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# Assessing the synergistic relationship of mycorrhiza and bacteria for the degradation of spent engine oil in maize grown soil

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## Abstract

**Background:** Pollution by waste hydrocarbons on soil and water bodies is an endemic problem in African countries, particularly in Nigeria. This has caused untold hardship and increase in poverty level of the people, due to the pollution of water and land; which had direct impacts on the livelihood of people. Several species of bacteria that can degrade hydrocarbon had been isolated from the root of plants. The aim of this work was to determine the ability of rhizosphere bacteria to degrade spent engine oil (SPO) in the presence of arbuscular mycorrhiza fungus.

**Results:** The total aerobic count in the eight treatments ranged between  $3.0 \times 10^6$  and  $1.18 \times 10^8$  CFU/g. No significant difference was observed in the total aerobic count among the treatments except when compared with the control experiment (M–C–). Isolated bacteria from the polluted soil samples were identified by 16S rRNA sequencing as *Bacillus cereus*, *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Lysinibacillus fusiformis*, *Bacillus enecimensis*, and *Paraclostridium benzoelyticum*. Four of the bacteria were able to utilize spent engine oil effectively to different degrees. However, *Lysinibacillus fusiformis* biodegraded spent engine oil by 40%, *Paraclostridium benzoelyticum* by 30% and *Bacillus enecimensis* by 20% after 28 days of incubation. The infrared analysis result revealed that *Lysinibacillus fusiformis* (P6) reduced the strong and band of alcohol by 44%, carboxylic acid group by 22% and carbonyl group by 27%, respectively. This study revealed that spent engine oil at a minimum value of 50 mg/kg with arbuscular mycorrhiza fungus in the soil samples resulted in better growth for maize plant and higher total aerobic count.

**Conclusions:** It can thus be concluded that arbuscular mycorrhiza fungus positively impacts the ability of rhizosphere bacteria in the degradation of spent engine oil and the growth of maize plant on contaminated soil.

**Keywords:** Biodegradation, Residual oil, Mycorrhiza, spent engine oil, contamination

## Introduction

The discharge of spent oil into the environment brings about pollution, which is an undesirable change in the physical, chemical and biological characteristics of all the components of an environment (Aboribo, 2001).

The increasing uses of automobiles and other heavy equipment have resulted in the increased consumption of hydrocarbon products; and invariably, the contamination of soil and water bodies. This often results in the distortion of soil physical, biological, and chemical properties (Stephen et al. 2013).

Soil contamination by hydrocarbons causes extensive damage to the ecosystems and leads to the accumulation of pollutants in animals and plants tissue, which may cause progeny's death or mutation (Alvarez et al. 1991). Spent engine oil on land may cause damage to the

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environment in many ways like the retardation of vegetation growth and cause of soil fertility loss for a long period of time until natural processes re-establish stability (Sada and Odemerho, 2000). Contaminated soil possess high concentrations of chemicals and other substances derived from various man's use of land, thereby influencing surface and ground water quality, the nature and viability of ecosystems and invariably plant, animal, and human health (Vegter et al. 2002).

Many bacteria are known to stimulate plant growth through direct or indirect interactions with plant roots (Hashem et al. 2019). Bioremediation involves indigenous oil-consuming microorganism by enhancing and fertilizing them in their natural habitats (Heider and Rabus, 2008). Microorganisms degrade pollutant compounds by using enzymes in their metabolism and can be useful in cleaning up contaminated sites (Alexander et al. 1997). Vazquez et al. (2000) reported that several rhizospheres colonizing bacteria such as *Azotobacter*, *Azospirillum*, *Bacillus*, *Clostridium*, and *Pseudomonas* were found to produce substances that stimulate plant growth or inhibit root pathogens. The establishment of Mycorrhiza implies profound morphological and physiological changes in the root which operates in an inclusive manner with the fungus, promoting increase adaptation, and survival of symbionts (Teste et al. 2009). A fungus of great importance for agriculture in this type of association is *Arbuscular Mycorrhiza* fungus (AMF). This study therefore aimed at determining the ability of rhizosphere bacteria to degrade spent engine oil in the presence of arbuscular mycorrhiza fungus.

## Materials and methods

### Sample collection

Uncultivated soil samples were collected from a part of Abogunde Sagba area (Latitude 7° 7' 38.58' N Longitude 4° 42' 10.21' E) in Ede, Osun State, Nigeria. This was done at a depth of 10 cm deep using the "V-shaped" soil sampling method, with the aid of a spade in a zig-zag manner to ensure homogeneity.

