Role of Microbial Inoculants and Organic Carriers in Enhancing the Biological Response of Grass Pea (Lathyrus sativus L.) under Diesel-Contaminated Soil Conditions

Zainab Adnan Hussein * [1], Faris Mohammed Suhail [2]
[1, 2] Department of Soil Sciences and Water Resources, College of Agriculture, University of Diyala, Diyala, Iraq
[1] Email: zainabadnan@uodiyala.edu.iq
[2] Email: farissuhail@uodiyala.edu.iq

Abstract

This study analyzed how microbial inoculants and organic carriers improve the biological response of grass peas (Lathyrus sativus L.) in soil polluted by gasoil (diesel). A Randomized Complete Block Design (RCBD) factorial field experiment used 24 treatments from interactions of biological treatments (Rhizobium and Bacillus on biochar or cocopeat, and the mycorrhizal fungus Glomus mosseae), two gasoil levels (0 and 1%), and two biochar levels (0 and 1%), in three replications (72 units). Results showed significant effects on most studied traits. The triple interaction among mycorrhizae, gasoil, and biochar yielded highest values in growth traits: plant height (100 cm), shoot dry weight (30.11 g. plant^{-1}), and root dry weight (1.81 g. plant^{-1}). Furthermore, Rhizobium on biochar combined with gasoil and biochar excelled in active root nodules (31.33 nodules plant^{-1}), while Rhizobium on cocopeat with gasoil and biochar recorded highest leaf nitrogen (5.94%). Mycorrhizae demonstrated clear superiority in colonization percentage (averaging 83.08%, increasing to 87.17% with gasoil and 85.83% with biochar). Biochar improved mycorrhizal colonization, whereas gasoil alone had no significant effect on it. Overall, integrating microbial inoculants and organic carriers particularly mycorrhizae and Rhizobium with biochar significantly improved grass pea growth and biological response under gasoil pollution, supporting their potential to enhance biological systems in petroleum-affected soils.

Keywords: Grass Pea, Microbial Inoculants, Biochar, Mycorrhizae, Gasoil Pollution

Introduction

By affecting microbial activity and organic matter dynamics, diesel pollution significantly deteriorates soil quality and capital within the context of soil security, while also endangering the ability of soils to provide critical agricultural and ecological services (1). In particular, the microbiological balance of soil, its physicochemical characteristics, and its general capacity to sustain vital ecosystem activities are severely harmed by petroleum derivatives because of their toxicity, mutagenicity, and capacity to interfere with biological processes (2). As a result, the physicochemical and biological composition of the soil is significantly altered when it is contaminated with these petroleum-derived compounds, which frequently results in a shortage of water and oxygen as well as a noticeable reduction in the amount of nitrogen and phosphorus that are accessible. Over time, natural attenuation processes in soil and water can reduce petroleum pollution; however, these intrinsic mechanisms are frequently slow and inadequate, especially under severe contamination conditions (4, 5) To overcome these limitations, the application of exogenous microbial inoculants can boost

ISSN 2072-3857

total petroleum hydrocarbon (TPH) degradation by over 30% relative to natural processes alone, while simultaneously enriching bacterial diversity and promoting effective soil restoration. Therefore, accelerated bioremediation serves as an indispensable tool for prompt environmental management to mitigate further ecological harm and restore soil functionality (6, 7) Similarly, previous investigations into petroleum-polluted soils have demonstrated that applying biological remediation agents significantly accelerates the proliferation of hydrocarbon-degrading microbial communities. These studies highlight that biological amendments provide long-term efficacy in restoring microbial diversity and enhancing the overall biological response of contaminated soils (8) A study on diesel-contaminated soil remediation showed that bioaugmentation achieved the highest TPH degradation efficiencies of 72.7% (light fractions) and 75.2% (heavy fractions), with natural attenuation outperforming biostimulation. Furthermore, dehydrogenase activity increased by 3.3 to 4.0-fold, leading to the conclusion that the most effective remediation approach relies on inoculating pre-selected indigenous microorganisms originating directly from the site (9)

A study evaluating the remediation of soils co-contaminated with heavy metals and diesel oil demonstrated the role of using a tailored native microbial formula (comprising ten heavy metal-resistant strains) in accelerating bioremediation. Over 42 days, the treatment achieved complete degradation of n-C_{12-20, total disappearance of phenanthrene, a 60% reduction in isoprenoids, and an overall diesel hydrocarbon reduction of approximately 75%, alongside abated ecotoxicity and increased microbial activity (10)

A comprehensive review study examined bioremediation strategies for petroleum hydrocarbon-contaminated soils, detailing biodegradation, biostimulation, bioaugmentation, plant-assisted remediation, and nano-remediation approaches. The findings concluded that maximizing remediation efficiency and sustainability depends fundamentally on understanding molecular metabolic pathways and optimizing key environmental variables such as soil composition, moisture, and temperature(11)

A review study examined petroleum hydrocarbon soil contamination and evaluated the application of microbial remediation and integrated strategies. The findings concluded that standalone microbial treatments face performance limitations, highlighting the superior efficacy of combined approaches such as microbe-biochar, microbe-nutrition, and microbe-plant technologies. The study emphasized that optimizing remediation success fundamentally relies on understanding pollutant sources, metabolic pathways and genes, and controlling key environmental factors (12).

A study on microbial inoculants derived from organic waste compost for remediating hydrocarbon-contaminated soils demonstrated their strong ability to stimulate biological activity. The findings concluded that these inoculants successfully removed over 99% of diesel and gasoline pollutants in clay soils, establishing them as an effective and reliable option for advancing bioremediation technologies (13).

A review study examining key remediation methods for petroleum-contaminated soils emphasized that selecting optimal treatments depends on pollutant type and soil characteristics, prioritizing eco-friendly, cost-effective, and efficient methods where in-situ treatments are generally more

ISSN 2072-3857

economical, and contaminant removal is favored over containment (14). In this context, evaluating the phytoremediation of diesel-contaminated soil using grass pea (Lathyrus sativus) showed that maximum diesel removal was achieved using treated wastewater, reaching 44% in 2018 and 41% in 2019, while plant growth decreased with higher concentrations, and removal rates positively correlated with plant height and shoot dry weight (15). Furthermore, a study demonstrated the efficacy of phytoremediation in removing TPH, achieving a maximum removal efficiency of 50.66% at 50,000 ppm (16). To enhance these processes, another study evaluated the role of Rhizobia and Bacillus inoculants with grass pea, showing that Rhizobia isolates significantly increased shoot absorption by 230.12% and achieved the highest diesel degradation in soil—a 58.33% increase—confirming the high efficacy of bacterial inoculants in enhancing bioremediation (17). These findings are complemented by research indicating that microbial inoculants serve as vital biological catalysts to optimize organic soil amendments, offering a sustainable approach for agricultural waste recycling and the restoration of degraded soils (18), particularly highlighting the crucial role of Rhizobium as a sustainable alternative to chemical fertilizers, alongside the necessity of developing effective, low-cost carrier materials to protect inoculants from desiccation and ensure their efficiency under abiotic stress conditions (19). Despite the documented potential of bioremediation and plant-based strategies in addressing petroleum pollution, the synergistic interaction between microbial inoculants and organic carriers—particularly under diesel stress conditions—remains underexplored. Therefore, this study aims to evaluate the role of microbial inoculants and organic carriers in enhancing the biological response of grass pea (Lathyrus sativus L.) under diesel-contaminated soil conditions, and to demonstrate the efficiency of their interaction in improving plant response and adaptability to pollution conditions.

Materials and Methods

Soil samples collection

Four root samples of leguminous plants (broad bean, grass pea, cowpea) were collected from various locations across Diyala Governorate for the purpose of isolating Rhizobium bacteria. Additionally, three petroleum derivative-contaminated soil samples were collected from different sites within the governorate to isolate Bacillus bacteria. Table 1 illustrates the sample numbers, their sources, and the names of the isolated bacteria. Soil sampling was conducted randomly from multiple sites and then thoroughly mixing them to form a representative sample for the study area. The samples were placed in sterilized plastic bags and stored in a refrigerator until use.

