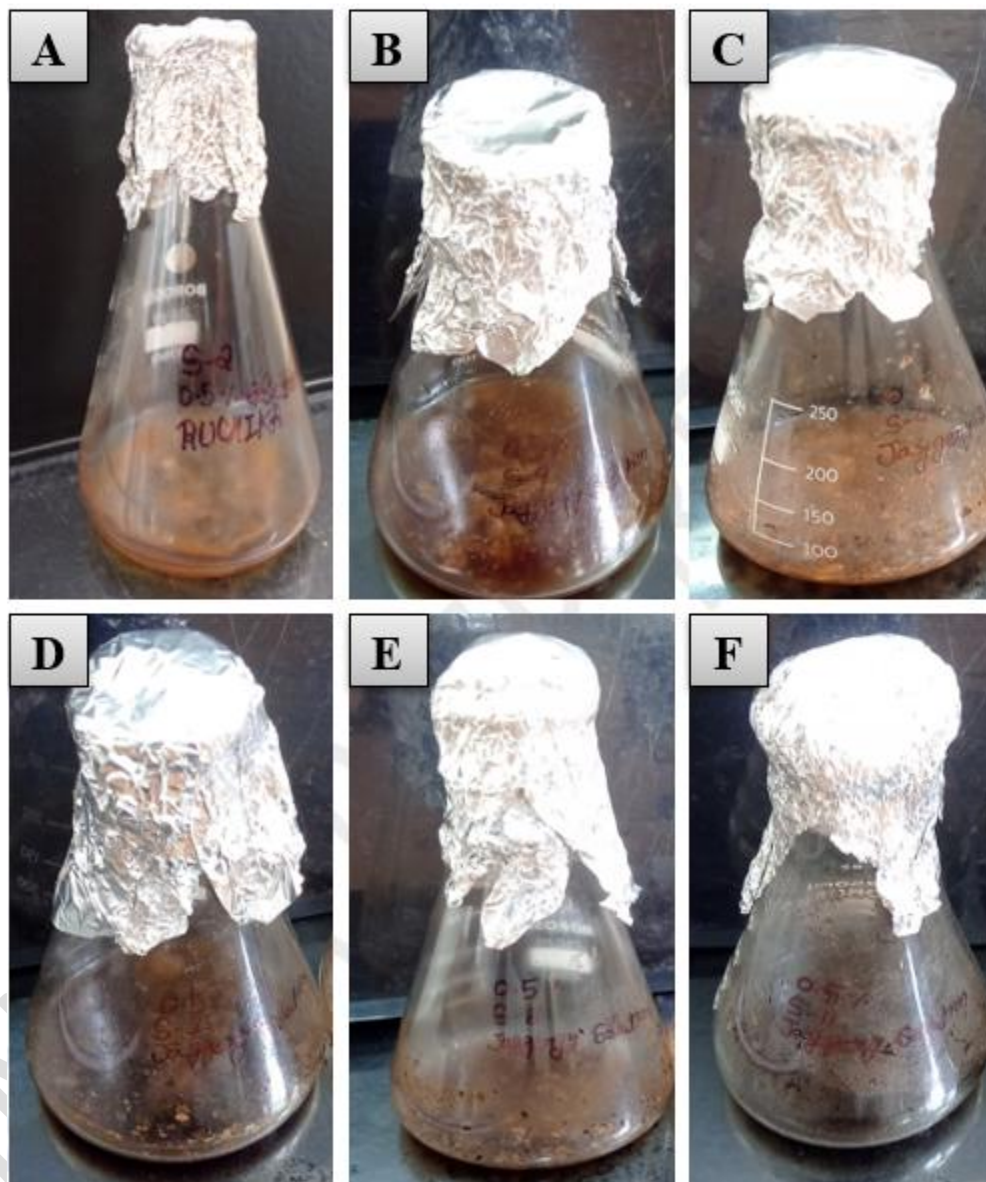

Fig. 1:Production of extracellular enzymes (A) *Aspergillus luchuensis*(B) *Aspergillus violaceofuscus*(C) *Aspergillus niveus*(D) *Aspergillus terreus*(E) *Aspergillus japonicus*(F) *Aspergillus ustus on* 1.0 % Jaggery and 10 % dieseloil after 21-day of incubation.