### Experimental design

After a thorough mixing of the soil samples, 3 kg each was transferred into different nursery planting bags. Two experimental soil groups were set up (one with mycorrhiza and the other without mycorrhiza) for three treatments of different spent engine oil concentrations added (50, 75, and 100 mg/kg). Two controls (one with mycorrhiza only and one without any treatment) were also set-up. The experimental soil samples were then planted with maize seeds (*Zea mays*). Soil samples were taken from the maize plant root area at a space of 0, 7, 14, 21, 28, 35, and 42 days. Total aerobic counts of the samples were carried out. The experimental set-up consisted of contamination with spent engine oil as the first

factor designated C at two levels (contaminated C+ and uncontaminated C-). The second factor was the inoculation with mycorrhiza (*Glomus muse*) designated M also at two levels (inoculated M+ and not inoculated M-). The two factors were arranged in factorial combination to give four treatments each, namely, (in milligram per kilogram): M+C-, M+C+50, M+C+75, M+C+100, M-C-, M-C+50, M-C+75, and M-C+100.

### Total aerobic count

Soil samples were serially diluted and plated out on Nutrient agar using the pour plate method (Nwite and Alu, 2015). Plates were inoculated with 1 ml of diluents and incubated at 35±2 °C for 24 h. Grown plates were counted under the colony counter.

### Bacteria isolation and characterization

Bacteria with persistent occurrence (based on morphological characteristics) were picked and streaked until pure colonies were obtained.

### Oil degrading test

Bacterial isolates obtained were grown in minimal salt medium (broth and agar) containing 10.0 g NaCl; 0.42 g MgSO<sub>4</sub>; 0.83 g KH<sub>2</sub>PO<sub>4</sub>; 1.25 g NaPO<sub>4</sub>; 0.42 g NaNO<sub>3</sub>; 0.29 g KCl; and 15 g Agar, while spent engine oil was added as the sole carbon source in the broth (Banks et al. 2003), filter paper soaked in spent engine oil were used on agar plates (Ajoy et al. 2012); these were incubated at 37 °C for 7 days.

### Bacterial characterization

Pure cultures of the bacterial isolates were subjected to morphological and biochemical characterization like citrate test, sugar utilization, motility test, and catalase test among others (Ajoy et al. 2012). The 16S rRNA of the bacterial isolates were amplified and sequenced (Amao et al. 2019) using universal primer 518F (CCAGCAGCCG CGGTAATACG) and 800R (TACCAGGGTATCTAAT CC). The sequence was performed using a Sanger sequencer. The bacterial sequences were submitted to the NCBI.

### Hydrocarbon degradation assay

The hydrocarbon degradation assay was carried out using the mineral salt medium (MSM) as adopted by Sepahi et al. (2008). After sterilization and cooling, 5 ml of sterile engine oil and 2 ml inoculums of the hydrocarbon utilizing bacteria were added to each bottle. Un-inoculated bottle of sterile MSM and spent engine oil were set up as control. The bottles were incubated at 37 °C for 21 days as described by Ukaegbu and Mbakwem (2014).

**Table 1** Effect of spent engine oil and mycorrhiza on the number of leaves per plotted maize plant

Treatment	Number of leaves in maize plant in days					
	7	14	21	28	35	42
M-C-	3.00 ± 0.01	5.00 ± 0.01	6.00 ± 0.01	6.33 ± 0.47	7.00 ± 0.47	8.33 ± 0.47
M+C	3.00 ± 0.01	5.00 ± 0.82	6.00 ± 0.47	7.60 ± 0.47	10.00 ± 0.47	11.00 ± 0.82
M+C+50	3.00 ± 0.01	5.00 ± 0.47	6.00 ± 0.47	6.00 ± 0.47	9.00 ± 0.47	13.00 ± 0.47
M+C+75	3.00 ± 0.47	5.00 ± 0.47	6.00 ± 0.94	6.00 ± 0.47	6.30 ± 0.47	12.00 ± 0.47
M+C+100	3.00 ± 0.58	5.00 ± 0.58	6.00 ± 0.58	6.00 ± 0.58	6.30 ± 1.53	8.33 ± 1.16
M-C+50	3.00 ± 0.47	4.30 ± 0.47	6.00 ± 0.47	6.30 ± 0.47	8.00 ± 0.47	9.30 ± 0.47
M-C+75	3.30 ± 0.47	4.30 ± 0.47	5.60 ± 0.47	6.60 ± 0.47	7.00 ± 0.47	8.30 ± 0.47
M-C+100	2.60 ± 0.47	3.60 ± 0.47	5.00 ± 0.47	5.60 ± 0.47	7.30 ± 0.47	7.60 ± 0.47

**Extraction of residual oil**

The residual spent engine oil in the experimental and the control set up after the incubation period were extracted by the liquid-liquid solvent extraction method using chloroform. Twenty milliliter of chloroform was measured into each of the bottles and the content of each bottle was transferred into a separating funnel (Ayandele et al. 2012). The funnel was allowed to stand for 30 min, the layer of the organic solvent and oil was emptied into sterile Petri dishes, and the MSM-organism layer was discarded. The organic solvent was allowed to evaporate from the residual oil and the residual oil was measured using a pipette.