Table (1): Sample sources, collection locations, and names of isolated bacteria.

No.	Collection Site	Bacteria	Sample Source
1	Diyala University	*Rhizobium* spp.	Faba bean root nodules
2	Diyala University	*Rhizobium* spp.	Grass pea root nodules
3	Jadidat Al-	*Rhizobium*	Cowpea root nodules

ISSN 2072-3857

	Shatt	spp.	
4	Muqdadiya	*Rhizobium* spp.	Faba bean root nodules
5	Khanaqin	*Bacillus* spp.	Soil contaminated with petroleum derivatives (Generator site)
6	Muqdadiya	*Bacillus* spp.	Soil contaminated with petroleum derivatives (Fuel station site)
7	Diyala University	*Bacillus* spp.	Soil contaminated with petroleum derivatives (Generator site)

Studied Traits and Measurements

Plant Measurements

1. Plant Height (cm): The average was taken from six plants.
2. Shoot Dry Weight (g. plant $^{-1}$): The dried parts were placed in thermal bags, then dried in an electric oven at 60 ◦C until weight stabilization, after which the weight was measured using a sensitive balance.
3. Root Dry Weight (g. plant $^{-1}$): The dried parts were placed in thermal bags, then dried in an electric oven at 60 ◦C until weight stabilization, and weighed using a sensitive balance.
4. Number of Root Nodules (nodules per plant): The roots were carefully uprooted after moistening to count the active root nodules. They were placed on a metal sieve to preserve the root nodules, washed by passing a gentle stream of water to remove soil from the roots, and then counted.
5. Leaf Nitrogen Concentration (%): Plant leaves were collected cleared of dust, dried in an oven at 65 ◦C, and then ground. Plant samples were digested by taking 0.2 g of the sample into a digestion flask, adding 4 ml of concentrated sulfuric acid, and leaving it for 24 hours. Then, 1 ml of perchloric acid was added, and it was placed on a hot plate, continuing heating until a clear color appeared. The digestion product was transferred to a 50 ml volumetric flask, and the volume was made up to the mark with distilled water. Nitrogen content was then measured by adding sodium hydroxide to the extracted plant sample using the Micro-Kjeldahl device, according to the method described by (20).

Biological Soil Traits

Percentage of Mycorrhizal Colonization (%): The percentage of colonization was estimated at the end of the experiment according to the method of (21). Plant roots were carefully uprooted from the soil, rootlets from each experimental unit were taken and placed in Petri dishes, and the roots were washed thoroughly with water. They were then transferred to small 20 ml glass vials containing 10% potassium hydroxide (KOH) solution and placed in a water bath at 90 ◦C for one hour. The KOH solution was decanted through a fine-mesh sieve, and the rootlets were washed with distilled water. Afterwards, 10% alkaline hydrogen peroxide solution was added for one minute to depigment the roots, followed by 10% hydrochloric acid for 3 minutes. The rootlets were then transferred to Petri dishes, and 0.05% Trypan Blue stain in lactophenol solution (consisting of 33 ml lactic acid + 33 ml glycerol + 34 ml distilled water) was added to the roots for 5 minutes to prepare them for microscopic examination using a light microscope with a minimum magnification of 40×. Twenty-five rootlet pieces, each 1 cm in length, were placed on a glass slide. The percentage of colonization was calculated using the equation no. 1:

$$\text{Colonization Percentage (\%)} = \left(\frac{Total\ Number\ of\ Segments}{Number\ of\ Infected\ Segments}\right) \times 100 \quad \ldots\ldots\ldots\ldots\ldots (1)$$

ISSN 2072-3857

Isolation of Rhizobium Bacteria

Rhizobium bacteria were isolated from healthy root nodules collected from broad bean, grass pea, and cowpea plants. The root nodules were separated using a sterilized scalpel, cutting a small portion of the root attached to the nodule. The root nodules were washed several times with tap water to remove adhering soil particles, followed by surface sterilization. They were immersed in a 0.1% mercuric chloride, $HgCl_2$ solution for 5 minutes, then in 95% ethyl alcohol for 3 minutes, and rinsed several times with sterile distilled water to remove all traces of ethyl alcohol. Under sterile conditions, the root nodules were crushed inside a Petri dish using a sterilized glass rod. The suspension was streaked onto the surface of Petri dishes containing Yeast Extract Mannitol Agar (YEMA) medium using the streaking method. The plates were incubated in an incubator at at 28°C ± 2°C for 72 hours, as described by (22).

Characterizations of Rhizobium Bacteria.

1. Cultural Properties of Rhizobium Bacteria.

The cultural properties of Rhizobium bacterial isolates growing on the selective solid medium (Yeast Extract Mannitol Agar; YEMA) were recorded. This included studying colony shape, size, color, margins, elevation, consistency, transparency, and pigment production capacity, based on the morphological characteristics of colonies growing on the nutrient medium according to (22).

2. Microscopic Properties of Rhizobium Bacteria.

Eamination of Rhizobium bacterial isolates was performed after Gram staining to study their microscopic properties. The shape of the bacterial cells, their arrangement pattern, and staining reaction were observed. Slides were examined using a light microscope under an oil immersion lens (100 x). Microscopic characteristics for isolating and identifying the isolates were based on (22).

The Ability of Isolates to Grow in Gasoil

The ability of bacterial isolates to degrade gasoil was tested using Yeast Extract Mannitol Broth (YEMB) supplemented with gasoil as the sole carbon and energy source. Pure bacterial isolates were inoculated into the nutrient medium containing 1% gasoil, and then incubated at 28°C ± 2°C for 5–7 days with continuous agitation or periodic shaking. The capability of the isolates to degrade gasoil was determined by observing bacterial growth and increased turbidity of the medium. Growth (+) indicated the ability of the bacteria to grow in gasoil, whereas (-) indicated no growth (23).

Isolation of Bacillus Bacteria

Dilutions were prepared for each soil sample by adding 10 g of soil to 90 ml of sterile physiological saline solution in 250 ml flasks, and then mixed thoroughly. Serial dilutions from 10^{-1} to 10^{-7} were made by transferring 1 ml of the soil suspension into test tubes containing 9 ml of sterile saline solution. Nutrient Agar solid medium was used to isolate the bacteria, where 1 ml from the final dilutions was inoculated into tubes containing liquid nutrient medium in three replications for each dilution. The tubes were incubated at 28 °C for 2–3 days, and bacterial growth was monitored. Afterward, 0.1 ml from the tubes showing bacterial growth was taken and spread onto Petri dishes containing Nutrient Agar solid medium, then incubated at 28 °C for 2–3 days. Sub-culturing was repeated several times to obtain pure colonies.

Characterizations of Bacillus spp.

1. Cultural Properties of Bacillus spp.

The cultural properties of the bacterial isolates growing on Nutrient Agar (NA) solid medium were recorded, including colony shape, size, color, margins, elevation, consistency, transparency, and pigment

ISSN 2072-3857

production capacity, based on the morphological characteristics of colonies growing on the nutrient medium according to (24).

2. Microscopic Properties of Bacillus spp.

Microscopic examination of the Bacillus bacterial isolates was performed after Gram staining to determine the shape of the bacterial cells, their arrangement pattern, staining reaction, and ability to form endospores. This was conducted using a light microscope under an oil immersion lens (100 x), relying on the diagnostic keys in (24).

The Ability of Bacterial Isolates to Grow in Gasoil

The ability of the bacterial isolates to utilize gasoil as a carbon source was tested by inoculatin g. pure bacterial isolates into Mineral Salt Medium containing 1% gasoil as the sole carbon source. They were then incubated at 28 ± 2°C for 5–7 days. Following the incubation period, the test results were evaluated based on growth in the medium: the appearance of growth was considered positive (+), indicating the bacteria's ability to grow in gasoil as the sole carbon source, while the absence of growth was considered negative (−) (24).