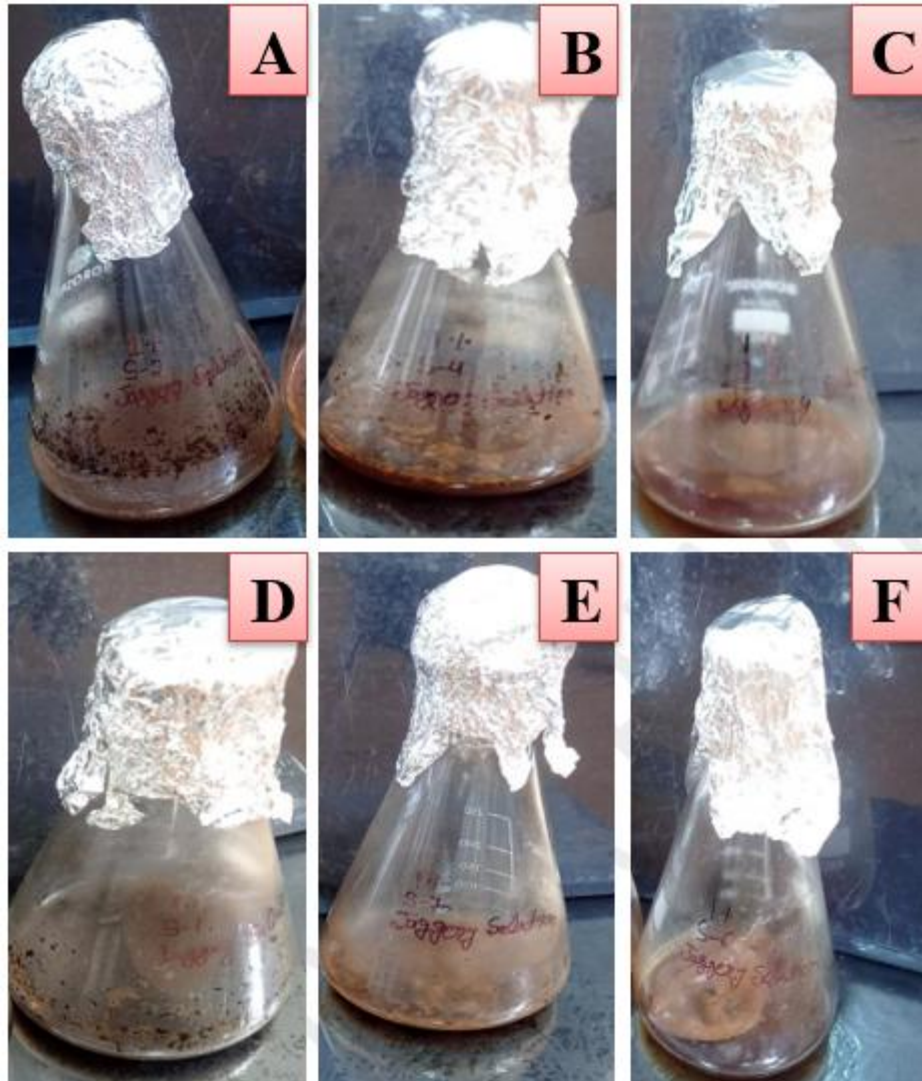

Fig 2: Production of extracellular enzymes (A) *Aspergillus luchuensis*(B) *Aspergillus violaceofuscus*(C) *Aspergillus niveus*(D) *Aspergillus terreus*(E) *Aspergillus japonicus*(F) *Aspergillus ustus on* 1.0 % Jaggery and 10 % dieseloil after 21-day of incubation.

Table 1: Radial enzyme activity (mm) of 7 different extracellular cell free enzymes secreted by *Aspergillusluchuensis*in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (days)	Enzymes activity(mm)													
	Manganese peroxidases		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5%	1.0 %	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%
7	1.19	2.04	2.02	2.78	3.0	3.67	4.19	5.17	7.82	7.91	3.18	3.66	4.89	4.95
14	2.21	3.31	3.09	3.45	3.91	4.45	6.19	7.73	9.12	9.92	4.61	5.01	6.99	7.56
21	3.69	4.99	5.09	6.89	4.01	5.61	8.79	9.97	11.02	13.01	5.68	6.66	8.69	9.79

