*Plant Archives* Vol. 19, Supplement 2, 2019 pp. 322-326 e-ISSN:2581-6063 (online), ISSN:0972-5210

# THE ROLE OF *GLOMUS MOSSEAE* IN THE BIOCHEMICAL RECOMMENDATION OF CONTAMINATED HYDROCARBON (BLACK OIL) SOILS

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## Abstract

The aim of this study is to determine the ability of the *Glomus mosseae* in collaboration with the carrot *Daucas carota* L.in biochemical biodegradation and reduce the proportion of hydrocarbons from contaminated soil, A factorial experiment was carried out in pots with two factors: first use of the inoculum *Glomus mosseae* gets from the German company Bio-Vita (M1 or M0), second factor using 3 concentration of black oil (0, 1 and 2%) (H0, H1 and H2) and the cultivation of carrot *Daucas carota* L to recommendation soil contaminated with black oil. The results showed a significant difference in the number of mycorrhiza spores in the soil and the percentage of infected carrot roots with *G. mosseae.* Significant increase was also found in wet and dry weight of vegetative and root parts at *G. mosseae* treatment and the addition of black oil at treatment H1. There was also a significant increase in the amount of hydrocarbons absorbed in the root and vegetative of the carrot plant in the inoculum and black oil treatment at the concentration of 2% and the interaction of the treatments was greater than the treatment H2M1 (concentration of 2% with *G. mosseae*) on other treatments. An acceptable decrease was observed in the amount of residual hydrocarbons in the soil. In the M1 treatment 78.60% and 74.60% for the concentrations of 1% and 2% compared to the treatment M0 (71.40 and 69.70%) respectively, We conclude that the use of carrot and *G. mosseae* in the removal of black oil compounds from the soil was effective with a concentration of 2%.

***Keywords*****:** *G. mosseae,* black oil, carrot plant, contaminate.

## Introduction

The soil pollution of hydrocarbons is one of the problems facing the world and it is common in industrialized and producing countries and other factors due to the leakage of large amounts of these polluting compounds to the soil. This is reflected in microorganisms in the soil and thus on their fertility and on plants and animals. Through the transmission of these pollutants to plants from soil and then to humans and other organisms (Toledo *et al*., 2006). The addition of black oil to the soil to change some of the characteristics of water conservation through the packaging of granules, so that the soil becomes hydroponic water, then hater to keep water from entering the soil granules, there are also harmful and negative effects on the plant when the contamination of the soil with black oil, including direct and indirect effects in addition to the impact. Various methods are used to treat oil pollution from the environment. The use of microbes is an environmentally safe way to remove oil pollution. The importance of microorganisms for the decomposition of organic pollutants is based on the use of petroleum products as a source of carbon and energy (Okerentugba *et al*., 2003). Microbial use is of economic importance in the agricultural field by increasing the availability of some nutrients or by analyzing organic wastes, as well as their role in the secretion of certain growth regulators and enzymes, and conservation of soil properties (Cicatelli *et al*., 2014). The use of *G. mosseae* is a promising strategy for promoting plant growth and protecting it from environmental pressures because it acts as fertilizer, protection and bio treatment (Shaker-Koohi, 2014). The *G. mosseae* has ability to detoxify materials and has been used to treat hydrocarbon contaminants. Increase surface area of plant roots and thus aid in treatment (Gao *et al*., 2010). There are uses to add some of the oil compounds not considered by the planters of the carrot to fight the bushes associated with the plant. The carrot plant is a candidate crop for genetic engineering applications. It has important characteristics such as resistance to agricultural pests and environmental stresses (Braden *et al*., 2002). Research determination of the ability of fungi and carrot plant to remove soil pollution with black oil.

