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Bioremediation Potential of Selected Rhizosphere Fungi of *Tridax Procumbens* Linn. and *Chromolaena odorata* (L.) R.M. King & H. Rob

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Abstract

Co-metabolism between plants and rhizosphere microbes is the mainstay of rhizoremediation of contaminated soils. The aim of the current study was to isolate and screen selected rhizosphere micro-fungi of two Asteraceae (Tridax procumbens and Chromolaena odorata) collected from the wild in University of Lagos, Akoka, Lagos State for bioremediation potential. Rhizosphere fungi were isolated, identified and evaluated for crude oil myco-remediation. The inhibitory effect of different concentrations of crude oil on mycelial growth of the most abundant fungi was determined with poisoned plate assay method. The most abundant and recurring fungi around C. odorata and T. procumbens were determined using the serial dilution and plating methods. Analysis of the rhizosphere soil of T. procumbens and C. odorata showed they were sandy loam type. C. odorata soil had higher moisture, organic carbon, and acid content than T. procumbens. Aspergillus flavus and Trichoderma harzianum were the most abundant and recurring fungi in C. odorata and T. procumbens, respectively. Thirty-nine micro-fungi belonging to twenty genera were isolated from the test plants' rhizosphere. A. flavus and T. harzianum tolerated 2.50 to 10.00% crude oil contamination assessed with their mycelial growth inhibition reducing with time. A. flavus and T. harzianum caused a 68.45% and 86.71% reduction of crude oil contamination respectively, in a time dependent manner. The filamentous fungi ~A. flavus and T. harzianum can potentially be used to simultaneously ameliorate crude oil contaminated soils in conjunction with C. odorata and T. procumbens, respectively in the innovative technology termed rhizoremediation.

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Introduction

The contamination of soil with crude oil and allied products is a major problem for an oil producing country like Nigeria and the world at large. The remediation technologies vary from mechanical and chemical treatments to biological means. Phytoremediation is one of the popular biological remediation options which uses plants for treatment of contaminated soils that is environment friendly, and studies show that it is safe and sustainable (Hussain, et al., 2018). Rhizoremediation is a new biotechnology that combines phytoremediation and bioaugmentation and makes use of the symbiotic relationship between plants and their associated soil microorganism in the root zone (Hoang, et al., 2021). The microbes in soil create a nutritious and safe environment for the plants whilst removing harmful organisms and compounds (Dawar, et al., 2014; Hesammi, et al., 2014; Dindar, et al., 2015). The fact previously stated is the basis for research interest on rhizosphere organisms and technologies. The zone of soil affected by a plant's root activity with the highest concentration of carbon and altered microbial diversity is called the rhizosphere and has lower pH, oxygen, and carbon dioxide than bulk soil (Brady and Buckman, 1990; Slaughter, 2021). To ensure successful decontamination of crude oil contaminated soil with plants, the microbial population around the root zone specifically the petroleum hydrocarbon degrading species must be stimulated to grow or their population increased by augmenting soils with the species (Ruley, et al., 2022). It is important that the identity of rhizosphere fungi around potential plants to be used for rhizoremediation should be investigated and

their physiology and responses to contaminants known (Zuzolo, et al., 2021). Wild plants like weeds support more fungi with high degradative potentials and their use for phytoremediation is well documented (Ogbo, et al., 2009a; Ogbo et al., 2009b; Zhou, et al., 2011; Raju, et al., 2015). The microorganisms particularly fungi around the root zone of the plants do the actual break down of contaminants and through sequestration remove the contaminants, sending less harmful compounds into the plant's system (Khatoon, et al., 2021). Fungal bioremediation is the best biotechnology-based method for cleanup of contaminated sites because of the array of pathways and enzymes inherently at their disposal and combining this with phytoremediation will enhance and cause total removal of the contaminants from the soil (Tomer, et al., 2021). In the current study, micro-fungi associated with two Asteraceae weeds (*Tridax procumbens* and *Chromolaena odorata*) were isolated from different sites at University of Lagos, Akoka, Yaba, Lagos and screened for crude oil remediation potential.

Tridax procumbens Linn (Asteraceae) also known as coat button plant is widespread in the tropical and subtropical regions of the world and used for the treatment of various diseases such as dysentery, malaria, diarrhea, high blood pressure and alopecia (Edeoga, et al., 2005).

Chromolaena odorata (L) King & H. E. Robinson on the other hand commonly called Siam weed is another Asteraceae species, and a tropical weed considered medicinal by most locals with antipyretic, antimicrobial, and anti-inflammatory activities attributed to it (Vijayaraghavan, et al., 2017). *C. odorata* has shown potentials for decontamination of crude oil and heavy metal polluted soils and even nuclear wastes contaminated sites (Singh, et al., 2009; Atagana, 2011). *T. procumbens* and *C. odorata* are considered noxious weeds in the environment to be removed and disposed of despite their medicinal values. They can however become useful if they are rechanneled into useful purposes like decontamination of crude oil polluted soils. It is important to establish the right plant and microbes balance in the soil to ensure complete or effective removal of petroleum hydrocarbons in soil by the process of rhizoremediation (Hoang, et al., 2021). The aim of the current study was to identify the rhizosphere fungi of the weeds *T. procumbens* and C*. odorata* and find out if representative fungi can be used for remediation of crude oil substrates or soils.

Materials and Methods

Collection of Samples

Soil samples were collected from the rhizosphere of *Tridax procumbens* and *Chromolaena odorata* from ten separate locations at the Akoka Campus of the University of Lagos, Lagos, Nigeria. The test plants were harvested, roots shaken down and a total of four samples collected at a depth of 0-20cm and composite soil samples were taken for each site and poured into labelled Ziploc bags (Hazzat, et al., 2018).