Table 2: Radial enzyme activity (mm) of 7 different extracellular cell free enzymes secreted by *Aspergillusviolacefuscus*in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (Days)	Enzymes activity(mm)													
	Manganese peroxidases		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5%	1.0 %	0.5 %	1.0%	0.5%	1.0 %	0.5%	1.0%	0.5 %	1.0%	0.5 %	1.0 %	0.5 %	1.0%
7	4.11	4.54	3.82	3.93	1.29	1.51	7.22	7.35	5.05	5.67	2.87	3.05	3.54	3.79
14	5.11	6.73	5.71	6.02	2.79	4.91	9.03	9.25	7.5	7.76	4.07	5.47	6.59	7.70
21	7.81	8.91	8.92	9.99	6.09	7.91	11.21	13.21	9.65	11.15	6.97	7.73	8.51	9.71

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Table 3: Radial enzyme activity (mm) of 7 different extracellular cell free enzymes secreted by *Aspergillusniveus*in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (Days)	Enzymes activity(mm)													
	Manganese peroxidases		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5%	1.0 %	0.5%	1.0 %	0.5 %	1.0 %	0.5 %	1.0 %	0.5 %	1.0 %	0.5 %	1.0 %	0.5 %	1.0%
7	1.99	2.29	2.89	2.34	1.93	2.45	1.92	2.02	1.01	1.87	1.1	1.45	1.99	2.45
14	3.40	3.70	4..01	5.33	2.93	4.93	1.02	3.07	1.22	1.92	2.31	3.02	2.09	3.67
21	5.49	6.79	5.59	7.59	4.53	6.53	4.92	5.97	2.02	3.12	3.91	6.92	5.69	7.69

Table 4: Radial enzyme activity (mm) of 7 different extracellular cell free enzymes secreted by *Aspergillusterreus*in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (Days)	Enzymes activity(mm)													
	Manganeses peroxide		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5 %	1.0 %	0.5%	1.0 %	0.5%	1.0%	0.5%	1.0%	0.5%	1.0%	0.5 %	1.0 %	0.5 %	1.0 %
7	2.91	3.04	3.00	3.32	7.19	7.22	6.19	6.24	5.52	5.61	2.17	2.26	2.79	2.89
14	4.01	7.01	5.80	6.69	9.19	11.90	9.29	8.94	7.72	8.27	4.67	5.57	3.77	5.75
21	8.21	9.51	7.00	8.09	12.09	13.49	10.09	11.59	10.12	11.32	6.97	7.67	6.79	7.97

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Table 5: Radial enzyme activity (mm) of 7 different extracellular cell free enzymes secreted by *Aspergillus japonicus* in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (Days)	Enzymes activity(mm)													
	Manganese peroxidases		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5%	1.0 %	0.5%	1.0 %	0.5%	1.0 %	0.5 %	1.0%	0.5 %	1.0%	0.5 %	1.0 %	0.5 %	1.0%
7	4.81	4.95	4.65	4.70	3.49	3.52	4.91	4.98	4.12	4.23	2.51	2.65	4.78	6.84
14	7.61	7.85	5.95	6.50	5.48	7.11	6.91	6.69	5.92	5.28	3.59	4.03	6.08	8.88
21	9.01	10.41	7.05	8.25	8.41	9.11	8.01	9.21	8.32	10.02	5.51	6.31	9.78	10.08

Table 6: Radial enzyme activity of 7 different extracellular cell free enzymes secreted by *Aspergillusustus*in0.5% - 1.0% Jaggery + 10% dieseloil broth on 7, 14 and 21-day incubation using agar plate assay method.

Incubation (Days)	Enzymes activity(mm)													
	Manganese peroxidases		Cellulases		Pectinases		Amylases		Proteases		Lipases		Gelatinases	
	0.5%	1.0 %	0.5%	1.0 %	0.5%	1.0%	0.5 %	1.0%	0.5 %	1.0 %	0.5 %	1.0 %	0.5 %	1.0 %
7	3.78	3.82	3.92	4.27	6.02	6.11	5.10	5.18	3.65	3.78	2.12	2.23	3.87	4.98
14	5.08	5.64	4.85	6.47	7.02	8.92	7.20	8.18	5.95	6.05	5.10	5.21	4.07	5.09
21	8.78	9.38	7.92	8.91	10.02	11.32	9.10	10.10	8.65	9.75	7.12	8.22	7.87	8.89