Field Experiment

Experimental Factors

A factorial field experiment was conducted using a Randomized Complete Block Design (RCBD) at the research station of the Department of Soil Sciences and Water Resources, College of Agriculture, University of Diyala. The study aimed to investigate the role of bioremediation using a local isolate of Rhizobium, Bacillus, mycorrhizal fungi, and vegetation in removing gasoil from the soil. The experiment included 24 treatments resulting from the interaction of three factors:

Factor One: Bacterial and fungal inoculation across six groups:

1. Uninoculated (carrier only).
2. Rhizobium bacteria (R3) inoculated on biochar.
3. Rhizobium bacteria (R3) inoculated on cocopeat.
4. Bacillus bacteria (B3) inoculated on biochar.
5. Bacillus bacteria (B3) inoculated on cocopeat.
6. Mycorrhizal fungus (Glomus mosseae).

Factor Two: Gasoil pollution (0, 1%).

Factor Three: Biochar (0, 1%).

Each treatment was replicated three times, making a total of 72 experimental units (6×2×2×3 = 72).

Preparation of Bacterial Inoculants

Rhizobium Bacterial Inoculant

A Rhizobium isolate designated with the symbol (R3) was selected for use in the field experiment due to its high efficiency in growing on gasoil-containing media. The isolate was grown on Yeast Extract Mannitol Broth (YEMB) and incubated at 28 ± 2°C for 3–5 days to obtain dense bacterial growth. Subsequently, the bacterial inoculant was mixed separately with the two sterilized carriers (biochar and cocopeat) at a 1:1 (volume/weight) ratio to obtain a solid bio-inoculant. The mixture was left for two hours to ensure inoculant homogeneity and distribution of bacterial cells within the carrier. For seed inoculation, a 10% Arabic gum solution was used as an adhesive material. Seeds were moistened with the solution, then the bacterial inoculant was added and thoroughly mixed to ensure the inoculant adhered to the seed surface, and left to dry in the shade immediately before planting. The density of the bacterial inoculant used was $6.1x10^{9}$, CFU ml^{-1}.

Bacillus Bacterial Inoculant

An isolate of Bacillus spp. designated with the symbol (B3) was selected for the field experiment due to its efficiency in growing

ISSN 2072-3857

on gasoil-containing media. The isolate was grown on Nutrient Broth and incubated at 28 ± 2°C for 24–48 hours to obtain dense bacterial growth. Afterward, the bacterial inoculant was mixed separately with the two sterilized carriers (biochar and cocopeat) at a 1:1 (volume/weight) ratio to obtain a solid bio-inoculant. The mixture was left for two hours to ensure inoculant homogeneity and distribution of bacterial cells within the carrier. For seed inoculation, a 10% Arabic gum solution was used as an adhesive material. Seeds were moistened with the solution, then the bacterial inoculant was added and thoroughly mixed to ensure adherence to the seed surface, and left to dry in the shade immediately before planting. The density of the bacterial inoculant used was $5.4x10^9$, CFU ml^{-1} (25, 26).

Mycorrhizal Inoculant

Mycorrhizal inoculant of the species Glomus mosseae was obtained from the Ministry of Science and Technology / Directorate of Agricultural Research. The inoculant was in the form of a mixture of soil and infected roots with a density of 50 spores. g^{-1} of inoculant. The inoculant was added at a rate of 50 g. per planting row in the form of a cushion supporting the seeds to ensure direct contact between the inoculant and the roots and to enhance the efficiency of fungal colonization.

Addition of Biochar to the Field

Biochar (pomegranate wood) was added to the field soil prior to planting according to the treatment levels: non-addition (0%) and addition (1%). The first level was used without biochar supplementation, while the second level was added at a rate of 6 k g. per experimental unit (plot), and then thoroughly mixed with the surface layer of the soil using a hand hoe to ensure uniform distribution prior to planting.

Soil Contamination with Gasoil

Gasoil was obtained from the fuel station affiliated with the General Company for Oil Products in Diyala Governorate. It was stored in airtight, opaque plastic bottles and kept at room temperature until use. Table (1) illustrates some of the physical and chemical properties of the gasoil used in the study. The soil of the plots was contaminated on November 18, 2025 —two days prior to planting—using gasoil at concentrations of 0% and 1%, calculated based on the ratio of gasoil volume to dry soil weight according to the experimental treatments. The quantity of gasoil added to each experimental unit (plot) was calculated based on its soil weight, adding an amount of 6 liters per plot, noting that the area of each plot was 3 m^2. Gasoil was added manually with thorough soil mixing to ensure complete homogeneity between the soil and the pollutant within each experimental unit. The same addition procedure was followed for the biochar used in the experiment.

Execution of the Experiment

The soil site designated for cultivation was prepared by plowing, smoothing, and leveling. The land was divided into three blocks, with each block containing 24 experimental units. The length of each experimental unit was 1.5 m and its width was 2 m. Each experimental unit contained three rows; the distance between adjacent rows was 40 cm, the distance between plants within a row was 25 cm, and the number of seedlings per experimental unit was 21 plants. The distance between blocks was 1 m, and the distance between adjacent experimental units was 0.5 m. A drip irrigation system was installed by placing the main pipe along the side of the experimental field, from which sub-main pipes of 16 mm diameter and 30 m length (GR tape type) were distributed. Soil samples were randomly collected from various representative sites across the field

ISSN 2072-3857

soil at a depth of 0–30 cm, air-dried, crushed with a wooden mallet, and passed through a 2 mm sieve to conduct physical and chemical analyses. Table (2) shows the chemical and physical properties prior to planting. The plants were harvested on March 29, 2026.

Table (2): Chemical, Physical, and Biological Properties of the Soil Prior to the Study

Properties		Unit	Value
Electrical Conductivity		dSm^{-1}	7.35
pH (Reaction Degree)		-	7.62
Organic Matter		$g\ kg^{-1}$	1.85
Soluble Magnesium		$mmol\ L^{-1}$	4.25
Soluble Calcium			27.4
Available Nitrogen		$mg\ kg^{-1}$	28.15
Available Phosphorus			14.2
Available Potassium			215
Texture			Clay Loam
Sand	Soil Separates	$g\ kg^{-1}$	230
Silt			390
Clay			380
Total Bacteria		C.F.U g^{-1} dry soil	35×10^6
Phosphorus-Solubilizing Bacteria			10×10^6
Nitrogen-Fixing Bacteria			12×10^6

Results and Discussion

Effect of Biological Treatments, Mycorrhizal Fungi, Gasoil Levels, and Biochar on Plant Height.

The results in Table (3) indicate that the addition of biological treatments led to a significant increase in plant height compared to non-addition. However, the mycorrhizal fungus treatment recorded the highest mean plant height of 87.83 cm compared to the control treatment, which gave the lowest mean of 69.16 cm, with an increase rate of 26.99%. This was followed by the Rhizobium treatment inoculated on cocopeat with a mean of 85.16 cm.

The addition of gasoil had a significant effect on plant height, recording the highest mean plant height of 86.50 cm compared to non-addition, which gave 75.39 cm, with an increase rate of 14.74%. Similarly, the addition of biochar significantly affected plant height, giving the highest mean height of 89.78 cm compared to non-addition, which reached 72.11 cm, with an increase of 24.49%. The interaction between biological treatments and gasoil levels had a significant effect on plant height; the addition of biological treatments led to a significant increase in plant height both with and without gasoil addition compared to non-addition. However, the mycorrhizal fungi treatment with gasoil addition recorded the highest plant height of 94.50 cm compared to the control, which recorded the lowest mean of 69.00 cm, with a significant increase of 36.95%. This was followed by the Rhizobium treatment inoculated on cocopeat with gasoil addition, reaching

ISSN 2072-3857

91.00 cm. The two-way interaction between biological treatments and biochar levels significantly affected plant height; the addition of biological treatments with and without biochar addition led to a significant increase in plant height compared to non-addition. However, the mycorrhiza treatment with biochar addition recorded the highest mean of 95.50 cm compared to the control treatment, which gave 53.50 cm, with a significant increase of 78.5%.

The two-way interaction between gasoil levels and biochar significantly affected plant height, as the addition of gasoil with biochar addition recorded the highest mean plant height of 93.44 cm compared to the absence of gasoil and biochar, which was 64.67 cm, with a significant increase of 44.51%.