Soil Analysis

Particle size distribution was determined with the Bouyoucos Hydrometric Method (Bouyoucos, 1962). The pH was recorded for each soil sample after making solutions with sterile distilled water using standard methods (Bouyoucos, 1962). Soil moisture was obtained after oven drying and the weight loss method was used to determine the moisture and organic carbon content of the soil (Sparks, et al., 1996).

Isolation of Rhizosphere Myco-flora

The Serial Dilution Assay method was used for isolation of rhizosphere fungi by adopting methods by Aneja, (2001) and Dawar, et al. (2014) with slight modifications. Soil sample (10 mg) was mixed into 1 ml distilled water and 9 ml of distilled water was added and four-fold serial dilutions made. One milliliter of soil solution was dispensed into Malt Extract Agar (MEA) filled Petri plates with antibiotics (Penicillin). Aliquots were taken from each dilution and plated with three replicates each. The plates were incubated at 30˚C, and total fungal colonies formed were recorded and pure culture isolates were got by subculturing from the mixed cultures into new Malt Extract Agar (MEA) culture plates.

Identification of Rhizosphere Fungi

Rhizosphere fungi were identified using standard manuals (Thom and Raper, 1945; Barnett, 1960; Nelson, et al., 1983; Gams and Bissett, 1998). The colony characteristics, colour and spore formed, hyphal structures and conidia aggregations were observed macroscopically and microscopically.

The number of plants stands from which fungal Species were isolated was used to calculate the species percentage incidence around the rhizosphere of each plant Species (Dawar, et al., 2014).

Percentage Incidence of each rhizosphere fungus = $\frac{\text{Number of Plant Specimens having a particular fungus}}{\text{Total number of Plant Specimens analyzed}} \times 100$

The fungal species with the highest incidence in all sampled plant species was regarded as the dominant strain and the species that recorded near 100% incidence for each plant's rhizosphere were selected for crude oil degradation potential.

Crude Oil Tolerance Test for Rhizosphere Fungi

The ability of the selected rhizosphere fungi to tolerate diverse levels of crude oil contamination was evaluated by growing them in Petri plates filled with crude oil contaminated Bushnell Haas mineral agar medium (BHA) using a modification of methods by Asemoloye, et al. (2019) and Moslem and El-Kholie, (2009). The Bushnell Haas (BH) broth and agar (Sigma-Aldrich, Amsterdam, Germany) is a basal medium composed of only minerals and no carbon source were prepared following manufacturer's instructions. The BH agar was contaminated with various levels of crude oil contamination (2.5, 5, 7.5 and 10%) and plates augmented with dextrose as carbon source served as control. The treatments and control were replicated three times and the crude tolerance levels were investigated by taking radial mycelial growth measurements to the nearest mm every 24 h and comparing with mycelial growth in control plates.

Mycelia Growth Inhibition (MGI) = $\frac{\text{Radial Mycelial Extension in crude oil contaminated plate}}{\text{Radial Mycelial Extension in control plate}} \times 100$

The mycelial growth inhibition of the fungus is equivalent to the dose (concentration of crude oil) inhibition response (DIR) to crude oil contamination.

The mycelial growth inhibition of *T. harzianum* was calculated by evaluating the percentage coverage of the mycelia on the Petri dish (Gams and Bissett, 1998). This is because it was noticed that the growth of *T. harzianum* was not radial, submerged in parts of the growth medium and shooting off aggregations of conidiophores in separate sections of the Petri plates.

Crude Oil Degradation Efficiency of Selected Rhizosphere Fungi

The crude oil degradation potential of the two selected rhizosphere fungi (*Aspergillus flavus* and *Trichoderma harzianum*) was determined after growing them submerged in crude oil contaminated Bushnell Haas (BH) broth in 250 ml Erlenmeyer flasks. One hundred milliliter of Bushnell Haas (BH) broth contaminated with 1% crude oil was inoculated with a four-day old pure culture of the two test fungi from the periphery of actively growing cultures. The control experiments had crude oil contaminated broth but not seeded with any fungus. They were incubated in the dark in an incubator with a shaker set at 100rpm at 30˚C. The degree of crude oil bioremediated was determined gravimetrically adopting methods by Al-Hawash, et al. (2018).

The residual crude oil was extracted from the broth with chloroform with an equal volume of the growth media. The extracted crude oil was dehydrated with anhydrous sodium sulphate after evaporating the chloroform with a rotary evaporator under pressure in a water bath at 55˚C (Al-Hawash, et al., 2018).

Gravimetric Analysis

The residual crude oil from both control and treated samples was estimated by weighing. The degradation efficiency of the test fungi was calculated using the formular

Bioremediation/Biodegradation Efficiency (%) = $\frac{\rho 0 - \rho 1 - \rho 2}{\rho 0} \times 100$

Where ρ^0 = concentration of the crude oil in control; ρ^1 = residual crude oil after different incubation periods (4, 8, 12 and 16 days) in fungi treatments; ρ^2 = concentration of crude oil loss due to abiotic factor or extraction process.

Data Analysis

Analysis of Variance (One-Way ANOVA) was conducted with the Statistical analysis software -SPSS Version 26 program for Windows (SPSS Inc., Chicago, IL, US). Duncan's Multiple Range Test was used to determine significant difference between treatments $p \geq 0.05$.

RESULTS AND DISCUSSION

The wild Asteraceae plants used in this study (*Tridax procumbens* and *Chromolaena odorata*) had rhizosphere soils that were sandy loam (Table 1). There was no significant difference between the organic carbon content of the two plants, as it varied between 3.94-4.43 (Table 1). The *T. procumbens* rhizosphere soil had more moisture content than that of *C. odorata* from analysis of soil around it (Table 1). The rhizosphere soil of *C. odorata* was slightly more acidic than that of *T. procumbens* and as such could have contributed to its ability to host more fungal species (Yamanaka, 2003). The textural classification of both Soil Types places them into nutritionally rich soil but prone to run off or drainage and might also explain the abundance of micro-fungi (Bhattacharyya, et al., 2009).