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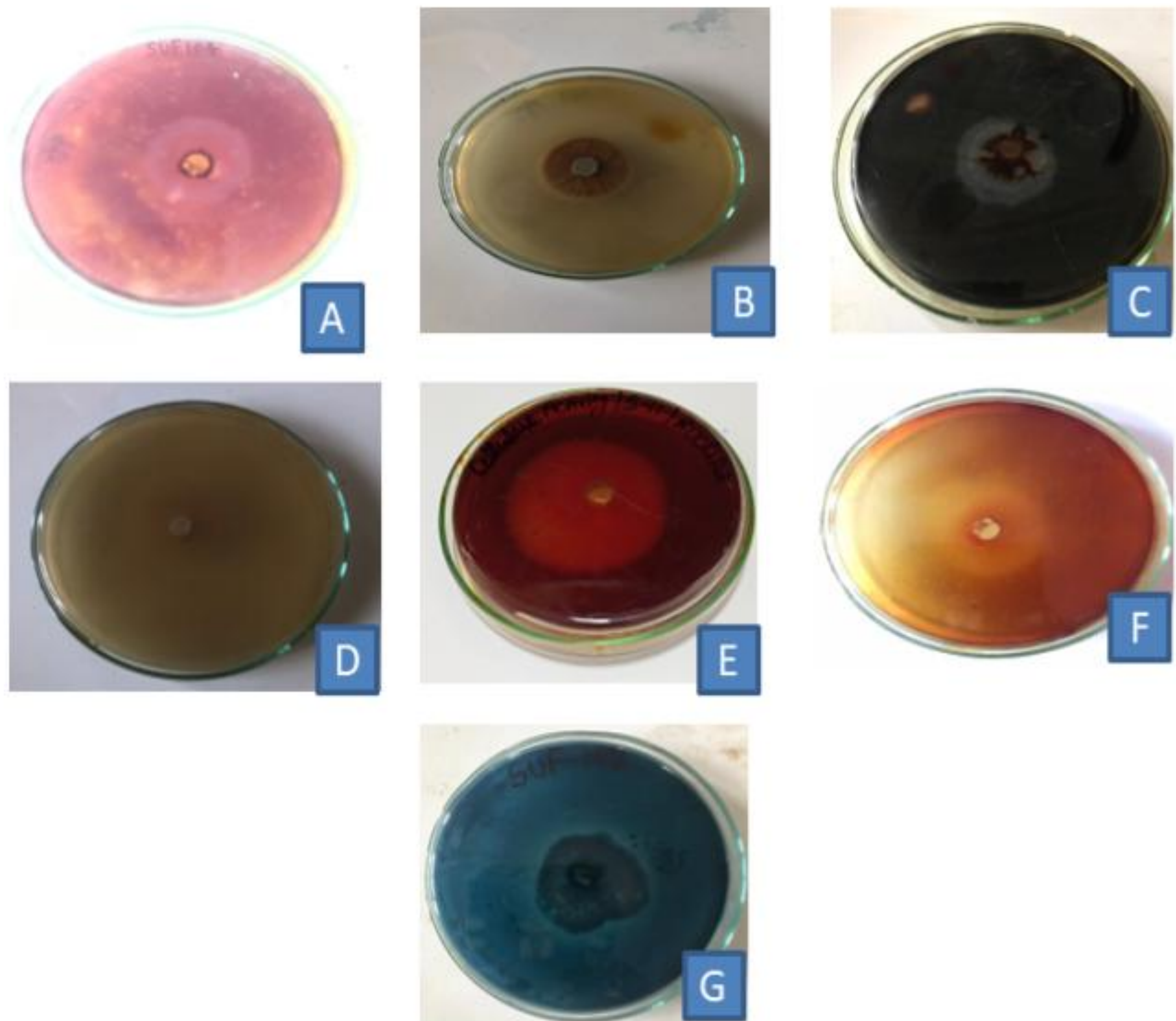

Fig 3: Different fungal enzymes (produced on 0.5% jaggery + 10% diesel oil) activities on agar plate (A) Manganese peroxidases (B) Pectinases, (C) Gelatinases, (D) Amylases, (E) Cellulases, (F) Proteases, (G) Lipases

The gelatinases are responsible for thedegradation of hydrocarbon bonds and were produced in varying amounts by all 6 species, however, *Aspergillus japonicus* produced very high activities of gelatinases. The manganese peroxidasesis one of the major and very significant enzyme for the degradation of O_2 and H_2O during oil breakdown process.