The triple interaction between biological treatments, gasoil levels, and biochar showed a significant effect on plant height. The addition of biological treatments led to a significant increase in plant height with and without the addition of gasoil and biochar. The addition of gasoil led to a significant increase in plant height with and without biochar addition for all biological treatments. However, the mycorrhiza treatment with gasoil and biochar addition recorded the highest plant height of 100.00 cm, compared to the control treatment with gasoil addition and no biochar addition, which recorded 56.33 cm (an increase of 77.52%). Meanwhile, the treatment combining no biological treatment with gasoil addition and no biochar addition recorded the lowest mean of 50.67 cm.

Table (3): Effect of Biological Treatments, Gasoil Levels, and Biochar on Plant Height (cm)

Biological Treatments	Gasoil	Biochar 0%	Biochar 1%	Bio. x Gasoil Mean
Control (Carrier only)	0%	56.33 n	81.67 i	69.00 h
	1%	50.67 o	88.00 ef	69.33 h
Rhizobia on Biochar	0%	63.33 m	87.00 f	75.17 f
	1%	84.33 gh	91.67 d	88.00 c
Rhizobia on Cocopeat	0%	69.33 k	89.33 e	79.33 e
	1%	87.00 f	95.00 b	91.00 b
Bacillus on Biochar	0%	62.00 m	82.67 hi	72.33 g
	1%	82.67 hi	92.33 cd	87.50 c
Bacillus on Cocopeat	0%	65.67 l	85.00 g	75.33 f
	1%	83.67 gh	93.67 bc	88.67 c
Mycorrhiza fungi	0%	71.33 j	91.00 d	81.17 d
	1%	89.00 e	100.00 a	94.50 a
Means of bio-treatments and biochar				Biological Treatments Means
		53.50 j	84.83 e	69.17 E
		73.83 h	89.33 c	81.58 C
		78.17 g	92.17 b	85.17 B
		72.33 i	87.50 d	79.92 D
		74.67 h	89.33 c	82.00 C
		80.17 f	95.50 a	87.83 A
Means of biochar and diesel oil				Means of diesel oil

ISSN 2072-3857

Gasoil Levels	0%	64.67 d	86.11 b	75.39 B
	1%	79.56 c	93.44 a	86.50 A
Biochar Average	-	72.11 b	89.78 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effect of Biological Treatments, Gasoil Levels, and Biochar on Shoot Dry Weight.

From the results in Table (4), it is evident that the addition of biological treatments led to a significant increase in shoot dry weight compared to non-addition. However, the mycorrhizal treatment recorded the highest mean of 26.63 g. plant $^{-1}$ compared to the control treatment, which recorded 22.86 g. plant $^{-1}$, with a significant increase rate of 16.46 % .The addition of gasoil significantly affected shoot dry weight, recording the highest mean of 26.24 g. plant $^{-1}$ compared to non-addition, which was 24.02 g. plant $^{-1}$, with a significant increase of 9.25 % .The addition of biochar significantly outperformed in shoot dry weight, recording the highest mean of 27.11 g. plant $^{-1}$ compared to non-addition, which was 23.15 g. plant $^{-1}$, with an increase rate of 17.12 % .The interaction between biological treatments and gasoil levels significantly affected shoot dry weight. The addition of biological treatments led to a significant increase in dry weight with and without gasoil addition compared to non-addition. However, the mycorrhizal treatment with gasoil addition recorded the highest mean of 27.89 g. plant $^{-1}$ compared to the control treatment, which recorded 22.58 g. plant $^{-1}$, with an increase of 23.50 % .The two-way interaction between biological treatments and biochar levels significantly affected shoot dry weight. The addition of biological treatments led to a significant increase in dry weight with and without biochar addition compared to non-addition. The addition of biochar led to a significant increase in dry weight compared to non-addition across all biological treatments. However, the mycorrhizal treatment with biochar recorded the highest mean of 28.81 g. plant $^{-1}$ compared to the control treatment, which recorded 20.20 g. plant $^{-1}$, with an increase of 42.62 % .The two-way interaction between gasoil levels and biochar showed a significant effect on shoot dry weight, as the addition of the gasoil treatment with biochar recorded the highest mean of 28.40 g. plant $^{-1}$ compared to the control treatment, which recorded 22.21 g. plant $^{-1}$, with an increase of 27.87 % .The triple interaction between biological treatments, gasoil levels, and biochar showed a significant effect on shoot dry weight. The addition of biological treatments with and without gasoil and biochar led to a significant increase in dry weight compared to non-addition. The addition of gasoil led to a significant increase in dry weight with and without biochar addition for all biological treatments. However, the mycorrhizal treatment with the addition of gasoil and biochar recorded the highest mean of 30.11 g. plant $^{-1}$, compared to the control treatment, which recorded 20.94 g. plant $^{-1}$, with a significant increase of 43.79 % . Meanwhile, the treatment combining no biological treatment with gasoil addition and no biochar addition recorded the lowest mean of 19.45 g. plant $^{-1}$.

ISSN 2072-3857

Table (4): Effect of Biological Treatments, Gasoil Levels, and Biochar on Shoot Dry Weight (g. plant $^{-1}$)

Biological Treatments	Gasoil	Biochar 0%	Biochar 1%	Bio. x Gasoil Mean
Control (Carrier only)	0%	20.94 r	24.22 m	22.58 i
	1%	19.45 s	26.83 g	23.14 h
Rhizobia on Biochar	0%	21.78 q	25.56 j	23.67 g
	1%	24.50 l	28.06 d	26.28 d
Rhizobia on Cocopeat	0%	22.89 o	25.83 i	24.36 f
	1%	25.06 k	29.11 b	27.09 b
Bacillus on Biochar	0%	21.89 q	25.67 ij	23.78 g
	1%	24.72 l	27.78 e	26.25 d
Bacillus on Cocopeat	0%	22.56 p	26.17 h	24.36 f
	1%	25.11 k	28.50 c	26.81 c
Mycorrhiza fungi	0%	23.22 n	27.50 f	25.36 e
	1%	25.67 ij	30.11 a	27.89 a
Means of bio-treatments and biochar				Biological Treatments Means
		20.20 h	25.53 d	22.86 E
		23.14 g	26.81 c	24.97 D
		23.97 f	27.47 b	25.72 B
		23.31 g	26.72 c	25.01 D
		23.84 f	27.33 b	25.58 C
		24.45 e	28.81 a	26.63 A
Means of biochar and diesel oil				Means of diesel oil
Gasoil Levels	0%	22.21 d	25.82 b	24.02 B
	1%	24.08 c	28.40 a	26.24 A
Biochar Average	-	23.15 b	27.11 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effect of Biological Treatments, Gasoil Levels, and Biochar on Root Dry Weight.

The results in Table (5) showed that the addition of biological treatments led to a significant increase in root dry weight compared to non-addition. The mycorrhizal treatment recorded the highest mean of 1.52 g. plant $^{-1}$ compared to the control treatment, which recorded 1.25 g. plant $^{-1}$, with an increase of 21.60 % .The addition of gasoil significantly affected root dry weight, as the 1% level recorded the highest mean of 1.49 g. plant $^{-1}$ compared to the 0% level, which was 1.33 g. plant $^{-1}$, with an increase of 12.03 % .The addition of biochar significantly affected root dry weight, recording the highest mean of 1.51 g. plant $^{-1}$ compared to non-addition, which was 1.31 g. plant $^{-1}$, with an increase of 15.27 % .The interaction between biological treatments and gasoil levels significantly affected root dry weight. The addition of biological treatments led to a significant increase in root dry weight with and without gasoil addition compared to non-addition. However, the mycorrhizal treatment with gasoil recorded the highest mean of 1.62 g.