Table 1: Physicochemical Properties of Rhizosphere Soil of Two Asteraceae Weeds

Soil Characteristics	*Tridax procumbens*	*Chromolaena odorata*
Soil texture	Sandy loam	Sandy loam
pH	7.84±0.26[a]	6.8 ±0.35[a]
Moisture Content	27.33±8.62[b]	20±5.41[c]
Organic carbon	4.43±0.31[d]	3.94±0.31[d]

*Superscript with same letters along the same column are not significantly different (p≥0.05)

A total of thirty-nine fungal species belonging to twenty genera was isolated from the rhizosphere of both plants (Table 2). *Tridax procumbens* recorded fifteen rhizosphere fungal Species while *C. odorata* had thirty Species (Table 2). The fact that *C. odorata* recorded a higher population of micro fungi can be partly attributed to the more acidic nature of its rhizosphere soil. Previous results show that microbial activity and population is fundamentally influenced by the plant species, fertility, and pH status of the soil (Hauchhum and Tripathi, 2019). Secondly, the presence of scores of saprophytic filamentous fungal in the *Tridax* and *Chromolaena* species rhizosphere can also be attributed to the rhizodeposition of organic matter and root exudates into the soil (Hauchhum and Tripathi, 2019). Rhizodeposition is a term that refers to root exudates released from plants into the soil in the form of organic carbon compounds and metabolic exudates fuel soil microflora which in turn, participate in the cycling dynamics on nutrients, pollutants and soil borne pathogens (Nguyen, 2019). The composition of the fungal components of the rhizosphere of the two plants is consistent with data in literature which shows that filamentous fungi like *Aspergillus flavus, Penicillium citrinum* and *Trichoderma harzianum* are among the most abundant in plant rhizosphere (Merckx, et al., 1987 ; (Darwar et al., 2014).

Table 2: Fungal Isolates from the rhizosphere of two wild plants on the Akoka Campus, University of Lagos

Fungal Species	Family	*Tridax procumbens*	*Chromolaena odorata*
Absidia glauca Hagem	Cunninghamellaceae	+	-
Aspergillus aculeatus lizuka	Aspergillaceae	+	+
Aspergillus fumigatus Fresen	Aspergillaceae	-	+
Aspergillus niger Tiegh	Aspergillaceae	+	+
Aspergillus flavus Link	Aspergillaceae	+	+
Acremonium falciforme (Carriòn) W. Gams	Hypocreaceae	-	+
Arthrobotrys conoides Drechsler	Obiliaceae	+	-
Botrytis cinerea Pars.	Sclerotiniaceae	-	+
Bjerkandera adjusta (Willd.) P. Karst	Meruliaceae	+	+
Chaetomium funicola Cooke	Chaetomiaceae	-	+
Cladosporium cladosporioides (Fresen.) G. A. de Vries	Cladosporiaceae	+	-
Chrysonilia sitophila (Mont.) Arx. Sydowia	Sordariaceae	+	-
Curvalaria lunata (Wakker) Boedijn	Pleosporaceae	-	+
Cunninghamella elegans Lendner	Cunninghamellaceae	+	+
Fusarium oxysporum Schltdl.	Nectriaceae	+	+
Fusarium solani (Mart.) Sacc.	Nectriaceae	-	+
Geotrichum silvicola Pimenta, G. S. Prasad, Lachance & C. A. Rosa	Dipodascaceae	+	-
Clonostachys rosea (Link) Schroers, Samuel, Seifert & W. Gams	Bionetriaceae	+	-
Gliocardium viride Matr.	Hypoceaceae	-	+

Helminthosporium acalyphae (Thüm.) Cif.	Massarinaceae	-	+
Laccaria laccata (Scop.) Cooke	Hydnangiaceae	-	+
Macrophomina phaseolina (Tassi) Goid.	Botryosphaeriaceae	+	-
Mucor mucedo L.	Mucoraceae	+	+
Mucor irregularis Stchigel. Cano, Guarro & Ed. Àlvarez	Mucoraceae	-	+
Pichia occidentalis (Kurtzman, M. J. Smiley & C. J. Johnson	Saccharomycetaceae	+	+
Penicillium brevicompactum Dierckx.	Aspergillaceae	+	-
Penicillium citrinum Thom.	Aspergillaceae	-	+
Penicillium chrysogenum Thon.	Aspergillaceae	-	+
Penicillium nigricans Bainier ex Thom	Aspergillaceae	+	-
Penicillium sp. Link	Aspergillaceae	-	+
Phoma eupyrena Sacc.	Didymellaceae	-	+
Talaromyces purpurogenus Samson, Yilmaz	Trichocomaceae	-	+
Trichoderma harzianum Rifai	Hypocreaceae	+	+
Trichoderma viride Pers.	Hypocreaceae	+	+
Trichoderma sp.	Hypocreaceae	+	+
Rhizoctonia solani J. G. Kühn	Ceratobasidiaceae	-	+
Rhizopus arrhizus var arrhizus	Mucoraceae	+	+
Rhizopus stolonifer (Ehrebb.) Vuill.	Mucoraceae	-	+
Verticillium dahlia Kleb.	Plectosphaerellaceae	-	+

*- Absent; + Present

The most recurring fungal specimens in order of increasing percent incidence for the plant *Tridax procumbens* were *Trichoderma harzianum*, *Aspergillus aculeatus, Aspergillus flavus*, *Aspergillus niger,* and *Trichoderma viride* (percent incidence ~99.79%, 75.78%, 60.76%, 55.66% and 55.64% respectively (Table 3).