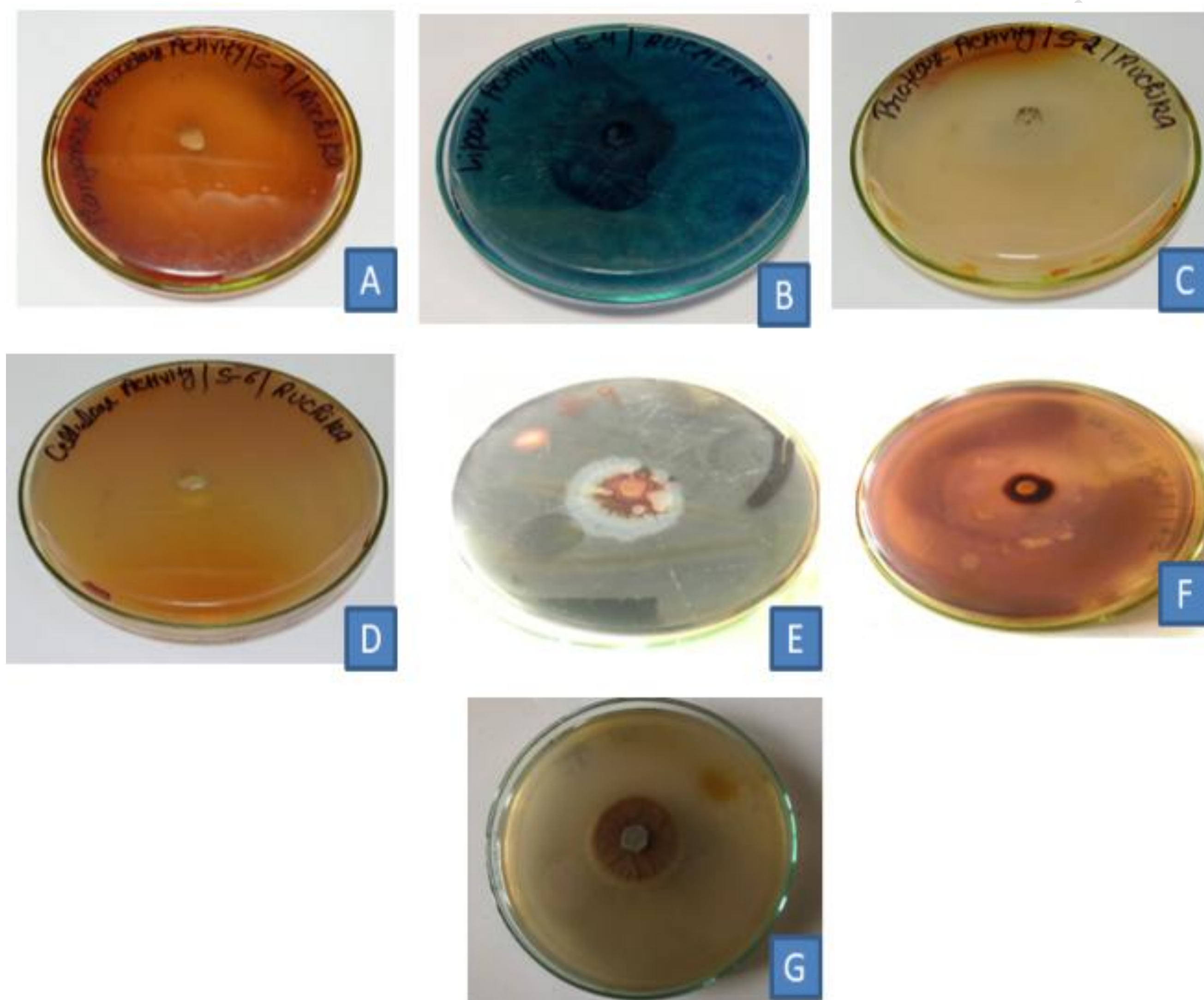

Fig. 4:Different fungal enzymes (produced on 1.0% jaggery + 10% diesel oil) activities on agar plate (A) Manganese peroxidases, (B) Lipases(C) Proteases (D) Cellulases (E) Amylases (F) Pectinases (G) Gelatinases