## Materials and Methods

### Field Experiment

The experiment was carried out at the site of the Center for Desert Studies at Anbar University, on 2017/10/12. Pots were organized with 3 replicates per treatment, According to the design of RCBD (complete random block) and the factorial treatments (concentration of oil 0,1 and 2%,: inoculum *G. mosseae*). The number of experimental units was 18 pots, brought the soil from a field in the Sufia area of the Ramadi city. The soil was dehydrated and then was contaminated with black oil, Table (1) shows the chemical, physical and biological properties of the soil used in the study. The soil was placed in plastic pots 25 cm high and 28 cm in diameter, And inoculated soil *G. mosseae*, planted pots with carrot seeds local class *Daucas carota* L. M0,M1 at a rate of 10 seeds per pot, and irrigation was done with water. After two weeks of growth, the plants were reduced by 5 plants per pot. After plant harvesting, the traits included were measured: Number of spores in soil, percentage of roots of carrots with *G. mosseae*, Wet and dry weight of the vegetative part and the root part, The amount of hydrocarbons in the vegetative and root regions, The amount of residual hydrocarbons in the soil. Insulate *G. mosseae* fungi from soil

The wet sieve method was used to isolate the spores mycorrhiza from the soil samples (Brundrett *et al*., 1994), taking 100 g of soil surrounding the roots of plants after air drying and estimate the moisture content of weight, Placed in a 1 liter flask containing 500 ml distilled water and then well left for 15-20 minutes, swipe from a range of sieves with 250, 180, 45 and 38 microns, The washing water was collected from each sieve in a sterile clean petri dish, The samples are now ready for microscopic examination. Percentage of infusion with *G. mosseae* (%) of carrot roots, Phillips and Haymann (1970) were used to examine the incidence of roots, by washing the roots well from the residue of the soil, addition of 10% potassium hydroxide solution (minutes from melting 10 KOH in 100 ml distilled water, the tubes were then placed in a water bath at 90° C for 60-30 minutes. The root was then washed with sterile water and the hydrochloric acid was added at a concentration of 10% for 2-3 minutes. The acid was then poured without washing the roots. A trypan blue dye was added to the root pieces in the test tubes and then transferred to a 90 m water bath for 20-15 minutes, Add the lactic acid to the root pieces after extracting the root pieces of the tubes and put them on the buckle, dried and ready to be examined, the incidence of mycorrhiza was estimated by estimating the percentage of infection in the root pieces that were pigmented and by the method of examining the stem of the roots according to Gerdemann and Nicolson (1963).

**Table 1 :** Chemical, physical and biological properties of soil used in the study

| Properties | Units | Value |
|---|---|---|
| EC | $Ds.m^{-2}$ | 3.45 |
| PH | - | 7.56 |
| $CaCO_3$ | | 223 |
| (OM.)organic mater | | 6.20 |
| sand | $g\ kg^{-1}$ | 194 |
| Loom | | 436 |
| Clay | | 370 |
| texture | - | Loom |
| CEC | cmol(+)/kg | 18.69 |
| N total | | 168.40 |
| P a valuable | $mg\ kg^{-1}$ | 7.32 |
| K a valuable | | 136.60 |
| Hydrocarbons | $ml\ kg^{-1}$ | 0.0 |
| PSB | Log cfu $g^{-1}$ | $1.2x10^4$ |
| Spores | Spore $g^{-1}$ | 10 |

## Results

### 1. The number of spores in the soil and the infection percentage of *G. mosseae* of carrot roots

Table (2) shows a significant difference in the number of. *G. mosseae* spores in the soil for the treatment of *G. mosseae* supplementation compared with the inoculum non-addition treatment which reached 41.44 and 15.33 spore $g^{-1}$. The treatment of addition of black oil only rates of 30.50 and 29.50 and 25.17 $spore^{-1}$ levels of H0 and H1 and H2 respectively, moral superiority was also observed in interference treatment. M0H0 gave the lowest rate of 12.33 spore $g^{-1}$, while the highest interference rate was 44.33 spore $g^{-1}$ for M1H2. As for the rate of infection.

Table (2) confirms that there is a significant superiority in the root infection ratio of treatment M1 averaging 67.01% compared to treatment M0 without inoculum, which averaged 42.11. As well as the treatment of the addition of black oil, which reached the highest rate of 60.30% at treatment H1, While the lowest average was 50.83% at H0. In the treatment of interaction between treatments, there was also a significant difference in interaction (inoculum and 1% black oil), the highest value was 72.17%, while the lowest value was at interference (without inoculum and 0%) at 36.90%.

### 2. Wet and dry weight of the vegetative part ($pot^{-1}$)

Table (3) shows significant differences in the treatment of G. mosseae, which reached 220.31 and 60.62 g, respectively, for wet and dry, respectively, compared with the control treatment of 185.09 and 44.47 $g^{-1}$ respectively, In the treatment of addition of black oil, treatment (H1) was higher in wet weight while treatment (H2) was higher in dry weight. Interaction treatment achieved significant differences in the combination of H1M1 with the highest rate of 255.55 $g^{-1}$ in the wet weight of the vegetative group, In the dry weight, the combination of H2M1) and the highest rate of 72.71 $g^{-1}$.