On the other hand, the most abundant micro-fungi in the rhizosphere of the plant *Chromolaena odorata* in order of increasing percent incidence were *Aspergillus flavus*, *Aspergillus niger, Trichoderma harzianum, Laccaria laccata, Mucor irregularis,* and *Rhizoctonia solani* (Percent incidence ~99.50%, 75.55%, 65.68%, 50.79%, 50.76%, and 50.52% respectively) (Table 3). The fungi~ *Absidia glauca, Aspergillus fumigatus, Acremonium falciforme, Botrytis cinerea, Chaetomium funicola, Cladosporium cladosporioides Cunninghamella elegans, Fusarium solani, Gliocardium viride, Helminthosporium acalyphae, Laccaria laccata, Mucor irregularis, Pichia occidentalis, Penicillium chrysogenum, Penicillium nigricans, Talaromyces purpurogenus* and *Rhizoctonia solani* were found in the rhizosphere soil of *Chromolaena odorata* but absent in that of *Tridax procumbens* (Table 3). *C. odorata* supported the growth and abundance of micro-fungi species than *T. procumbens.*

The study of Mapook et al. (2020), shows that there are even more micro fungi in the rhizosphere of *C. odorata* than the thirty microfungal species recorded in the current study (Table 2). Mapook et al. (2020) recorded that, the micro-fungal community of the rhizosphere of Siam weed (*C. odorata*) had a total of 130 species with *Pseudocercospora* being the dominant genus contrary to the current study, the plant had thirty-nine rhizosphere species and *Aspergillus flavus* as the most abundant species (Table 3).

Research indicate that weeds play particularly important roles in restoring soil after disruptive events like floods or crude oil pollution, with a high degree of specificity. They do this in conjunction mostly with the rhizosphere fungal community during remediation or degradation activities. Wild plant species tend to have more microbes in their rhizosphere, and they use the beneficial ones to promote nutrient appropriation, tolerate changes without any adaptative

features formed and develop disease resistance (Chang, et al., 2018; Chang, et al., 2021). This therefore explains the substantial number of rhizosphere fungi encountered in the current study.

Table 3: Incidence of Rhizosphere Fungi of Two Wild Asteraceae Species

Fungal Species	*Tridax procumbens*		*Chromolaena odorata*	
	Incidence (N=20)	Percent Incidence (%)	Incidence (N=20)	Percent Incidence (%)
Absidia glauca	0.00±0.00	0.00±0.00	2.87±0.71	15.53±0.61
Aspergillus aculeatus	5.91±0.36	75.78±0.27	8.97±0.72	40.55±0.64
Aspergillus fumigatus	0.00±0.00	0.00±0.00	3.63±0.60	15.72±0.29
Aspergillus niger	1.68±0.75	55.66±0.53	15.64±0.58	75.55±0.52
Aspergillus flavus	2.82±0.27	60.76±0.42	19.73±0.51	99.50±0.25
Acremonium falciforme	0.00±0.00	0.00±0.00	2.47±0.42	10.76±0.35
Arthrobotrys conoides	6.71±0.31	31.02±0.66	0.00±0.00	0.00±0.00
Botrytis cinerea	0.00±0.00	0.00±0.00	5.57±0.48	30.65±0.42
Bjerkandera adjusta	2.57±0.45	10.61±0.41	4.60±0.45	20.80±0.23
Chaetomium funicola	0.00±0.00	0.00±0.00	3.70±0.36	15.84±0.33
Cladosporium cladosporioides	0.00±0.00	0.00±0.00	4.61±0.49	20.88±0.30
Chrysonilia sitophila	2.67±0.40	11.13±0.61	0.00±0.00	0.00±0.00
Curvalaria lunata	2.55±0.40	10.70±0.53	3.71±0.64	15.64±0.52
Cunninghamella elegans	0.00±0.00	0.00±0.00	6.54±0.52	30.85±0.27
Fusarium oxysporium	1.70±0.30	5.94±0.40	2.57±0.47	21.10±0.40
Fusarium solani	0.00±0.00	0.00±0.00	3.56±0.48	15.79±0.41
Geotrichum silvicola	3.78±0.28	15.74±0.34	0.00±0.00	0.00±0.00
Gliocardium viride	0.00±0.00	0.00±0.00	7.66±0.42	36.04±0.41
Helminthosporium acalyphae	0.00±0.00	0.00±0.00	2.49±0.49	10.61±0.47
Laccaria laccata	0.00±0.00	0.00±0.00	10.45±0.55	50.79±0.23
Macrophomina phaseolina	1.58±0.38	5.74±0.36	0.00±0.00	0.00±0.00
Mucor mucedo	4.59±0.47	20.55±0.47	7.63±0.52	35.64±0.41
Mucor irregularis	0.00±0.00	0.00±0.00	10.67±0.36	50.76±0.25
Pichia occidentalis	0.00±0.00	0.00±0.00	3.47±0.56	15.69±0.64
Penicillium brevicompactum	2.57±0.45	10.80±0.55	9.77±0.78	45.57±0.46
Penicillium citrinum	2.50±0.46	10.73±0.40	0.00±0.00	0.00±0.00
Penicillium chrysogenum	0.00±0.00	0.00±0.00	4.55±0.42	20.76±0.33
Penicillium nigricans	0.00±0.00	0.00±0.00	2.48±0.48	10.65±0.42
Penicillium sp.	0.00±0.00	0.00±0.00	5.69±0.49	25.64±0.55
Phoma eupyrena	5.70±0.55	25.70±0.53	6.68±0.38	30.72±0.40
Talaromyces purpurogenus	0.00±0.00	0.00±0.00	2.79±0.26	10.99±0.56
Trichoderma harzianum	19.58±0.56	99.79±0.85	13.79±0.24	65.68±0.42
Trichoderma viride	11.81±0.32	55.64±0.54	1.68±0.41	5.79±0.46
Trichoderma sp.	5.62±0.49	25.82±0.26	8.71±0.40	40.81±0.22
Rhizoctonia solani	0.00±0.00	0.00±0.00	4.59±0.51	50.52±0.45
Rhizopus arrhizus	0.00±0.00	0.00±0.00	3.56±0.41	15.69±0.29
Rhizopus stolonifer	4.77±0.23	20.61±0.40	9.65±0.32	45.85±±0.25
Verticillium dahlia	0.00±0.00	0.00±0.00	7.63±0.38	35.81±0.33

