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Effect of *Bacillus megaterium* and *Pseudomonas aeruginosa* bacteria on the biodegradation of hydrocarbon pollutants and NPK availability in soil

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Abstract

The field experiment was conducted during the spring season of 2025, at Al-Muthanna Governorate, Research Station, College of Agriculture, Al-Muthanna University, to study the effect of adding a bioinotrope of *B. megaterium* and *P. aeruginosa* bacteria, and different levels of black oil, on the growth and yield of maize. The experiment was implemented using a randomized complete block design (RCBD). The first control was the *B. megaterium* and *P. aeruginosa* bioinotrope, as follows: no inotrope (B0), *B. megaterium* bioinotrope (B1), *P. aeruginosa* bioinotrope (B2), and a double crossover of the bacteria (B3). The second control was as follows: no contamination (C0), 2% black oil (C1), 3% black oil (C2), and 4% black oil (C3). The results showed that the treatment with the addition of the bacterial bioinotrope B3 was significantly superior to the control treatment, recording the highest values in most of the studied traits, including soil hydrocarbon concentration, phosphorus, and available soil potassium after harvest. Treatment B2, however, recorded the highest soil phosphorus value. The superiority of the C3 black oil treatment over the control treatment, as it recorded the highest values in soil hydrocarbon concentration. The results of the statistical analysis showed the superiority of the B3C3 treatment in the interaction of the bacterial bioinoculation and the addition of black oil in both soil nitrogen and potassium concentrations, total bacterial counts, and Pseudomonas bacteria at flowering, while the B1C1 treatment had the highest soil phosphorus value.

Keywords: *Bacillus megaterium*, *Pseudomonas aeruginosa*, hydrocarbon pollutants, NPK availability, soil.

Introduction

Hydrocarbons are primarily composed of hydrogen and carbon, they can be classified into alkanes, cyclic alkanes, aromatic compounds, asphalts, and resins. Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds. They are found in protein-rich foods and are formed during certain cooking and grilling processes. Benzo(a)pyrene is one such compound, containing five aromatic rings linked together in its chemical structure (Kothari *et al*., 2014).

Using certain plants capable of breaking down hydrocarbon pollutants in bioremediation can rid the environment of a wide range of these pollutants. This addresses, on a limited scale, pollution problems, including cleaning soil and groundwater contaminated with hydrocarbons and other organic materials (Al-Qarghouli, 2019).

Biodegradation in the root zone is one of the most important mechanisms for removing hydrocarbons from soil. The efficiency of reed and papyrus roots in removing polycyclic aromatic hydrocarbons (PAHs) from freshwater sediments was demonstrated (Al-Alwani, 2019). Sharma *et al.* (2020) used phytoremediation of soil contaminated with hydrocarbons and carbonates dumped from sludge treatment plants in India. The results showed that adding biochar (a carbon-rich plant product) to PAH-contaminated soil, led to positive removal effects by the plants and with the help of root microbes.

Rath *et al*. (2016) pointed out the ability of Pseudomonas aeruginosa bacteria to biodegrade crude oil. This type of bacteria has been extensively studied for its ability to break down hydrocarbons and produce biosurfactions that increase the solubility of hydrocarbons and break down the bonds between their atoms, thus degrading them. The resulting carbon is then used for growth.

This study aims to demonstrate the effect of *Bacillus megaterium* bacteria on the biodegradation of hydrocarbon pollutants, the effect of *Pseudomonas aeruginosa* bacteria on the biodegradation of hydrocarbon pollutants, and the interaction effect of *Bacillus megaterium* and *Pseudomonas aeruginosa* bacteria on NPK availability in the soil.

Material and methods

A field experiment was designed using a Randomized Complete Block Design (RCBD). After perpendicular tillage of the field soil with a moldboard plow and disc harrows, leveling, smoothing, and ridge preparation were carried out to create a suitable seedbed. The field was divided into 48 experimental units, each measuring 5 m^2. The units were divided into rows with a spacing of 70 cm between rows. The spacing between planting holes was 25 cm. A distance of 1 m was left between experimental units and 1.5 m between sections. A calibration irrigation was administered, and the soil was left to dry until suitable conditions were met for planting. Then, on March 30, 2025, yellow maize seeds of the 'Layla' variety were sown at a rate of 4 seeds per hole, at a depth of 3 cm (Al-Sahouki, 1994). Irrigation and weeding were carried out as needed. The land was divided into three sections, each containing 16 experimental units. Each experimental unit measured 5 square meters. The distance between sections was 2.5 meters, and the distance between experimental units was also 2.5 meters. There were a total of 48 experimental units. Each experimental unit contained three rows, and the maize variety "Lila" was planted. After the black oil injection process was completed in the experimental units, and before the plants reached physiological maturity, the plants were covered with netting to protect them from birds. The land was harvested on July 15, 2025, when the plants showed signs of maturity and complete yellowing. Samples were taken from each experimental unit, table (2) shows the soil analysis before planting.