Six species of *Aspergillus* isolated from naturally degrading diesel oils sites, were assessed for qualitative production ofoil degrading enzymes using agar plate assay method for the production of manganese

peroxidases, pectinases, proteases, amylases, lipases, cellulases and gelatinases activities (Fig 3 and 4). Lipases are one of the most important groups of biocatalysts for industrial applications, such as manufacturing of detergents, production of food ingredients, treatment of wastewater, paper processing, production of pharmaceuticals, fine chemical synthesis and manufacturing of pesticides, cosmetics as well as single cell proteins[23]. Fungal species producing lipases are routinely used for cleaning oil spillages and the prolific role of lipase-producing fungi has also been widely reported in the process of bioremediation of hydrocarbons [24]. Correa-Garca et al. [25], reported that laccases, manganese peroxidases and lignin peroxidasesdegrade diesel oil in nature as well as in laboratory conditions. This study confirms earlier reports of bioremediation of hydrocarbons by fungi[26], however, the activities reported in this paper are based on isolation of species of *Aspergillus* from naturally degrading diesel sites.Different fungi have a variety of processes that they can be used to secrete enzymes that can break down petroleum hydrocarbonswith industrial and commercial applications as bioremediation agents[1]. The outcome of the present study is that the species of Aspergillus can need carbon source for initial growth and thereafter are able to produce a variety of oil degrading enzymes to breakdown the diesel into simple carbon and nitrogen sources to serve them as nutrients for their growth and survival, thus, by converting petroleum to simpler absorbable forms for their nutrition and growth [5]. Various species of fungi *Aspergillus flavus* and *Saccharmyces cerevisiae* [27], *Alternaria alternata*and *Aspergillus ustus* [28], *Aspergillus* and *Penicillium* [29] have been isolated from various habitats that are able to actively degrade oil, diesel oil, petroleum and hydrocarbons in natural soils. Manjunatha and Praveen Kumar [30], have isolated 20 indigenous fungal isolates from diesel, oil engine and crude oil contaminated soils in India and have assessed their bioremediation potential. Hydrocarbon degrading enzyme activities of *Aspergillus oryzae* and *Mucor irregularis* with 1% engine oil from Nigerian crude oil polluted sites are reported by Asemoloye et al [31]but in our studies we have demonstrated that all 6 test species of *Aspergillus* are producing high activities of different oil degrading enzyme at a very high concentration of 10% (w/v) of the diesel oil. *Aspergillus* sp. has also been used to remove different petroleum hydrocarbons from soils[32]. *Aspergillus, Fusarium, Cladosporium, Penicillium, Rhizopus and Alternaria* species are isolated from sludge contaminated soils of refinery in Haryana [9]. The use of microorganisms for remediation of hydrocarbons, and oil contaminated soils is an economically effective solution and *Aspergillus ustus* and *Purpureocilliumlilacinum* has been effectively used [6]. Candida strains have been demonstrated morphological changes / adaptations when cultivated under oil rich medium[14] and we also found morphological variations in the species of *Aspergillus* isolated from diesel contaminated soils, the changes were noticed in colony morphology as well as the size of vesicles and the hyphal breadth.

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Mangrove fungi isolated from red sea coast of Saudi Arabia[33], consortium of fungi from polluted soils[34] also have capabilities to degrade diesel contaminated soils. Hydrocarbon degrading potential of fungi has recently been reviewed by Mahmud et al. [1] and have concluded that filamentous fungi have high potential for commercial exploitation for remediation of oil pollutants and oil spill sites.

Conclusions-

Fungi have capabilities to degrade diesel oil in soil and can be used as potential organisms for bioremediation of hydrocarbons from polluted sites. The species of *Aspergillus* produce a set of different enzyme complexes that are able to degrade hydrocarbons. Supplementation of jaggery in oil broth enhance the growth of fungi. Jaggery serve as source of energy for initial growth and thereafter the fungi are able to secrete the metabolites that can degrade the diesel oil into mono and oligomers which are then absorbed by the fungi to produce specific enzymes to degrade diesel oil. Our study revealed that*A. luchuensis* produced highest activities of proteases, *A. violacefuscus* - amylases, *A. niveus* - gelatinases, *A. terreus*and *A. ustus* - pectinases, and *A. japonicus* produced highest activities of manganese peroxidases, though, all species produced all 7 enzymes in varying amounts.

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