ISSN 2072-3857

plant $^{-1}$ compared to the control treatment, which recorded 1.19 g. plant $^{-1}$, with an increase of 36.13 % .The two-way interaction between biological treatments and biochar levels significantly affected root dry weight. The addition of biological treatments led to a significant increase in root dry weight with and without biochar addition compared to non-addition. The addition of biochar led to a significant increase in dry weight compared to non-addition across all biological treatments. However, the mycorrhizal treatment with biochar recorded the highest mean of 1.68 g. plant $^{-1}$ compared to the control treatment, which recorded 1.19 g. plant $^{-1}$, with an increase of 41.18 % .The two-way interaction between gasoil levels and biochar showed a significant effect on root dry weight, as the addition of gasoil with biochar recorded the highest mean of 1.63 g. plant $^{-1}$ compared to the control treatment, which recorded 1.25 g. plant $^{-1}$, with an increase of 30.40 % .The triple interaction between biological treatments, gasoil levels, and biochar showed a significant effect on root dry weight. The addition of biological treatments with and without gasoil and biochar led to a significant increase in root dry weight compared to non-addition. The addition of gasoil led to a significant increase in root dry weight with and without biochar addition for all biological treatments. However, the mycorrhizal treatment with the addition of gasoil and biochar recorded the highest mean root dry weight of 1.81 g, compared to the control treatment, which recorded 1.21 g, with a significant increase of 49.58 %. Meanwhile, the treatment combining no biological treatments with gasoil and no biochar recorded the lowest mean root dry weight of 1.16 g. plants $^{-1}$.

Table (5): Effect of Biological Treatments, Gasoil Levels, and Biochar on Root Dry Weight (g. plant $^{-1}$)

Biological Treatments	Gasoil	Biochar 0%	Biochar 1%	Bio. x Gasoil Mean
Control (Carrier only)	0%	1.21 kl	1.18 lm	1.19 h
	1%	1.16 m	1.44 fg	1.30 g
Rhizobia on Biochar	0%	1.23 k	1.38 hi	1.30 g
	1%	1.36 i	1.60 d	1.48 d
Rhizobia on Cocopeat	0%	1.29 j	1.46 f	1.38 f
	1%	1.42 g	1.70 b	1.56 b
Bacillus on Biochar	0%	1.23 k	1.38 hi	1.30 g
	1%	1.36 i	1.56 de	1.46 d
Bacillus on Cocopeat	0%	1.27 j	1.47 f	1.37 f
	1%	1.41 gh	1.66 c	1.53 c
Mycorrhiza fungi	0%	1.30 j	1.55 e	1.43 e
	1%	1.43 fg	1.81 a	1.62 a
Means of bio-treatments and biochar				Biological Treatments Means
		1.19 g	1.31 f	1.25 D
		1.29 f	1.49 c	1.39 C
		1.36 de	1.58 b	1.47 B
		1.29 f	1.47 c	1.38 C
		1.34 e	1.56 b	1.45 B

ISSN 2072-3857

		1.37 d	1.68 a	1.52 A
Means of biochar and diesel oil				Means of diesel oil
Gasoil Levels	0%	1.25 d	1.40 b	1.33 B
	1%	1.36 c	1.63 a	1.49 A
Biochar Average	-	1.31 b	1.51 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

Effect of Biological Treatments, Gasoil Levels, and Biochar on the Number of Active Nodules.

The results in Table (6) indicated that the addition of biological treatments led to a significant increase in the number of active nodules compared to the control treatment. However, the Rhizobium treatment inoculated on biochar recorded the highest mean of 19.67 nodules plant $^{-1}$ compared to the control treatment, which recorded 7.42 nodules plant $^{-1}$, with an increase of 165.09 % . This was followed by the Rhizobium treatment inoculated on cocopeat with a mean of 17.08 nodules plant $^{-1}$ .The addition of gasoil led to a significant increase in the number of active nodules, as the 1% level recorded the highest mean of 17.50 nodules plant $^{-1}$ compared to non-addition, which recorded 11.31 nodules plant $^{-1}$, with an increase of 54.73 % .The addition of biochar significantly affected the number of active nodules, as the 1% level recorded the highest mean of 18.50 nodules plant $^{-1}$ compared to non-addition, which recorded 10.31 nodules plant $^{-1}$, with an increase of 79.44 % .The two-way interaction between biological treatments and gasoil significantly affected the number of active nodules; the addition of biological treatments with and without gasoil led to a significant increase in the number of active nodules compared to non-addition. However, the Rhizobium treatment inoculated on biochar with gasoil addition recorded the highest mean of 24.83 nodules plant $^{-1}$ compared to the control treatment, which recorded 7.00 nodules plant $^{-1}$, with an increase of 254.71 % , followed by the Rhizobium treatment inoculated on cocopeat with gasoil addition with a mean of 21.00 nodules plant $^{-1}$ .The two-way interaction between biological treatments and biochar significantly affected the number of active nodules, as the addition of biological treatments with and without biochar led to a significant increase in the number of active nodules compared to non-addition. The addition of biochar led to a significant increase in the number of nodules compared to non-addition across all biological treatments. However, the Rhizobium treatment inoculated on biochar with biochar addition recorded the highest mean of 25.33 nodules plant $^{-1}$ compared to the control treatment, which recorded 3.83 nodules plant $^{-1}$, followed by the Rhizobium treatment inoculated on cocopeat with biochar addition with a mean of 21.00 nodules plant $^{-1}$ .The two-way interaction between gasoil and biochar had a significant effect on the number of active nodules, as the addition of gasoil with and without biochar led to a significant increase in the number of active nodules compared to non-addition. The combination of gasoil addition and biochar addition recorded the highest mean of 22.17 nodules plant $^{-1}$ compared to the control treatment, which recorded 7.78 nodules plant $^{-1}$, with an increase of 184.96 % .The triple interaction between biological treatments, gasoil, and biochar showed a clear and significant effect on the

ISSN 2072-3857

number of active nodules. The addition of biological treatments with and without gasoil and biochar led to a significant increase in the number of root nodules compared to non-addition. The addition of biochar with and without gasoil led to a significant increase in the number of active nodules compared to non-addition. However, the Rhizobium treatment inoculated on biochar with the addition of gasoil and biochar recorded the highest mean of 31.33 nodules pear plant compared to the control treatment, which gave 5.33 nodules plant $^{-1}$. Meanwhile, the treatment combining no biological treatments with gasoil addition and no biochar addition recorded the lowest mean of 2.33 nodules plant $^{-1}$.

Table (6): Effect of Biological Treatments, Gasoil Levels, and Biochar on the Number of Active Nodules (nodules plant $^{-1}$)

Biological Treatments	Gasoil	Biochar 0%	Biochar 1%	Bio. x Gasoil Mean
Control (Carrier only)	0%	5.33 m	8.67 k	7.00 i
	1%	2.33 n	13.33 ij	7.83 i
Rhizobia on Biochar	0%	9.67 k	19.33 ef	14.50 e
	1%	18.33 f	31.33 a	24.83 a
Rhizobia on Cocopeat	0%	9.33 k	17.00 g	13.17 f
	1%	17.00 g	25.00 b	21.00 b
Bacillus on Biochar	0%	7.00 l	14.33 hi	10.67 h
	1%	12.67 j	20.67 d	16.67 d
Bacillus on Cocopeat	0%	8.33 k	15.33 h	11.83 g
	1%	14.33 hi	23.00 c	18.67 c
Mycorrhiza fungi	0%	7.00 l	14.33 hi	10.67 h
	1%	12.33 j	19.67 de	16.00 d
Means of bio-treatments and biochar				Biological Treatments Means
		3.83 h	11.00 f	7.42 E
		14.00 e	25.33 a	19.67 A
		13.17 e	21.00 b	17.08 B
		9.83 g	17.50 d	13.67 D
		11.33 f	19.17 c	15.25 C
		9.67 g	17.00 d	13.33 D
Means of biochar and diesel oil				Means of diesel oil
Gasoil Levels	0%	7.78 d	14.83 b	11.31 B
	1%	12.83 c	22.17 a	17.50 A
Biochar Average	-	10.31 b	18.50 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

ISSN 2072-3857

Effect of Biological Treatments, Gasoil Levels, and Biochar on Leaf Nitrogen Concentration.