Table (2) Soil analyses before planting.

Items	Value	Unit
ECe	5.30	dsm^{-1}
pH	7.80	---
organic matter	0.50	%
Available nitrogen	11.30	$mg\ kg^{-1}$
Available phosphorus	25.40	
Available potassium	201.18	
Soluble calcium	200.00	$Mmol\ L^{-1}$
Soluble magnesium	209.84	
Soluble sodium	473.54	
Carbonates	Nill	
Bicarbonates	280.00	
Sulfates	37.80	
Chloride	303.80	
Bulk Density	1.17	$gm\ cm^{3}$
True Density	2.47	
Porosity	52.63	
Sand	184.50	$gm\ kg^{-1}$ soil
Clay	240.10	
Silt	575.40	
Soil texture	Clay	
Total bacterial counts	3.70×10^{6}	CFU gm^{-1} dry soil
Azoospermum counts	5.40×10^{3}	
Thiobacillus counts	2.50×10^{2}	

Fertilization:

The full fertilizer recommendation of 160 kg N/ha was adopted using urea fertilizer (46% N), using triple superphosphate (P_2O_5) (46%, using 75% of the fertilizer recommendation) at 100 kg P_2O_5 per hectare, using potassium sulfate (K_2O_5) at 160 kg/ha (Al-Abedi, 2011). Nitrogen fertilizer in the form of urea was applied in two applications: one at planting and another 45 days later (Al-Nuaimi, 2000). Phosphate fertilizer was applied in a single application one month before planting, and potassium fertilizer was applied in a single full application at planting.

Factors of the Experiment:

The factorial experiment included two factors, as detailed below:

Factor 1: A biological inoculation of *Bacillus megaterium* and *Pseudomonas aeruginosa*, designated B. The experiment was conducted with three replicates, resulting in 4 x 4 x 3 = 48 experimental units. The experiment included four levels:

B0: No inoculation. **B1:** *Bacillus megaterium*. **B2:** *Pseudomonas aeruginosa*. **B3:** Interfering between Bacillus and Pseudomonas bacteria

Factor 2: Four levels of black oil, designated C:

C0: No addition. **C1:** 2% concentration. **C2:** 3% concentration. **C3:** 4% concentration.

Results and Discussion

Soil Available Nitrogen Concentration (mg N kg soil^{-1}):

Table (1) shows the effect of the bioinotropic inoculants of *B. megaterium* and *P. aeruginosa* on soil available nitrogen concentration. The results indicated that treatment B3 was significantly superior, yielding the highest average of 35.68 mg N kg soil^{-1}, representing an increase of 179.6%. This was compared to the control treatment B0 (without the inoculant), which yielded the lowest average of 12.76 mg N kg soil^{-1}. This increase may be attributed to the fact that the bioinotropic inoculant enhances microbial activity in the soil. It stimulates the fixation of organic nitrogen and its breakdown into forms available for plant uptake. *B. megaterium* is known for its ability to secrete enzymes that degrade organic matter. *P. aeruginosa* contributes to stimulating bio-fixation of nitrogen and the release of nitrates and ammonia in the soil, thus increasing the nitrogen content available to plants (Baker, 2018).

The addition of different levels of black oil to soil nitrogen concentrations. The results indicated that treatment C3 was significantly superior, yielding the highest average of 28.16 mg kg^{-1} soil nitrogen, representing a 54.5% increase. This was in contrast to the control treatment C0, which yielded the lowest average of 18.22 mg/kg^{-1} soil nitrogen. This increase may be attributed to the hydrocarbon compounds in black oil acting as an additional carbon source for specialized biodegradable bacteria. This stimulates their metabolic activity and helps release organic nitrogen into forms usable by plants, such as nitrates and ammonia. Furthermore, the increased microbial activity leads to increased secretion of enzymes that degrade organic matter, which improves the soil nitrogen content (Backer, 2018).