### 3. Wet and dry weight of roots in carrot plant (g $pot^{-1}$)

Table 4 shows significant superiority of wet and dry root weight treatment compared with non-inoculom treatment. Significant increase in root weight ratio was observed for the addition of black oil to both weights. The highest wet and dry weight was obtained at the concentration of 2%, As for the treatment of overlap, H2M1 obtained the highest treatment parameters for both wet and dry. On the other hand, the phenotypic shape of the roots, as shown in Image 1, was observed in the addition of black oil, as we find the deformation of the roots, irregular shape, small size and irregularity of their secondary root branches as well as the abundance of root hairs in black treated roots compared to control treatment which did not Treated with black oil.

### 4. Concentration of absorbed hydrocarbons in vegetative and root groups

The results of the statistical analysis in Table (5) showed a significant difference (0.05) in the amount of hydrocarbons in the vegetative and root groups of *G. mosseae* treatment. It was also observed that there was a significant difference in the treatment of the addition of concentrations of black oil,.

Where the treatment was superior to the concentration H2 compared with the concentration of H1, and also observed moral superiority of the treatment of interaction between (M1 addition of black oil) gave the combination

H2M1 highest rate, while in the vegetative and root groups, while less The rate was at the H1M0 combination.

**5. Residual hydrocarbons in the soil of the carrot plant**

Table 6 showed significant difference in the amount of hydrocarbons remaining in the soil for the treatment of the addition of the inoculum. The treatment of the addition to the treatment without the inoculum was characterized by containing the lowest remaining amount of oil in the soil. A significant difference was also observed for the addition of oil. In H2 concentration, there was also a significant difference in the treatment of interaction between the treatments. The combination of H1M1 gave the lowest amount of hydrocarbons left in the soil, while more hydrocarbons were obtained in the soil at the H2M0 combination.

Table (7) shows the percentages of added black oil and decomposed oil removed by the plant and remaining in the soil and the percentage of total removal using Carrot plant and *G. mosseae*. It was found that the rate of removal of black oil compounds in the carrot plant at the rate of adding 100 ml 10 $kg^{-1}$ of soil amounted to 39.2 and 26.27 ml 10 $kg^{-1}$ soil with the use of *G. mosseae* or without it respectively. It was also found that the remaining in the soil of the black oil analyzer G. *mosseae* was 21.4 and 28.60 ml 10 $kg^{-1}$ soil with the vaccine and primer respectively. While the rate of decomposition of added black oil reached to 39.4 and 45.13 ml 10 kg $^{-1}$ soil by the activity of living and plant with the presence of *G. mosseae* and without them sequentially. The total removal was 78.6% and 70.4% or less. When adding 200 ml 10 $kg^{-1}$ of black oil, it reached to 48.39 and 35.85 ml 10 $kg^{-1}$ soil with the use of *G. mosseae* or without it respectively. It was also found that the remaining in the soil of the black oil analyzer *G. mosseae* was 50.8 and 60.6 ml 10 $kg^{-1}$ soil with the vaccine and primer respectively While the rate of decomposition of added black oil reached to 100.81 and 103.55 ml 10 kg $^{-1}$ soil by the activity of living and plant with the presence of G. *mosseae* and without them sequentially. The total removal was 74.6% and 69.7% or less.

**Table 2 :** Effect of inoculum *G. mosseae* and concentration of black oil in the number of spores in the soil of the carrot plant , Ratio of infection roots

| Number of spores in the soil | | | |
|---|---|---|---|
| **Average** | ***G. mosseae* M1** | **Without *G. mosseae* M0** | **Black OIL %** |
| 25.17 | 38.00 | 12.33 | H1 =0 |
| 29.50 | 42.00 | 17.00 | H2 =1 |
| 30.50 | 44.33 | 16.67 | H3 =2 |
| | 41.44 | 15.33 | Average |
| LSD(0.05) M=2.481 , H= 3.038, MH= 4.297 | | | |
| Ratio of infection roots % | | | |
| 50.83 | 64.77 | 36.90 | H1 =0 |
| 60.30 | 72.17 | 48.43 | H2 =1 |
| 52.55 | 64.10 | 41.00 | H3 =2 |
| | 67.01 | 42.11 | Average |
| LSD(0.05) M=2.416 , H= 2.958, MH= 4.148 | | | |