The fungi that were found present with over 90% incidence in all samples of rhizosphere soil of the two plants were used for the crude oil degradation study. *Aspergillus flavus*

recorded >99.50% incidence in the sampled rhizosphere soil of *C. odorata* while *Trichoderma harzianum* had >99.79% incidence in the rhizosphere soil of *T. procumbens* (Table 3).

Aspergillus flavus from the root zone of *C. odorata* and *T. harzianum* from the rhizosphere soil of *T. procumbens* tolerated all levels of crude oil (2.50-10.00% crude oil concentration) evaluated (Table 4). The mycelial growth inhibition of the two fungi increased with increasing concentrations of crude oil contamination. The inhibitory effects reduced with time (from 88.30% on day 2 to 11.84% on day 10 at 2.50% level of crude oil contamination) as the fungi got used to the crude oil contaminated environment and eventually began using it up as a carbon source for growth (Table 4). At the end of 10 days mycelial growth inhibition for *T. harzianum* varied between 5.52% in 2.5% level of crude oil contamination to 10.81% in 10.00% level of crude oil contamination (Table 4). On the other hand, mycelia growth inhibition for *A. flavus* varied from 11.84% in 2.5% crude oil contamination to 35.83% in 10.00% level of crude oil contamination (Table 4). The metabolization of crude oil by the two fungi led to a reduction in the concentration of crude oil in the medium as shown in table 5.

Table 4: Effect of Crude Oil on Mycelial Growth of Rhizosphere Fungi~ *Aspergillus Flavus* and *Trichoderma Harzianum*

	LEVEL OF CRUDE OIL CONTAMINATION (%)							
	2.50		5.00		7.50		10.00	
Time (Days)	A. f	T. h	A. f	T. h	A. f	T. h	A. f	T. h
2	88.30±2.42	76.78±3.18	90.58±4.73	78.96±2.87	92.64±0.97	85.43±2.75	95.75±3.34	88.53±2.59
4	76.39±1.05	58.85±0.21	83.94±1.03	60.74±0.66	76.18±0.64	60.47±1.25	89.33±0.76	69.45±0.71
6	65.81±0.47	32.71±0.53	71.12±0.69	39.62±0.75	59.73±0.47	48.46±0.93	75.85±0.59	47.18±3.09
8	47.89±0.66	17.31±0.49	55.81±0.25	20.84±0.27	43.87±0.21	17.93±0.44	63.85±0.59	28.91±0.65
10	11.84±0.38	5.52±0.57	14.75±0.29	7.75±0.35	18.92±0.64	7.29±0.52	35.83±0.77	10.81±0.52

*A. f- *Aspergillus flavus*; T. h – *Trichoderma harzianum*

The final degradation efficiency of *A. flavus* and *T. harzianum* were 68.45% and 86.71% respectively at 5.00% level of crude oil contamination (Figure 1). The incubation period affected the degree of degradation as the concentration of crude removed increased with time (Figure 1). This can be explained by the fact that there was prolonged contact with fungal mycelia and enzymatic process with time and as such more reaction with the increased volume of enzymes in the medium (Echeveria, et al., 2020). The fungus *T. harzianum,* has more enzymes that can effectively degrade crude oil than *A. flavus* (Lee, et al., 2017). *T. harzianum* also colonized the contaminated substrates faster than *A. flavus* as shown by mycelial growth inhibition (Table 4). The high degradative potential of *T. harzianum* is consistent with reports of (Daccò, et al., 2020) in which the fungus performed better than other species of *Trichoderma* in the degradation of a petroleum hydrocarbon complex mixture. *Trichoderma* species had previously been reported to tolerate high concentrations of crude oil contamination and can effectively and completely remove crude oil from soil if co-metabolized with *Tridax procumbens* as shown in the current study (Hamzah, et al., 2012). Crude oil degradation rate of 60% was reported for *Aspergillus flavus* by Al-Dossary, et al. (2019) consistent with the study here. The particularly significant role played by fungi within the root zone of plants with high phytoremediation potentials is displayed by the enhanced remediation potential of the plant *Verbascum sinuatum* when rhizosphere fungi were added exogenously (Zuzolo, et al., 2021). The main mechanism of action in rhizoremediation involves bio-stimulation of microbial action by the root exudates which leads to co-metabolism of contaminants in the rhizosphere emphasizing the role of microbes in bioremdiation with plants in soil (Infante, et al., 2017).