The results indicated that the B3C3 combination was significantly superior, yielding the highest average of 47.50 mg kg^{-1} soil, representing 334.8% increase. This was in contrast to the control treatment, B0C0, which yielded the lowest average of 10.93 mg kg^{-1} soil. This increase may be attributed to the fact that bioinoculation enhances overall microbial activity in the soil. It stimulates the fixation and decomposition of organic nitrogen into forms usable by plants, such as nitrates and ammonia. Furthermore, the presence of high levels of black oil (C_3) provides an additional carbon source for specialized bacteria, which increases their metabolic activity and stimulates nitrogen release and availability to plants. The synergistic interaction between the two species in bioinoculation and the high oil level enhances the biodegradation of organic matter and increases the production of available nitrogen. This explains the high nitrogen content of 47.50 mg kg^{-1} of soil in the B3C3 combination compared to the uninoculated and uncontaminated treatment (Backer, 2018).

Table (1) Effect of bioinoculation of *Bacllius megaterum* and *Pseudomonas aeruginosa* bacteria and different levels of black oil on the concentration of available nitrogen in the soil (mg kg soil^{-1}).

Bioinoculation	Hydrocarbons				Mean
	C0	C1	C2	C3	
B0	10.93	12.77	12.65	14.70	12.76
B1	18.13	14.73	18.87	17.73	17.37
B2	20.47	32.57	40.94	32.69	31.67
B3	23.33	28.70	43.20	47.50	35.68
Mean	18.22	22.19	28.91	28.16	
$L.S.D_{0.05}$	B		C		BC
	2.492		2.492		4.984

Soil Phosphorus Concentration (mg P kg soil^{-1}):

Table (2) shows the effect of the bioinopolymer of *B. megaterium* and *P. aeruginosa* bacteria on soil phosphorus concentration. The results showed that treatment B2 was significantly superior, reaching 32.16 mg P kg soil^{-1}, representing a 43.8% increase. This was compared to the control treatment B0, which gave the lowest average of 22.37 mg P/kg soil^{-1}. This may be attributed to the vital role of the bacteria used in the bioinopolymer. *B.*

megaterium and *P. aeruginosa* are among the most important phosphorus-solubilizing bacteria (PSB). These microorganisms secrete organic acids such as citric, oxalic, and lactic acids. These acids dissolve insoluble phosphates in the soil and convert them into mineral forms readily available for plant uptake. (Rodríguez, 1999). These bacteria also secrete enzymes such as organic phosphatases, which stimulate the mineralization of organic phosphorus in soil organic matter, thus increasing the concentration of available phosphorus (Richardson *et al*., 2009). *P. aeruginosa* is a root-promoting bacterium (PGPR), which contributes to increased nutrient uptake, by enhancing root growth and secreting compounds that activate beneficial microorganisms in the rhizosphere (Bhattacharyya and Jha, 2012).

The addition of different levels of black oil to the soil affected the available phosphorus content after plant growth. The results indicated that treatment C1 was significantly superior, reaching 32.73 mg P kg soil^{-1}, representing 51.2% increase. This was in contrast to treatment C2, which yielded the lowest average of 21.65 mg P kg soil^{-1}. This may be attributed to the effect of black oil as an additional source of organic carbon. This organic carbon can be utilized by soil microbes, thus increasing their biological activity. Specifically, phosphorus-solubilizing bacteria such as Bacillus and Pseudomonas, utilize organic carbon to stimulate their metabolic processes and produce organic acids. These acids dissolve unavailable phosphorus, such as calcium and iron phosphates, and convert it into a form available for plant uptake (Subba Rao, 2007).

The results indicate significant differences in the interaction between the bioinoculum of *B. megaterium* and *P. aeruginosa* and the addition of different levels of black oil on soil phosphorus content. The B1C1 combination outperformed the others, yielding the highest average phosphorus content of 39.56 mg kg soil^{-1}, representing 172.5% increase. This is in contrast to the control treatment B0C0 (without inoculum and without contamination), which yielded 14.52 mg kg soil^{-1}. This suggests that the B1C1 combination provided a healthy and balanced microbial environment, which significantly increased soil nutrient content compared to the uninoculumed and uncontaminated treatment. This may be attributed to the bioinoculum, which stimulated microbial activity in the soil and increased the rate of organic compound decomposition, thus improving nutrient availability for plants. Black oil at the C_1 level provides a source of organic carbon that is utilized by inoculating bacteria, thus enhancing their vital activity and their ability to break down complex hydrocarbon compounds and release nutrients in a form available to plants (Atlas, 1981).