**Infrared analysis**

The residual oil from both experimental and control bottles after the 28 days incubation period was subjected to infrared (IR) analysis at the Multi-disciplinary Central Research Laboratory of the University of Ibadan, Oyo State, Nigeria.

**Results**

The number of leaves in treatment M+C- increased to 11 leaves while that in M+C+50 increased to 13 at the expiration of the experiment. The ANOVA showed a significant

difference at  $P < 0.05$ , observed in the number of leaves in both treatments (M+C- and M+C+75) compared with the control (Table 1). There was a steady increase in the shoot height of the control treatment and the others throughout the planting period. The M+C- showed a rapid shoot growth till the expiration of the experiment with mean value of 65 cm in height. Both the control (M-C-) and M+C+50 had increased shoot height with different height at the end of the experiment (Table 2). Bacteria count in the control treatment (M-C-) ranged from  $3.0 \times 10^6$  to  $3.12 \times 10^7$  CFU/g. The bacteria count in the rhizosphere soil treatment inoculated with AM fungi M+C- ranged from  $2.23 \times 10^7$  to  $1.02 \times 10^8$  CFU/g count but later declined at 6 weeks planting period. The bacteria count in inoculated rhizosphere soil treatment with AM fungi and contamination with 50 mg/kg of spent oil ranged from  $2.13 \times 10^7$  to  $9.13 \times 10^7$  CFU/g, respectively (Fig. 1).

The bacteria count in soil with AM fungi and contaminated with 75 mg/kg of spent engine oil ranged from  $2.13 \times 10^7$  CFU/g to  $9.9 \times 10^7$  CFU/g. The bacteria count in M+C+75 began with  $2.13 \times 10^7$  CFU/g and increased gradually throughout the weeks of planting (Fig. 2). Three of the 8 bacterial isolates were

**Table 2** Effect of spent engine oil and mycorrhiza fungi on the shoot height per plotted maize plant

Treatment	Shoot height in maize plant in days					
	7	14	21	28	35	42
M-C-	7.66 ± 0.47	36.67 ± 1.25	36.67 ± 1.25	45.00 ± 0.82	50.33 ± 0.47	61.00 ± 0.25
M+C-	17.67 ± 0.47	34.67 ± 0.33	41.33 ± 1.89	49.33 ± 2.49	55.67 ± 2.06	65.00 ± 5.10
M+C+50	14.00 ± 1.49	33.33 ± 4.11	36.33 ± 2.63	44.67 ± 3.77	52.67 ± 5.44	55.67 ± 5.19
M+C+75	14.00 ± 0.82	35.00 ± 0.82	41.33 ± 1.88	46.33 ± 3.09	50.00 ± 0.82	55.00 ± 1.41
M+C+100	14.33 ± 0.57	23.33 ± 4.00	28.67 ± 4.00	30.67 ± 4.00	35.33 ± 1.57	40.00 ± 2.65
M-C+50	7.67 ± 0.47	36.00 ± 0.82	38.00 ± 0.81	47.33 ± 0.47	58.67 ± 0.47	73.33 ± 0.94
M-C+75	6.37 ± 0.01	32.33 ± 0.47	38.33 ± 0.47	46.67 ± 1.25	52.33 ± 0.94	59.67 ± 0.47
M-C+100	6.80 ± 2.70	35.67 ± 0.47	35.93 ± 0.47	36.33 ± 0.47	48.00 ± 0.12	50.00 ± 1.82