The results in Table (7) indicated that the addition of biological treatments significantly affected leaf nitrogen concentration. The addition of biological treatments led to a significant increase in leaf nitrogen concentration compared to non-addition. However, the mycorrhizal treatment recorded the highest mean of 4.37% compared to the control treatment, which recorded the lowest mean of 2.42%, with no significant difference from the Rhizobium treatment inoculated on cocopeat, which recorded 4.34%.

The addition of gasoil led to a significant increase in leaf nitrogen concentration compared to non-addition, recording the highest mean of 4.43% compared to the non-addition treatment, which was 3.37%.

The addition of biochar led to a significant increase in nitrogen concentration compared to non-addition, recording the highest mean of 4.49% compared to non-addition, which recorded 3.31%.

The interaction between biological treatments and gasoil levels significantly affected leaf nitrogen concentration. The addition of biological treatments led to a significant increase in nitrogen concentration compared to non-addition. However, the Rhizobium treatment inoculated on cocopeat with gasoil addition recorded the highest mean of 5.08%, compared to the control treatment, which recorded the lowest mean of 2.20%.

The interaction between biological treatments and biochar levels significantly affected nitrogen concentration. The addition of biological treatments with and without biochar led to a significant increase in nitrogen concentration compared to non-addition. Biochar addition led to a significant increase in leaf concentration compared to its absence across all biological treatments. However, the mycorrhiza treatment with biochar recorded the highest mean of 5.06% compared to the control treatment, which recorded 1.69%.

The interaction between biochar levels and gasoil significantly affected the nitrogen content in the plant, as the treatment combining biochar addition with gasoil recorded the highest mean of 5.16%, compared to the control treatment, which recorded the lowest mean of 2.92%.

The triple interaction between biological treatments, gasoil levels, and biochar showed a significant effect on nitrogen content. The addition of biological treatments with and without biochar and gasoil led to a significant increase in nitrogen concentration compared to non-addition. However, the Rhizobium treatment inoculated on cocopeat with the addition of gasoil and biochar recorded the highest mean of 5.94%, compared to the control treatment, which recorded 2.01%.

Table (7): Effect of Biological Treatments, Gasoil Levels, and Biochar on Leaf Nitrogen Concentration (%)

Biological Treatments	Gasoil	Biochar 0%	Biochar 1%	Bio. x Gasoil Mean
Control (Carrier only)	0%	2.01 k	2.39 j	2.20 f
	1%	1.37 l	3.90 g	2.63 e
Rhizobia on Biochar	0%	3.11 h	4.00 f	3.56 d
	1%	4.14 e	5.09 b	4.61 b
Rhizobia on Cocopeat	0%	3.17 h	4.03 f	3.60 d
	1%	4.22 d	5.94 a	5.08 a

ISSN 2072-3857

Bacillus on Biochar	0%	3.01 i	4.13 e	3.57 d
	1%	4.13 e	5.01 c	4.57 b
Bacillus on Cocopeat	0%	3.07 i	4.18 d	3.63 c
	1%	4.17 d	5.06 b	4.62 b
Mycorrhiza fungi	0%	3.15 h	4.19 d	3.67 c
	1%	4.20 d	5.93 a	5.07 a
Means of bio-treatments and biochar				Biological Treatments Means
		1.69 i	3.15 h	2.42 D
		3.62 f	4.55 d	4.08 C
		3.69 e	4.98 b	4.34 A
		3.57 g	4.57 d	4.07 C
		3.62 f	4.62 c	4.12 B
		3.67 e	5.06 a	4.37 A
Means of biochar and diesel oil				Means of diesel oil
Gasoil Levels	0%	2.92 d	3.82 b	3.37 B
	1%	3.70 c	5.16 a	4.43 A
Biochar Average	-	3.31 b	4.49 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

The results across Tables (3, 4, 5, 6, and 7) demonstrated that biological treatments significantly enhanced plant height, shoot and root dry weights, active nodule counts, and leaf nitrogen concentration. These improvements stem primarily from the pivotal role of biological inoculants in optimizing the rhizosphere microenvironment, enhancing nutrient availability, and boosting absorption efficiency. Specifically, Rhizobium inoculation contributes to atmospheric nitrogen fixation and the synthesis of plant growth regulators that stimulate root development and biomass accumulation, whereas Bacillus strains facilitate phosphorus solubilization and secrete organic acids, enzymes, and growth-promoting metabolites to boost vegetative and root growth (27).

The superiority of mycorrhizal fungi across most growth parameters is attributed to their extensive extraradical hyphal networks, which expand the absorption surface beyond the depletion zone, thereby heightening water, phosphorus, and immobile nutrient uptake, enhancing plant physiological status, and driving dry matter accumulation and tissue nitrogen enrichment (28). Conversely, Rhizobium excelled in active nodule formation due to its specific symbiotic capability with legume roots, where it initiates nodulation and activates nitrogenase-mediated nitrogen fixation, thereby upgrading plant nutritional status and nitrogen utilization efficiency (29).

Furthermore, the addition of gasoil significantly improved vegetative and root traits compared to the unpolluted control. This outcome indicates that the applied gasoil concentration remained below the threshold of severe growth inhibition, allowing plants to maintain physiological activity and adapt to mild pollutant stress. Low hydrocarbon levels may trigger

ISSN 2072-3857

adaptive mechanisms in plants, enhancing root proliferation and stress tolerance to increase overall biomass in both shoot and root systems (30). Additionally, the gradual partial degradation of gasoil attenuates its toxicity over time and optimizes the root-zone environment, facilitating water and nutrient uptake—particularly nitrogen, as reflected by its elevated foliar concentration—and promoting nodulation through enhanced root vigor, whereas higher petroleum concentrations typically disrupt plant physiological processes and stunt growth (31).

Biochar application similarly led to significant increases in vegetative traits by improving soil structural stability and aggregate formation, which promotes root penetration and nutrient acquisition efficiency (32). Biochar also elevates the soil's cation exchange capacity (CEC), enhances nutrient retention, and adsorbs organic contaminants to alleviate root-zone toxicity, thereby preserving physiological efficiency and stimulating plant growth (33).

Finally, factor interactions significantly boosted plant traits, with the triple interaction outperforming all dual combinations. This synergy arises from the functional integration among biological treatments, gasoil, and biochar. Microorganisms not only improved nutrient availability and growth factor synthesis, but also accelerated hydrocarbon biodegradation, mitigating toxic effects. Plants and rhizosphere microbes interact symbiotically via root exudates and hydrocarbon substrates, stimulating pollutant degradation and infection processes (34). This was further supported by Rhizobium isolates supplying essential elements and expanding microbial populations (27), and Bacillus strains fostering a dense, deep root system with hormone-like activity (30). Biochar amplified these benefits by providing a porous refuge for microbial colonization, improving water and nutrient retention, and trapping petroleum fractions to maximize biodegradation efficiency (33). Simultaneously, low-level gasoil served as an organic carbon source for adapted hydrocarbon-degrading communities, boosting metabolic activity and rhizosphere vitality (32). Collectively, these findings confirm that successful bioremediation in gasoil-polluted soils relies not on isolated factors, but on the functional integration of microorganisms, biochar, and adapted hydrocarbon levels, creating a stable root habitat that optimizes soil biological processes and enhances plant performance (31).

Effect of Biological Treatments, Gasoil Levels, and Biochar on Mycorrhizal Colonization Percentage.