Table (2) Effect of bioinoculation of *Bacillus megaterium* and *Pseudomonas aeruginosa* bacteria and different levels of black oil on the concentration of available phosphorus in the soil (mg kg soil^{-1}).

Bioinoculation	Hydrocarbons				Mean
	C0	C1	C2	C3	

B0	14.52	34.92	15.14	24.90	22.37
B1	35.77	39.56	24.94	27.45	31.93
B2	31.86	35.17	29.25	32.34	32.16
B3	34.47	21.25	17.28	21.28	23.57
Mean	29.16	32.73	21.65	26.49	
$L.S.D_{0.05}$	B		C		BC
	5.219		5.219		10.438

Soil Potassium Concentration (mg K kg soil^{-1}):

Table (3) shows a significant increase in soil potassium concentration following the addition of the bioinoculant of *B. megaterium* and *P. aeruginonsa*. Treatment B3 yielded the highest average, reaching 204.9 mg K kg soil^{-1}, representing 73.4% increase. This was compared to the control treatment B0 (without inoculant), which yielded the lowest average, at 118.1 mg K kg soil^{-1}. This may be attributed to the bioinoculant's ability to enhance the release of bound nutrients in the soil through microbial metabolic activity. *B. megaterium* is known for its ability to solubilize insoluble phosphorus and potassium by secreting organic acids (such as oxalic and citric acids) and mineral-degrading enzymes, converting them into forms available for absorption by plants and other microorganisms. *P. aeruginosa* bacteria also contribute to enhancing the metabolic activity of other bacteria. They stimulate the decomposition of minerals, thereby increasing the bioavailability of potassium in the soil (Kpomblekou and Tabatabai, 1994).

The addition of different levels of black oil significantly increased potassium concentration in the soil. Treatment C3, which yielded the highest average of 180 mg K kg soil^{-1} (35% increase), outperformed the control treatment, which yielded the lowest average of 133.7 mg K kg soil^{-1}. This increase may be attributed to the hydrocarbon compounds present in black oil, stimulating microbial activity in the soil. Specialized bacteria break down the complex organic compounds in the oil into simpler compounds. This releases bound nutrients such as potassium, making them more available to plants. The increased microbial activity also stimulates the secretion of organic acids and enzymes, which dissolve non-absorbable potassium, thus increasing its bioavailable concentration (Kpomblekou and Tabataba, 1994).

Significant differences were found in the interaction between bioinoculant and different levels of black oil on soil potassium availability. The B3C3 combination showed the highest average potassium availability at 287.4 mg K kg soil^{-1}, representing 146.3% increase. This was in contrast to the control treatment, which yielded the lowest average potassium availability at 116.7 mg K kg soil⁻¹. This increase may be attributed to bioinoculant enhancing overall microbial activity in the soil. It stimulates the secretion of organic acids and mineral-degrading enzymes, leading to the release of bound potassium from the soil and making it available to plants. Simultaneously, higher levels of black oil (C3) provide additional carbon sources for microbes, further increasing their activity and stimulating the biodegradation of minerals and organic matter. Therefore, the positive interaction between the bioinoculant and the high black oil level resulted in a significantly higher level of available potassium compared to the uninoculant and uncontaminated treatment. (Kpomblekou and Tabatabai, 1994).

Table (3) Effect of bioinoculation of *Bacillus megaterum* and *Pseudomonas aeruginosa* bacteria and different levels of black oil on the concentration of available potassium in the soil (mg kg soil^{-1}).

Bioinoculation	Hydrocarbons				Mean
	C0	C1	C2	C3	
B0	116.7	117.0	120.7	118.0	118.1
B1	121.3	119.1	126.4	142.3	127.3
B2	147.4	151.6	166.9	174.4	160.1
B3	149.4	174.4	208.5	287.4	204.9
Mean	133.7	140.6	155.6	180.5	
L.S.D$_{0.05}$	B		C		BC
	10.79		10.79		21.58

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