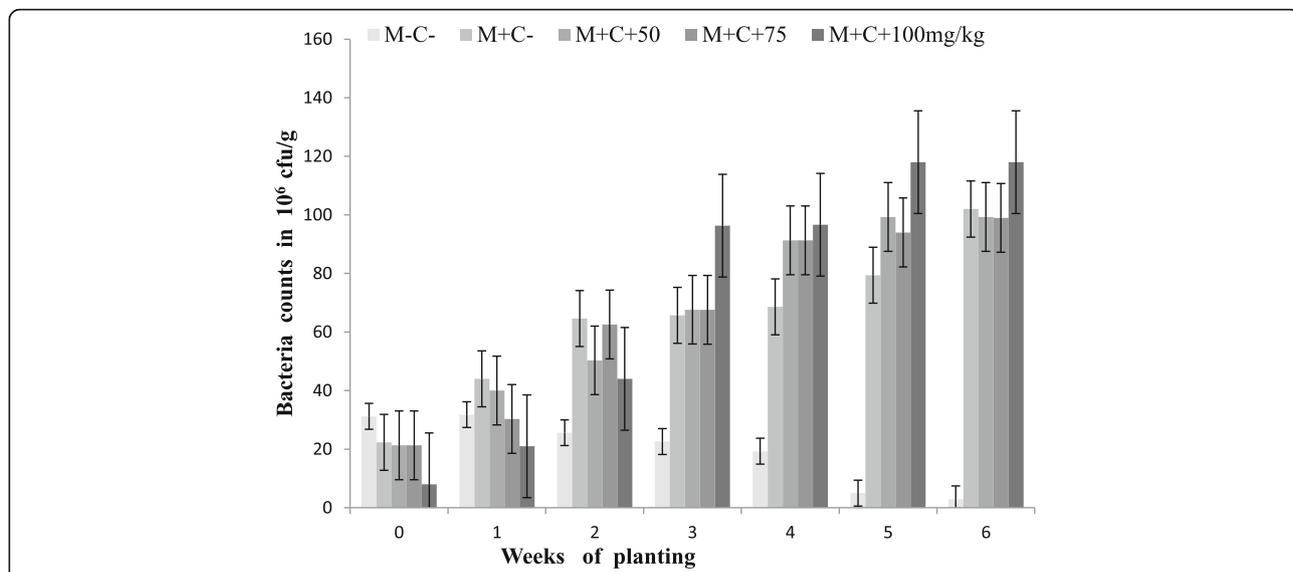


Fig. 1 Effect of inoculation with AM fungi and contamination with spent engine oil on the mean counts of bacteria in CFU/g dilution

Gram-negative. Seven were positive for motility test, and 6 were positive for catalase test (Table 3). Four were identified as members of the genus *Bacillus*, two were *Pseudomonas*, one was *Lysinibacillus*, and the remaining one was *Paraclostridium* (Table 4). *Bacillus cereus*, *Lysinibacillus fusiformis*, *Bacillus encimensis*, and *Paraclostridium benzoelyticum* utilized the spent oil effectively (Table 5). *Lysinibacillus fusiformis* utilized 40% of utilized engine oil, *Paraclostridium benzoelyticum* utilized engine oil up to 30%; while *Bacillus encimensis* utilized up to 20% of spent

engine oil and *Bacillus cereus* was able to utilize 12% of spent engine oil (Table 6).

The control contained carbonyl group C=O (1700 cm<sup>-1</sup>) with 74% absorbance (26 %T) or C=C stretch of ketone and alkenes SP<sup>2</sup> C-H stretch of substituted benzene (728 cm<sup>-1</sup>) with 80% absorbance (20%T) respectively. The IR bands of 3405 cm<sup>-1</sup> with 20%T, 2733 cm<sup>-1</sup> with 21%T, 1667 cm<sup>-1</sup> with 22%T and 728 cm<sup>-1</sup> with 18%T respectively were observed for *Bacillus cereus*. It also revealed that the injected sample with *Bacillus cereus* contained all