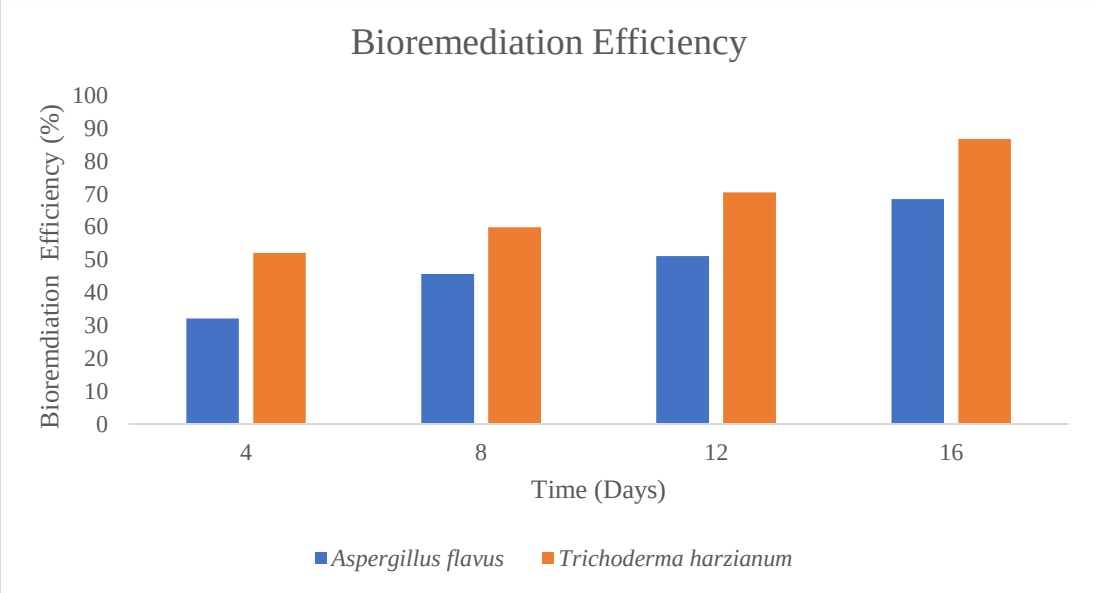

Figure 1: Bioremediation Efficiency of Two Rhizosphere Fungi Isolated from *Tridax procumbens* and *Chromolaena odorata* at 5.00% Level of Crude Oil Contamination

The fungi in a plant's rhizosphere play very crucial roles in its live as they affect its ability to acquire nutrients, and their susceptibility to pathogens and harmful elements in the soil (Chamkhi et al., 2022). The presence of these diverse micro-fungi in the root zones of plants makes their rhizosphere a hot bed of activities (Chamkhi et al., 2022). It is known that the health of the soil is linked to mycorrhizal associations, a mutually beneficial relationship which involves plant roots and fungal species. This therefore means that the fungal community form beneficial relationships with plants to ensure continued survival of the plants and health of the soil ecosystem.

Rhizoremediation of crude oil contaminated soils is a new biotechnological approach to safe clean-up of environmental contamination by petroleum hydrocarbons and heavy metal. Rhizoremediation employs the organisms within the rhizosphere of plants particularly weeds like *Chromolaena odorata* and *Tridax procumbens*. For successful rhizoremediation to be achieved, it is important to identify the soil microbes and know what roles they play as done here in this investigation. The microbes with crude oil degradation potential if identified, can be used with the plants as a co-metabolite for cleaning up crude oil contaminated soils effectively.

Conclusion

Rhizoremediation of crude oil contaminated soils is a new biotechnological approach to safe clean-up of environmental contamination by petroleum hydrocarbons and heavy metal. Rhizoremediation employs the organisms within the rhizosphere of plants particularly weeds like *Chromolaena odorata* and *Tridax procumbens* evaluated in the current study. For successful rhizoremediation to be achieved, it is important to identify the soil microbes and know what roles they play as done here in this investigation. The rhizosphere fungi, *Aspergillus flavus* and *Trichoderma harzianum* has crude oil remediation potential and can be used with the plants ~*T. procumbens* and *C. odorata* for simultaneous degradation of petroleum-based compounds from crude oil contaminated soils.

References

Al-Dossary, M. A., Abood, S. A., and Al-Saad, H. T. (2019). Biodegradation of Crude Oil Using *Aspergillus* species. JBAH*, 9*(4): 60-64. DOI: 10.7176/JBAH

Al-Hawash, A. B., Alkooorane, J. T., Abbood, H. A., Zhang, J., Sun, J., Zhang, X., and Ma, F. (2018). Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field, Iraq. Biotechnol. Rep., 17: 104-109. https://doi.org/10.1016/j.btre.2017.12.006

Aneja, K. R. (2001). Plant Pathology, and Botechnology, Experiments in Microbiology 4th Ed. New Age International Publishers pp. 157-162.

Asemoloye, M. D., Jonathan, S. G., and Ahmad, R. (2019). Degradation of 2, 2 - Dichlorovinyldimethylphosphate (Dichlorvos) through plant-fungi-SMC interactions in the rhizosphere of *Panicum maximus* Jacq. Chemosphere, 221: 403-411. DOI: 10.1016/j.chemosphere.2019.01.058

Atagana, H. I. (2011). Bioremediation of Co-contamination of Crude Oil and Heavy Metals in Soil by Phytoremediation Using *Chromolaena odorata* (L) King & H. E. Robinson. Water Air Soil Pollution. 215: 261-271. DOI: 10.1007/s11270-010-0476-z

Barnett, H. L. (1960). Illustrated genera of imperfect fungi. Burgess Pub. Co. Minneapolis US. 225pp.

Bhattacharyya, R., Prakash, V., Kundu, S., Srivastva, A. K., and Gupta, H. S. (2009). Soil Aggregation and organic matter in a sandy clay loam soil of the Indian Himalayas under different tillage and crop regimes. Agric Ecosyst Environ. 132: 126-134. https://doi.org/10.1016/j.agee.2009.03.007

Bouyoucos, G. J. (1962). Hydromethod Method Improved for Making Particle Size Analysis of Soil. J. Agron. 54: 464-465.

Brady, N. C. and Buckman, H. O. (1990). *The Nature and Properties of Soils* 10th Edn. New York: McMillan Publishing Company, Cranbury. 550pp.