The results in Table (8) show that biological treatments significantly affected the percentage of mycorrhizal colonization; the addition of biological treatments led to a significant increase in the colonization percentage compared to non-inoculation. However, the mycorrhizal treatment recorded the highest mean of 83.08 % compared to the control treatment, which recorded 27.67 % , with an increase of 200.25 % .The addition of gasoil did not significantly affect the percentage of mycorrhizal colonization compared to its absence. The addition of biochar significantly affected the percentage of mycorrhizal colonization, recording the highest mean of 52.28 % compared to its absence, which was 48.31 % , with an increase of 8.22 % .The interaction between biological treatments and gasoil showed a significant effect; the addition of biological

ISSN 2072-3857

treatments with and without gasoil led to a significant increase in colonization percentage compared to non-addition. However, the mycorrhizal treatment with gasoil recorded the highest mean of 87.17 % compared to the control treatment, which recorded 26.33 % .The interaction between biological treatments and biochar significantly affected the percentage of mycorrhizal colonization; the addition of biological treatments with and without biochar led to a significant increase in colonization percentage compared to non-addition. However, the mycorrhizal treatment with biochar recorded the highest mean of 85.83 % compared to the control treatment, which recorded 23.50 % , with an increase of 265.23 % .The interaction between gasoil and biochar had a significant effect, as the addition of biochar led to a significant increase in colonization percentage with and without gasoil addition, with no significant differences in colonization percentage when adding gasoil compared to its absence across biochar levels. However, the treatment combining no gasoil addition with biochar addition recorded the highest mean of 52.61 % compared to the control treatment, which recorded 48.11 % , with an increase of 9.35 % .The triple interaction significantly affected the percentage of mycorrhizal colonization, as the addition of biological treatments with and without gasoil and biochar led to a significant increase in colonization percentage compared to non-addition. However, the mycorrhizal treatment with the addition of gasoil and biochar recorded the highest mean of 91.00 % compared to the control treatment, which recorded 26.67 % , while the treatment combining no biological treatments with gasoil addition and no biochar recorded the lowest mean of 20.33 % .

Table (8): Effect of Biological Treatments, Gasoil Levels, and Biochar on Mycorrhizal Colonization Percentage (%)

Biological Treatments	Gasoil	Charcoal levels		Mean of interaction between bio-treatments & gasoil
		0%	1%	
Control (carriers only)	0%	26.67 q	31.33 p	29.00 i
	1%	20.33 r	32.33 p	26.33 j
Rhizobia loaded on charcoal	0%	42.00 m	41.33 mn	41.67 g
	1%	51.33 hi	45.00 l	48.17 f
Rhizobia loaded on cocopeat	0%	54.00 fg	47.67 jk	50.83 e
	1%	54.00 fg	52.00 gh	53.00 d
Bacillus loaded on plant charcoal	0%	39.67 n	54.67 f	47.17 f
	1%	36.33 o	42.00 m	39.17 h
Bacillus loaded on cocopeat	0%	49.00 j	60.00 e	54.50 c
	1%	45.67 kl	49.33 ij	47.50 f

ISSN 2072-3857

Mycorrhiza fungi	0%	77.33 d	80.67 c	79.00 b
	1%	83.33 b	91.00 a	87.17 a
Means of bio-treatments and plant charcoal				Biological Treatments Means
		23.50 j	31.83 i	27.67 E
		46.67 f	43.17 g	44.92 C
		54.00 c	49.83 d	51.92 B
		38.00 h	48.33 e	43.17 D
		47.33 ef	54.67 c	51.00 B
		80.33 b	85.83 a	83.08 A
Means of biochar and diesel oil				Means of diesel oil
Gasoil Levels	0%	48.11 b	52.61 a	50.36 A
	1%	48.50 b	51.94 a	50.22 A
Biochar Average	-	48.31 b	52.28 a	-

Values followed by the same lowercase letters within interactions or uppercase letters for main effects are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$).

The data presented in Table (8) reveal that biological treatments significantly promoted mycorrhizal colonization percentages through the restructuring of the rhizosphere microbiome and the amplification of functional diversity, wherein Mycorrhiza Helper Bacteria (MHB) critically facilitated spore germination and hyphal elongation. Furthermore, non-inhibitory, safe-threshold gasoil concentrations served as a supplementary organic carbon source that stimulated microbial metabolism and accelerated hydrocarbon biotransformation, thereby re-establishing ecological equilibrium within the root zone. Concurrently, biochar functioned as an efficient microbial carrier, providing a protective micro-habitat that sustained cell viability, augmented nutrient availability, and optimized overall soil fertility (35, 36). Ultimately, the robust triple interaction among biological inoculants, gasoil, and biochar engineered an optimal synergistic microenvironment, concurrently maximizing pollutant degradation, nutrient cycling, and plant-fungal symbiosis to achieve a maximum colonization peak of 91.00%.

Conclusion

This study demonstrates that the integrated application of microbial inoculants and organic soil conditioners specifically Glomus mosseae and Rhizobium combined with biochar effectively mitigates the adverse impacts of diesel contamination on grass pea (Lathyrus sativus L.). The synergistic interactions among these factors substantially enhanced plant growth, root nodulation, nitrogen accumulation, and mycorrhizal colonization under stress conditions. Consequently, the integration of these biological treatments with biochar offers a robust, eco-friendly biotechnological strategy for the remediation of petroleum-polluted soils and the

enhancement of agricultural productivity in contaminated environments.

References

1. Styc Q, Evangelista SJ, Watt D, Wensley P, Heuser I, Pachón Maldonado JC, Feeth J, Francos N, Hunakunti A, Jayasekara T, Borrelli P, Field D, Irving D, Minasny B, Moreira FMS, Morgan CLS, Ng W, O'Donoghue T, Román Dobarco M, Sharififar A, Thiene M, Wiese L, Yang JE and McBratney A (2025) Aroura – the soil and soil security think tank. Front. Environ. Sci. 13:1654863. doi: 10.3389/fenvs.2025.1654863
2. Borowik, A., Wyszkowska, J., Zaborowska, M., & Kucharski, J. (2025). Regulation of the Microbiome in Soil Contaminated with Diesel Oil and Gasoline. International Journal of Molecular Sciences, 26(13), 6491. https://doi.org/10.3390/ijms26136491
3. Brejea, R., Boroș, M., Roșca, S., Traian, J. E., Budău, R., Borza, I. M., & Păcurar, I. (2023). Bioremediation of Oil Contaminated Soil and Restoration of Land Historically Polluted with Oil Products in the Agricultural Circuit in the Plain and Western Hills, Romania. Applied Sciences, 13(18), 10245. https://doi.org/10.3390/app131810245
4. Gruiz, K. Natural attenuation in contaminated soil remediation. Eng. Tools Environ. Risk Manag. 2019, 4, 95–201
5. Lv, H.; Su, X.; Wang, Y.; Dai, Z.; Liu, M. Effectiveness and mechanism of natural attenuation at a petroleum-hydrocarbon contaminated site. Chemosphere 2018, 206, 293–301.
6. Sun, S.; Su, Y.; Chen, S.; Cui, W.; Zhao, C.; Liu, Q. Bioremediation of oil-contaminated soil: Exploring the potential of endogenous hydrocarbon degrader Enterobacter sp. SAVR S-1. Appl. Soil Ecol. 2022, 173, 104387
7. Guarino, C.; Spada, V.; Sciarrillo, R. Assessment of three approaches of bioremediation (Natural Attenuation, Landfarming and Bioagumentation—Assistited Landfarming) for a petroleum hydrocarbons contaminated soil. Chemosphere 2017, 170, 10–16.
8. Evans, Fubara & Nkalo, Ukoima & Amachree, Dokuboba & Raimi, Morufu. (2024). Evaluating Bioremediation Strategies on Microbial Diversity in Crude Oil-Contaminated Soil Over Three to Six Months in Port Harcourt, Nigeria.. 10.21203/rs.3.rs-4409893/v1.
9. Bento, Fatima & Camargo, Flavio & Okeke, Benedict & Frankenberger-Júnior, Willian. (2003). Bioremediation of soil contaminated by diesel oil. Brazilian Journal of Microbiology. 34. 65-68. 10.1590/S1517-83822003000500022.
10. Alisi, Chiara & Musella, Rosario & Tasso, Flavia & Ubaldi, Carla & Manzo, Sonia & Cremisini, Carlo & Sprocati, Anna. (2009). Bioremediation of Diesel Oil in a Co-Contaminated Soil by Bioaugmentation with a Microbial Formula Tailored with Native Strains Selected for Heavy Metals Resistance. The Science of the total environment. 407. 3024-32. 10.1016/j.scitotenv.2009.01.011.