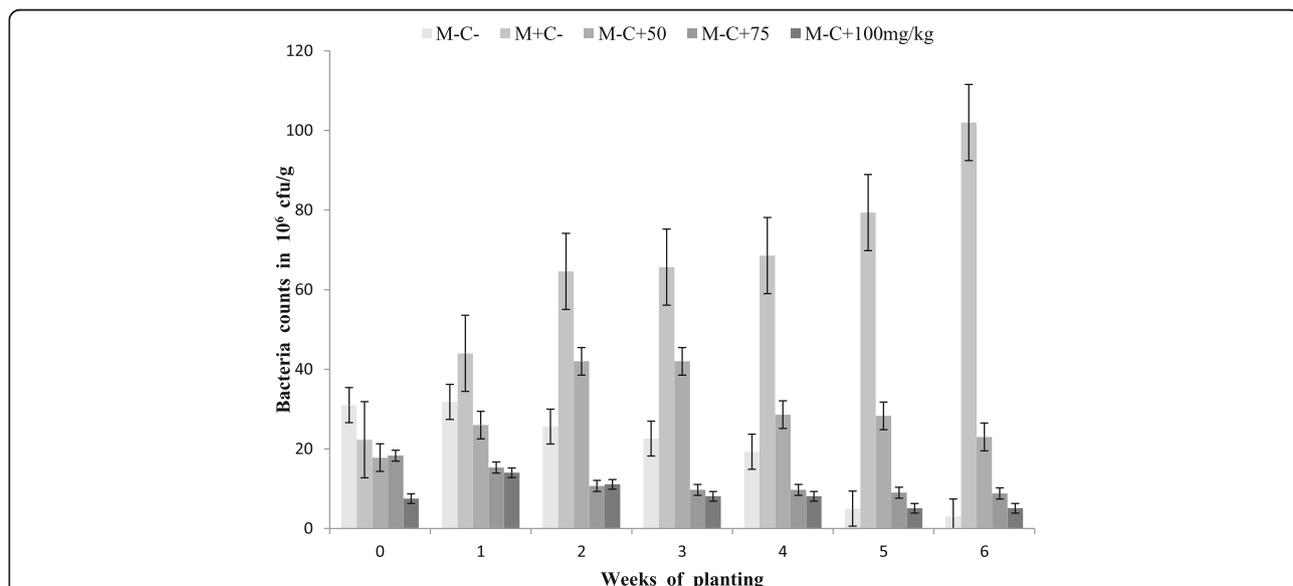


Fig. 2 Effect of contamination with spent engine oil on the mean counts of bacteria in CFU/g dilution

**Table 3** Biochemical characterization of the bacterial isolates

Isolate	Color/pigment	Gram reaction	Cellular morphology	Catalase test	Oxidase test	Indole test	Motility test	Methyl-red	VP-Proskueur	Citrate utilization	Urease activity	Starch hydrolysis	Gelatin hydrolysis	Casein hydrolysis	NO <sub>3</sub> reduction
Isolate	Spore test	Coagulase test	Glucose	Sucrose	Lactose	Xylose	Maltose	Manitol	Salicin	Sorbitol	Inositol	Galactose	Raffinose		
														P1	+
P2	-	-	+	+	+	+	+	-	-	-	-	+	+		
P3	+	-	+	+	+	+	-	+	-	-	-	-	-		
P4	-	-	+	+	+	+	+	-	-	-	-	+	+		
P5	+	-	+	-	-	+	-	+	-	-	-	+	-		
P6	+	+	+	+	+	-	+	+	-	-	+	-	+		
P7	+	-	+	+	-	+	-	+	-	+	-	-	+		
P8	+	-	+	-	+	+	+	-	-	-	+	+	-		

**Table 4** Isolates' identities and accession number from NCBI

Isolates	Identity	Accession number
P1	<i>Bacillus thuringiensis</i>	MK875170
P2	<i>Pseudomonas aeruginosa</i>	MK875171
P3	<i>Bacillus amyloliquefaciens</i>	MK875172
P4	<i>Pseudomonas aeruginosa</i>	MK875173
P5	<i>Bacillus cereus</i>	MK875174
P6	<i>Lysinibacillus fusiformis</i>	MK875175
P7	<i>Bacillus encimensis</i>	MK875176
P8	<i>Paraclostridium benzoelyticum</i>	MK875177

the functional groups in the control sample but at a reduced percentage. The strong and broad of alcohol was reduced by *Lysinibacillus fusiformis* by 44%, carboxylic acid group or aliphatic asymmetric group was reduced by 22% while the carbonyl group or C=C stretch has a reduction of 35%. There was more efficient reduction in all the functional groups by *Bacillus encimensis* than *Bacillus cereus* but not as efficient as *Paraclostridium benzoelyticum* and *Lysinibacillus fusiformis* (Fig. 4aa–e).

## Discussion

Plants laden with mycorrhizal fungi have earlier been reported to enhance the phytoremediation efficiency of oil contaminated soils with high concentrations of heavy oil (Kuo et al. 2014). Interactions between plants and their attendant microbes (bacteria and fungi) need to be better understood to improve the effectiveness and feats of phytoremediation, in addition to the close interactions between bacteria and fungi (Jambon et al. 2018). Bell et al. (2014) reported that fungal associations may affect the condition of plants and the success of co-inoculated microbial strains during phytoremediation.

Results from this study showed that the inoculation of AM fungi into the soil promotes growth of maize plants on both uncontaminated soil and contaminated soil with the

**Table 5** Hydrocarbon utilization by the bacteria isolates

Bacteria	Growth in oil
<i>Bacillus thuringiensis</i>	–
<i>Pseudomonas aeruginosa</i>	+
<i>Bacillus amyloliquefaciens</i>	+
<i>Pseudomonas aeruginosa</i>	–
<i>Bacillus cereus</i>	+++
<i>Lysinibacillus fusiformis</i>	+++
<i>Bacillus encimensis</i>	+++
<i>Paraclostridium benzoelyti</i>	+++