Chamkhi, I., El Omari, N., Balahbib, A., El Meryiy, N., Benali, T., Ghoulam, C. (2022). Is the rhizosphere a source of applicable multi-beneficial microorganisms for plant enhancement? Saudi J Biol Sci. 29(9): 1246-1259

Chang, C., Chen, W., Luo, S., Ma, L., Li, X. and Tian, C. (2018). Rhizosphere microbiota assemblage associated with wild and cultivated soybeans grown in three types of soil suspensions. Arch. Agron. Soil Sci., 65: 74-87. http://doi.org/10.1080/03650340.2018.1485147

Chang, J., Sun, Y., Tian, L., Ji, L., Luo, S., Nasir, F., Kramae, E. E. and Tian, C. (2021). The Structure of Rhizosphere Fungal Communities of Wild and Domesticated Rice: Changes in Diversity and Co-occurrence Patterns. Front. Microbiol. 12: 610823. https://doi.org/10.3389/fmicb.2021.610823

Daccò, C., Nicola, L., Temporiti, M. E., Mannucci, B., Corana, F., Carpani, G., and Tosi, S. (2020). *Trichoderma*: Evaluation of Its Degrading Ability for the Bioremediation of Hydrocarbon Complex Mixtures. Appl. Sci. 10(9): 3152. DOI: 10.3390/app10093152

Dawar, S., Batool, M., Tariq, M. and Zaki, M. J. (2014). Mycoflora in the rhizosphere of some wild plants around Karachi University Campus. Pak. J. Bot. 46(1): 369-373.

Dindar, E., Sagban, F. O. T. and Baskaya, H. S. (2015). Variations of soil enzyme activities in petroleum-hydrocarbon contaminated. Int. Biodeter. Biodegr., 105: 268-275. https://doi.org/10.1o16/j.ibiod.2015.09.011

Echeveria, L., GIlmore , S., Harrison, S., Heinz, K., Chang, A., Nunz-Conti, G., Cosi, F., Singh, P. and Bond, T. (2020). Versatile bio-organism detection using mirosphere for future biodegradation and bioremediation studies. In: A. V. Kudryashov, A. H. Paxton, V. S. IIchenko, and A. M. Armani, Laser, resonators, microresonators, and beam control XXII, SPIE Proceedings Vol. 112: p66. Bellingham: SPIE Proceedings. pp.8. DOI: 10.1117/12.2543800

Edeoga, H. O., Okwu, D. E., and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4(7): 685-688. DOI:10.5897/AJB2005.000-3127

Gams, W. and Bissett, J. (1998). Morphology and identification of *Trichoderma*. In G. E. Harman, and C. P. Kubicek, *Trichoderma and Gliocladium.* Volume 1. Genetics. CRC Press, London, UK. pp. 3-32.

Hamzah, A., Zarin, M. A., Hamid, A. A., Omar, O. and Senafi, S. (2012). Optimal physical and nutrient parameters for growth of *Trichoderma virens* UKMP-1M for heavy crude oil degradation. Sains Malays., 41: 71-79.

Haucchum, R and Tripathi, S. K. (2019). Carbon and Nitrogen Differences in Rhizosphere Soil of Annual Plants in Abandoned Lands Following Shifting Agriculture in Northeast India. *Nutr.* Cycl. Agroecosys. 113: 157-166

Hazzat, N. E., Artib, M., Touati, J., Chliyeh, M., Selmaoui, K., Touhami, A. O., Benkirane., and Douira, A. (2018). Diversity of Endomycorrizal Fungi in the Rhizosphere of Chickpea in Morocco. Acta Phytopathol. Entomol. Hung., 53(2): 1-14. DOI: 10.1556/038.53.2018.011

Hesammi, E., Farshidi, A., Sadatebrahimi, F. and Talebi, A. B. (2014). The Role of Soil Organisms on Soil Stability (A Review). *Int. J. Curr. Life Sci.* 4(11): 10328-10334.

Hoang, S. A., Lamb, D., Seshadri, B., Sarkar, B., Choppala, G., Kirkham, M. B. and Bolan, N. S. (2021). Rhizoremediation as a green technology for the remediation of petroleum hydrocarbon-contamianted soils. J. Hazard. Mater.. 401: 123282. https://doi.org/10.1016/j.jhazmat.2020.123282

Hussain, I., Puschenreiter, M., Gerhard, S., Schöftner, P., Yousaf, S., Wang, A., Syed, J. H., and Reichenauer, T. G. (2018). Rhizoremediation of petroleum hydrocarbon-contaminated soils: Improvement opportunities and field applications. Environ. Exp. Bot. 147: 202-219. https://doi.org/10.1016/j.envexpbot.2017.12.016

Infante, C., Harnández-Valencia, I., López, L. and Toro, M. (2017). Phytoremediation of Petroleum Hydrocarbon-Contaminated Soils in Venezuela. In: N. A. Anjum., M. E. Pereira., I. Ahmad., A. C. Duarte., S. Umar., & N. A. Khan. Phytotechnologies- Remediation of Environmental Contaminants CRC Press. Taylor and Francis Group. New York. pp. 99-112.