ISSN 2072-3857

11. Citation: Kakde P, Sharma J. Microbial Bioremediation of Petroleum Contaminated Soil: Structural Complexity, Degradation Dynamics and Advanced Remediation Techniques. J Pure Appl Microbiol. 2024;18(4):2244-2261. doi: 10.22207/JPAM.18.4.28

12. Sui, X., Wang, X., Li, Y., & Ji, H. (2021). Remediation of Petroleum-Contaminated Soils with Microbial and Microbial Combined Methods: Advances, Mechanisms, and Challenges. Sustainability, 13(16), 9267. https://doi.org/10.3390/su13169267

13. Leal, A. J. , Rodrigues, E. M. , Fernandes, R. D. C. R. , Borges, A. C. , Júlio, A. D. L. , Freitas, F. D. S. and Tótola, M. R. (2018). Microbial Inoculants Development for Bioremediation of Gasoline and Diesel Contaminated Soil. Open Access Library Journal, 5, e04449. doi: http://dx.doi.org/10.4236/oalib.1104449.

14. Ali,I. Mahmood (2024). Remediation Techniques for Soil Contaminated with Petroleum Products: A Review. Iraqi Journal of Oil and Gas Research (IJOGR), 4(2), 25-38. doi: 10.55699/ijogr.2024.0402.1066

15. Shiva Mottaghi, Omid Bahmani, Vahid Atlasi Pak; Phytoremediation of diesel contaminated soil using urban wastewater and its effect on soil concentration and plant growth. Water Supply 1 November 2022; 22 (11): 8104–8119. doi: https://doi.org/10.2166/ws.2022.312

16. Al-Obaidy, A. H., Al-Anbari, R., & Hassan, S. (2018). Phytoremediation of soil polluted with Iraqi crude oil using grass plant. MATEC Web of Conferences, 162, 05019. https://doi.org/10.1051/matecconf/201816205019

17. Mohammed Suhail, F., Adnan Hussein, Z. Rule of Rhizobia and Bacillus in phytoremediation of contaminated soil with diesel oil. J. Saudi Soc. Agric. Sci. 23, 11–16 (2024). https://doi.org/10.1016/j.jssas.2023.08.003

18. Bo X, Liu Y, Zhang H, Su C and Miao Y (2025) Synergistic effects of beneficial microbial inoculants and SMS-amendments on improving soil properties and Pinus seedling growth in degraded soils. Front. Microbiol. 16:1608689. doi: 10.3389/fmicb.2025.1608689

19. Kenday Sivaram, Anithadevi & Abinandan, Sudharsanam & Chen, Chengrong & Kadiyala, Venkateswarlu & Mallavarapu, Megharaj. (2023). Microbial inoculant carriers: Soil health improvement and moisture retention in sustainable agriculture. Advances in Agronomy. 180. 35-91. 10.1016/bs.agron.2023.03.001.

20. Haynes., R. J. (1980). A comparison of two modified Kjeldahl digestion techniques for multi- element plant analysis with conventional wet and dry ashing methods. Communications in Soil Science and Plant Analysis, 11(5), 459–467. https://doi.org/10.1080/00103628009367053

21. Kormanik., P. P.; Bryan, W. C. and Schultz, R. C. 1980 . procedures and equipment for staining Large numbers of Plant root samples for endomycorrhizal assay.Canadian Journal of Microbiology , 26 : 536 – 538

ISSN 2072-3857

22. Beck., D. P.L.A. Materon and F.Afandi. 1993. Practical Rhizobium-legume technology manual. Practical Rhizobium-legume technology manual., 5(19): 44-53.
23. Atlas., R.M. (1981) Microbial Degradation of Petroleum Hydrocarbons: An Environmental Perspective. Microbiological Reviews, 45, 180-209.
24. Holt., J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T. and William, S.T. (1994) Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore, 786-788.
25. Ghazi., Azza. (2017). Potential for Biochar as an Alternate Carrier to Peat Moss for the Preparation of Rhizobia Bio Inoculum. Microbiology Research Journal International. 18. 1-9. 10.9734/MRJI/2017/30828.
26. Li, L., Liu, K., Lan, F., Xiao, W., Wang, B., Zhou, J., Zhang, J., Wen, Y., Wang, X., & Shi, X. (2025). A Study on the Preparation of Multifunctional Bacillus spp. Composite Inoculants and Their Ability to Promote Watermelon Growth. Agronomy, 15(2), 350. https://doi.org/10.3390/agronomy15020350
27. Ali, M. H., Khan, M. I., Naveed, M., & Tanvir, M. A. (2023). Microbe-Assisted Rhizoremediation of Hydrocarbons and Growth Promotion of Chickpea Plants in Petroleum Hydrocarbons-Contaminated Soil. Sustainability, 15(7), 6081. https://doi.org/10.3390/su15076081
28. Rajtor, M., & Piotrowska-Seget, Z. (2016). Prospects for arbuscular mycorrhizal fungi (AMF) to assist in phytoremediation of soil hydrocarbon contaminants. Chemosphere, 162, 105-116. https://doi.org/10.1016/j.chemosphere.2016.07.071
29. Eze, M.O., Thiel, V., Hose, G.C. et al. Bacteria-plant interactions synergistically enhance biodegradation of diesel fuel hydrocarbons. Commun Earth Environ 3, 192 (2022). https://doi.org/10.1038/s43247-022-00526-2
30. Anwar-ul-Haq M, Ibrahim M, Yousaf B, Al-Huqail AA and Ali HM (2022) Role of Bacterial Consortium and Synthetic Surfactants in Promoting the Phytoremediation of Crude Oil-Contaminated Soil Using Brachiaria mutica. Front. Energy Res. 10:874492. doi: 10.3389/fenrg.2022.874492
31. Leilei, Xiang & Harindintwali, Jean Damascene & Wang, Fang & Redmile-Gordon, Marc & Chang, Scott & fu, Yuhao & He 何 超, Chao & Bertrand, Muhoza & Brahushi, Ferdi & Bolan, Nanthi & Jiang, Xin & Ok, Yong Sik & Rinklebe, Jörg & Schaeffer, Andreas & Tiedje, James & Xing, Baoshan. (2022). Integrating Biochar, Bacteria, and Plants for Sustainable Remediation of Soils Contaminated with Organic Pollutants. Environmental Science and Technology. 56. 10.1021/acs.est.2c02976.
32. Li, X., Ma, J., Niu, Y., Ling, T., Xia, X., Zhang, X., & Cheng, H. (2025). Synergistic biochar-microbe interactions enhance petroleum contaminant removal and microbial community succession. Environmental Technology &

ISSN 2072-3857

Innovation, 40, 104465. https://doi.org/10.1016/j.eti.2025.104465

33. Bolan, S., Hou, D., Wang, L., Hale, L., Egamberdieva, D., Tammeorg, P., Li, R., Wang, B., Xu, J., Wang, T., Sun, H., Padhye, L. P., Wang, H., Siddique, K. H. M., Rinklebe, J., Kirkham, M. B., & Bolan, N. (2023). The potential of biochar as a microbial carrier for agricultural and environmental applications. Science of The Total Environment, 886, 163968. https://doi.org/10.1016/j.scitotenv.2023.163968
34. Guan S, Shao X, Liu R, Ge J, Song G, Yang Z. Interactions Between Mycorrhizal Fungi and Mycorrhiza Helper Bacteria: Mechanisms, Ecological Functions, and Potential Applications in Sustainable Agriculture and Ecological Restoration. Sustainability. 2026; 18(11):5643. https://doi.org/10.3390/su18115643
35. Mukherjee, S., Sarkar, B., Aralappanavar, V. K., Mukhopadhyay, R., Basak, B. B., Srivastava, P., Marchut-Mikołajczyk, O., Bhatnagar, A., Semple, K. T., & Bolan, N. (2022). Biochar-microorganism interactions for organic pollutant remediation: Challenges and perspectives. Environmental Pollution, 308, 119609. https://doi.org/10.1016/j.envpol.2022.119609
36. Li, X., Lyu, Q., Han, C. et al. Biochar immobilized microbes for sustainable soil remediation and agriculture enhancement: from lab to farmland. Biochar 8, 107 (2026). https://doi.org/10.1007/s42773-026-00613-z