Key: (–) no growth, (+) slight growth, and (+++) profuse growth

**Table 6** Percentage of residual oil as degraded by bacteria isolates

Bacterial isolates	Oil concentration (ml)	Non-degraded oil (ml)	Percentage degraded oil (%)
<i>Bacillus cereus</i>	5.00	4.4	12
<i>Lysinibacillus fusiformis</i>	5.00	3.0	40
<i>Bacillus encimensis</i>	5.00	4.0	20
<i>Paraclostridium benzoelyticum</i>	5.00	3.5	30
Control	5.00	5.0	0

highest concentration of spent engine oil at  $\leq 75$  mg/kg (Tables 1 and 2). Frey-Klett et al. (2005) reported *Pseudotsuga menziesii*–*Laccaria bicolor* symbiosis in the mycorrhizosphere, able to curb the formation of the Douglas-fir. Founoune et al. (2002) reported a robust association between the mycorrhizosphere source of fluorescent pseudomonads and the positive effect they have on the ectomycorrhizal symbiosis of *Acacia holosericea*–*Pisolithus* species. The shoot height and the number of leaves in maize plant grown in contaminated soil (M–C+100 mg/kg) and inoculated, contaminated soil (M+C+100 mg/kg) had a retarded growth after 6 weeks. This is in conformity with the report of Banks et al. (2003) who claimed that too much of contamination with spent engine oil significantly reduced plant height at every stages of growth. This finding was also supported by Abdulla (2010) who affirmed that AM fungi had more effect on peanut plants irrespective of substrate soil conditions. Table 3 revealed that four of the isolated bacteria were Gram-positive and four were Gram-negative.

The effect of contamination with spent engine oil and inoculation with AM fungi on the mean count of maize rhizosphere bacteria community obtained at 6 weeks of plating presented a total bacteria count ranging between  $7.5 \times 10^6$  and  $4.2 \times 10^7$  CFU/g for the M–C+ treatment with different concentration of spent engine oil and  $8.0 \times 10^6$  to  $1.18 \times 10^8$  CFU/g for the M+C+ treatment with different concentration of spent engine oil ranged (Figs. 1 and 2). This result is in agreement with the report of Onifade et al. (2007) and Stephen et al. (2013) who recorded high bacteria counts in crude oil polluted soils. Total bacterial counts in soil inoculated only with AM fungi (M+C–) ranged from  $2.23 \times 10^7$  to  $1.02 \times 10^8$  CFU/g while counts in the control (M–C–) ranged from  $3.0 \times 10^6$  to  $3.1 \times 10^7$  CFU/g, respectively (Figs. 1 and 2). Spini et al. (2018) reported that both bacterial and fungal communities found after 1 week of enrichment, resembled those detected after 4 weeks, while the soil depth did not affect the evolution of microbial populations. The co-inoculation of bacteria and fungi is more advantageous for restoring the fertility of soil and the organic matter

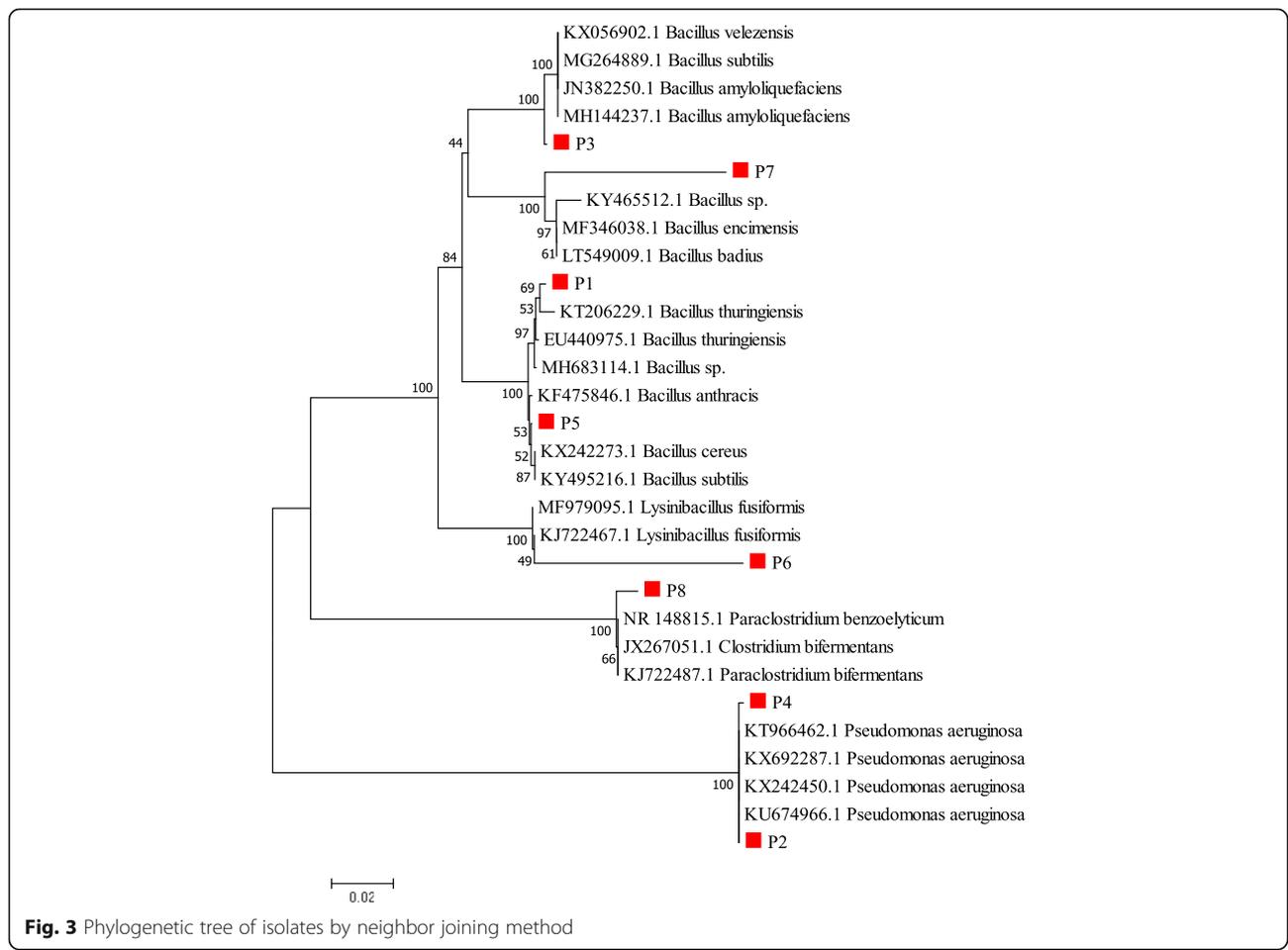
content with or without organic fertilizer than the use of single inoculum (Rashid et al. 2016).