Khatoon, H., Rai, J. P. and Jillani, A. (2021). Role of fungi in bioremediation of contaminated soil. In V. K. Sharma, M. P. Shah, S. Parmar, and A. Kumar (Eds.), Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-technology. Amsterdam: Academic Press. pp. 121-156. https://doi.org/10.1016/B978-0-12-821925-6.00007-1

Lee, H., Lee, Y. M., Heo, Y. M., Hong, J.-H., Jang, S., Ahn, B. J., Lee, S-S. and Kim, J.-J. (2017). Optimization of Fungal Enzyme Production by *Trichoderma harzianum* KUC1716 through Surfactant-Induced Morphological Changes. Mycobiology, **45(1):** 48-51. doi: 10.5941/MYCO.2017.45.1.48

Mapook, A., Hyde, K. D., Mckenzie, E. H., Jones, E. B., Bhat, D. J., Jeewon, R., Stadler, M., Samarakoon, M. C., Malaithong, M., Tanunchai, B., Buscot, F., Wubet, T., and Purahong, W. (2020). Taxonomic and phylogentic contributions to fungi associated with the invasive weed *Chromolaena odorata* (Siam weed). Fungal Divers., 101: 1-175. https://doi.org/10.1007/s13225-020-00444-8

Merckx, R., Dijkstra, A., Hartoh den, A. and Van Veen, J. A. (1987). Production of root derived material and associated microbial growth in soil at different nutrient levels. Biol. Fertil. Soils. 5:126-231. https://doi.org/10.1007/BF00257647

Moslem, M. A. and El-Kholie, E. M. (2009). Effect of neem (*Azadirachtha indica* A. Juss) seeds and leaves extract on some plant pathogenic fungi. Pak. J. Biol. Sci. 12 (14): 1045-1048. doi: 10.3923/pjbs.2009.1045.1048

Nelson, P. E., Toussoun, T. A. and Marasas, W. F. (1983). *Fusarium* species, An illustrated Manual of Identification. The Pennsylvania State University Press University Park, Pennsylvania. p 206.

Nguyen, C. (2009). Rhizodeposition of Organic C by Plant: Mechanisms and Controls. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Veronique, S., Alberola, C. *Sustainable Agriculture* Springer, Dordrecht. pp 97-123. https://doi.org/10.1007/978-90-481-2666-8_9

Ogbo, E. M., Avwerosovwe, U. and Odogu, G. (2009a). Screening of four common Nigerian weeds for use in phytoremediation of soil contaminated with spent lubricating oil. *Afr.* J. Plant Sci. 3(5): 102-106.

Ogbo, E. M., Zibigha, M. and Odogu, G. (2009b). The effect of crude oil on growth of the weed (*Paspalum scrobiculatum* L.)-phytoremediation potential of the plant. African J. Environ. Sci. Technol. 3(9): 229-233.

Raju, N. T., Madhavi, M. and Prakash, T. R. (2015). Bioremediation of Aquatic Environment Using Weeds. Int. J. Econ. Plants. 2(3): 148-152.

Ruley, J. A., Amoding, A., Tumuhairwe, J. B., and Basamba, T. A. (2022). Rhizoremediation of petroleum hydrocarbon-contaminated soils: A systematic review of mutualism between phytoremediation species and soil living microorganisms. In R. A. Bhat, F. M. Tonelli, G. H. Dar, and K. Hakeem, Phytoremediation: Biotechnological Strategies for Promoting Invigorating Environs. Academic Press, Amsterdam, Netherlands. pp. 263-296. https://doi.org/10.1016/B978-0-323-89874-4.00008-X

Singh, S., Thorat, V., Kaushik, C. P., Raj, K., Eapen, S. and D'Souza, S. F. (2009). Potential of *Chromolaena odorata* for phytoremediation of (137)Cs from solution and low level nuclaer wastes. J. Hazard. Mater. 162(2-3): 743-745. doi: 10.1016/j.jhazmat.2008.05.097

Slaughter, L. (2021). Rhizosphere. In T. J. Gentry, and D. A. Zuberer, *Principles and* Applications of Soil Microbiology. Amsterdam: Elsevier Inc. pp. 269-301. DOI: 10.1016/B978-0-12-820202-9.00011-3

Sparks, D. L., Page, A. L., Helmke, P. A., Loeppert, R. H., Soltanpour, P. N., Tabatabai, M. A., Johnston, C. T. and Sumner, M. E. (1996). *Methods of soil analysis: Part 3* Chemical Methods, SSSA Book Series 5.3). US: Soil Science Society of America Inc. (SSSA). 1390p DOI: 10.2136/sssabookser5.3

Thom, C. and Raper, K. B. (1945). A manual of the *Aspergilli.* Baltimore, MD: The Williams and Wilkins Company. p 363.

Tomer, A., Sigh, R., Dwivedi, S. A., Reddy, C. U., Keloth, M. R. and Rachel, R. (2021). Role of Fungi in Bioremdiation and Environmental Sustainability. In R. Prasad, S. C. Nayak, R. N. Kharwar, and N. K. Dubey (Eds.), Mycoremediation and Environmental Sustainability, Fungal Biology. Springer, Switzerland. pp. 187-200. http://doi.org/10.1007/978-3-030-54422-5_8

Vijayaraghavan, K., Rajkumar, J., Bukhari, S. N., Al-Sayed, B. and Seyed, M. A. (2017). *Chromolaena odorata*: A neglected weed with a wide spectrum of pharmacological activities (Review). Mol. Med. Rep., 15(3): 1007-1016. https://doi.org/10.3892/mmr.2017.6133

Yamanaka, T. (2003). The effect of pH on the Growth of Saprophytic and Ectomycorrhizal Fungi in vitro. Mycologia, 95(4): 584-589 doi:10.2307/3761934

Zhou, Q., Cai, Z., Zhang, Z. and Liu, W. (2011). Ecological Remediation of Hydrocarbon Contaminated Soils with Weed Plant. J. Resour. Ecol. 2(2): 97-105. https://doi.org/10.3969/j.issn.1674-764x.2011.02.001

Zuzolo, D., Sciarrillo, R., Postiglione, A. and Guarino, C. (2021). The remediation potentials of PAHs of *Verbascum sinuatum* L. combined with an enhanced rhizosphere landscape: A full-scale mesocosm experiment. Biotechnol. Rep. 31, e00657. https://doi.org/10.1016/j.btre.2021.e00657