**Table 3 :** Effect of *G. mosseae* and concentration of black oil in the wet and dry weight of the total vegetation of the carrot plant (g $pot^{-1}$)

| Wet weight | | | |
|---|---|---|---|
| **Average** | ***G. mosseae* M1** | **Without *G. mosseae* M0** | **Black OIL %** |
| 176.16 | 195.02 | 157.31 | H1 =0 |
| 235.50 | 255.55 | 215.46 | H2 =1 |
| 196.43 | 210.37 | 182.49 | H3 =2 |
| | 220.31 | 185.09 | Average |
| LSD(0.05) M=3.708 , H= 4.541, MH= 6.422 | | | |
| Dry weight | | | |
| 44.38 | 51.06 | 37.69 | H1 =0 |
| 51.73 | 58.10 | 45.39 | H2 =1 |
| 61.53 | 72.71 | 50.34 | H3 =2 |
| | 60.60 | 44.47 | Average |
| LSD(0.05) M=2.342 , H= 2.869, MH= 4.057 | | | |

**Table 4 :** The effect of *G. mosseae* and the concentration of black oil in the wet and dry weight of the root mass of the carrot plant (g $pot^{-1}$)

| Wet weight | | | |
|---|---|---|---|
| **Average** | ***G. mosseae* M1** | **Without *G. mosseae* M0** | **Black OIL %** |
| 144.43 | 148.32 | 140.54 | H1 =0 |
| 160.07 | 165.22 | 154.92 | H2 =1 |
| 171.68 | 181.03 | 162.34 | H3 =2 |
| | 164.86 | 152.60 | Average |
| LSD(0.05) M=3.563 , H= 4.364, MH= 6.171 | | | |
| Dry weight | | | |
| 38.80 | 41.18 | 36.42 | H1 =0 |
| 42.83 | 46.84 | 38.81 | H2 =1 |
| 46.42 | 48.69 | 44.15 | H3 =2 |
| | 45.57 | 39.79 | Average |
| LSD(0.05) M=3.264 , H= 3.998, MH= 5.654 | | | |

**Table 5 :** Effect of *G. mosseae* and H% in the proportion of hydrocarbons absorbed in the vegetative and root of carrot plant (%)

| wet weight, LSD(0.05) M=0.003 , H= 0.0032, MH= 0.004 | | | |
|---|---|---|---|
| **Average** | ***G. mosseae* M1** | **Without *G. mosseae* M0** | **Black OIL %** |
| 0.326 | 0.344 | 0.308 | H2 =1 |
| 0.340 | 0.360 | 0.320 | H3 =2 |
| | 0.352 | 0.314 | Average |
| Dry weight, LSD(0.05) M=0.005 , H= 0.0052, MH= 0.008 | | | |
| 0.367 | 0.410 | 0.319 | H2 =1 |
| 0.410 | 0.440 | 0.380 | H3 =2 |
| | 0.425 | 0.349 | Average |

**Table 6 :** The effect of *G. mosseae* and the concentration of black oil in the amount of residual hydrocarbons in carrot plant soil (ml-pot$^{-1}$ 10 soil)

| Average | *G. mosseae* M1 | Without *G. mosseae* M0 | Black OIL % |
|---|---|---|---|
| 25.18 | 21.77 | 28.59 | H1 =1 |
| 55.56 | 50.53 | 60.60 | H2 =2 |
| | 36.15 | 44.60 | Average |
| LSD(0.05) M=2.46 , H= 2.54, MH= 3.48 | | | |

**Table 7 :** Percentage of black oil added, removed, decomposed and remaining in the soil

| Rmoved total | | Decomposed in soil | | Remaining soil | | Removed plant | | Black OIL ml 10 kg soil$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| M0 | M1 | M0 | M1 | M0 | M1 | M0 | M1 | |
| 71.40 | 78.60 | 45.13 | 39.40 | 28.60 | 21.40 | 26.27 | 39.20 | H1 =100 |
| 69.70 | 74.60 | 103.55 | 108.81 | 60.60 | 50.80 | 35.85 | 48.39 | H2 =200 |