The isolated bacteria, with persistent occurrences were identified as *Bacillus thuringiensis* (MK875170), *Bacillus cereus* (MK875174), *Pseudomonas aeruginosa* (MK875171 and MK875173), *Bacillus amyloliquefaciens* (MK875172), *Lysinibacillus fusiformis* (MK875175), *Bacillus encimensis* (MK875176) and *Paraclostridium benzoelyticum* (Table 4). This result (Fig. 3) disagrees with the high occurrence of *Pseudomonas*, *Sphingobacterium*, *Bacillus*, *Stenothrophomonas*, *Achromobacter*, and *Serratia* reported by Spini et al. (2018).

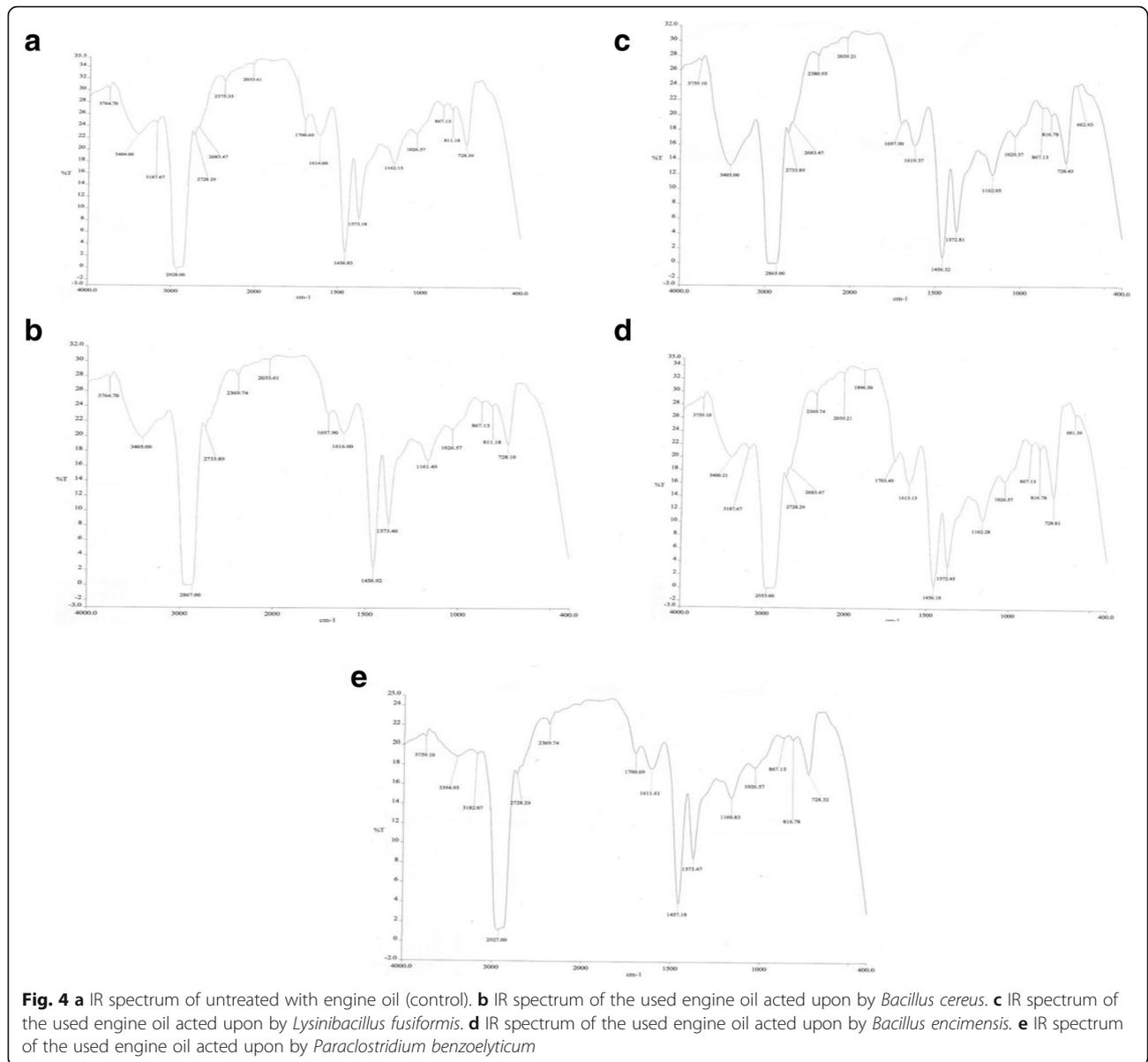
Four of the isolates were able to utilize spent engine oil to a different degree (Tables 5 and 6). *Lysinibacillus fusiformis* (P6) degraded spent engine oil by 40%, *Paraclostridium benzoelyticum* (P8) degraded spent engine oil by 30%, *Bacillus encimensis* (P7) by 20% while *Bacillus cereus* (P5) by 12% after 28 days of incubation. This finding is in line with the report of Onifade et al. (2007) and Ukaegbu and Mbakwem (2014), who noted that different bacteria species have different biodegradation capabilities. Vidalii (2001), Chaerun et al. (2004) and Nwaogu

et al. (2008), asserted that biodegradation of spent oil by bacteria is a natural process by organic carbon sources, causing breakdown of petroleum components to lower molecular compounds or transferred into other compounds, sources of energy, cell mass, biological product in respiration, and metabolism. However, high degradation potential recorded for *Lysinibacillus fusiformis* is in agreement with the reports of Ayed et al. (2015). This may be due to the fact that *Lysinibacillus fusiformis* has the ability to survive in contaminated soils (Table 6).

The result of IR analysis (Fig. 4a–e) showed that the residual oil from the bottles acted upon by *Bacillus cereus* (P6), *Pseudomonas aeruginosa* (P2 and P4), *Bacillus amyloliquefaciens* (P3), *Lysinibacillus fusiformis* (P6), *Bacillus encimensis* (P7), and *Paraclostridium benzoelyticum* (P8) have reduced alcohol, carboxylic acid, and carbonyl group after 28 days of incubation. This indicated that the four bacteria isolates utilized the spent oil component. This observation is in consonance with the report of Nwaogu et al. (2008), that many microorganisms have different rate at which they utilize hydrocarbons in soil and water.



**Fig. 3** Phylogenetic tree of isolates by neighbor joining method



**Conclusion**

This study revealed that spent engine oil at a minimum value of 50 mg/kg with presence of arbuscular mycorrhiza fungus in the soil samples resulted in better growth for maize plant and higher total bacterial count. Arbuscular mycorrhiza fungus could be concluded to positively impact on the ability of rhizosphere bacteria in the degradation of spent engine oil during the growth of maize plant on contaminated soil.

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**Authors' contributions**

ASB carried out the sample collection after obtaining the permission of the farm managers. He also carried out the lab work. MOL oversee the research work and also provided some of the materials required for the research. JAA also helped in the lab work, did the statistical analysis, and prepared the manuscript. AAA oversee the lab work, provided some of the materials used in the lab, and corrected the manuscript. The authors read and approved the final manuscript.

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**Availability of data and materials**

Data obtained in this study will be available on request.

**Ethics approval and consent to participate**

No ethical approval was required for this study.

**Consent for publication**

All authors agreed to publish this work in your reputed journal.

**Competing interests**

The authors declare that they have no competing interests.

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