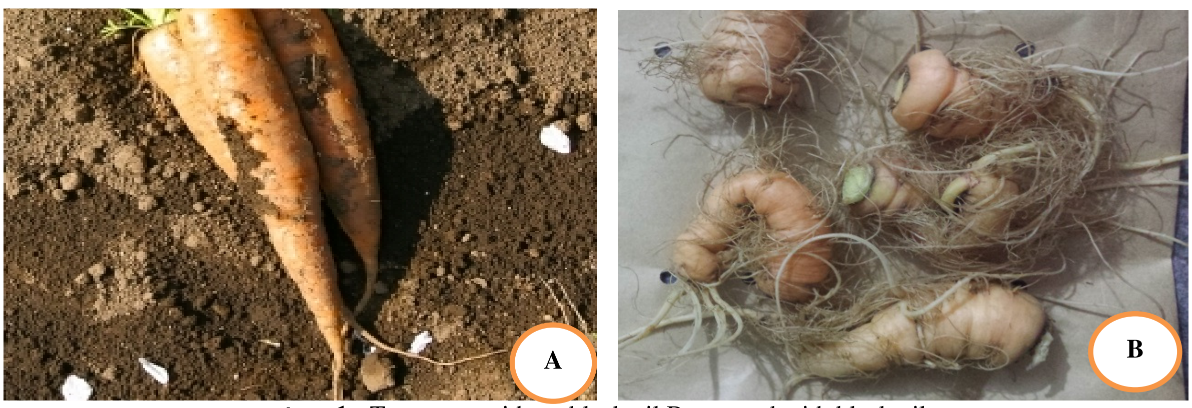


**Fig. 1** - Treatment without black oil B - treated with black oil

## Discussion

The reason for the increase in the number of spores in the soil for the treatment of Inoculum is due to the processing of M1 treatment, in addition to increasing the incidence of roots infected root fungi and the introduction of roots to the quantities of sugars and reducing the amino acids that lead to the formation of these fungi (Driai, 2015). Treatment of the addition of oil The increase in the number of blackboards is attributed to the roots of the plant is increasing its secretions in the presence of PAHs and this affects the amount of production of spores, Aranda and others (2013). The study shows the cause of the increase in the rate of infection of the roots of the carrot when adding *G. mosseae* as the fungi increases the secretion of amino acids and sugars reduced from the roots, which lead to the formation of fungi and thus increase the percentage of infection in the roots, (Samurai, 2003), while the treatment of oil studies have shown Mycorrhiza is not adversely affected by the presence of hydrocarbons, in addition to the role of root secretions that increase the incidence of infection( Geng *et al*., 2003). The reason for the increase in the total weight of the plant with the addition of *G. mosseae* to its efficiency in infecting the roots of the plant and adapting to the conditions of the soil, which helped to provide the essential elements as *G. mosseae* increase the readiness of phosphorus and other elements in the soil and its availability and ease of absorption by the plant and thus increase biomass (His Driai *et al*., 2015), The reason for the difference with the addition of oil levels and the treatment of interference is due to the presence of oil as an organic matter in the soil, which increases the production of the enzyme in the plant, which works on the division of the larger cell and thus increase the weight of the plant. which increases the production of the enzyme in the plant, which works on the division of the larger cell and thus increase the weight of the plant and corresponds to this study with Hamdi (2012). As for the wet and dry weight of the roots, due to the high moral differences in the treatment of the addition of fungal, works to improve metabolic processes, which reflects positively on the function and growth status of the plant, the secretion of growth organizations have an important role in the elongation of cells such as gibberellin, GA3, (Alguacil *et al*. 2011). The reason for the increase in root weight for the addition of oil is that most fungal species are able to grow in the medium containing crude oil as the sole source of carbon and energy (Luepromchai *et al*., 2007). The increase in root weight for the addition of black oil is due to the fact that the plants make IAA, and ethylene compound, in response to exposure to different types of environmental stresses (Glick, 2014). PAHs compounds stimulate the secretion of root secretions and increase the symbiotic relationship between *G. mosseae* and plants in the exchange of benefit and nutrients between them and thus increase the soft and dry weight of the roots by Chibuike *et al*. (2013). The reason for the significant difference in the treatment of *G. mosseae* in the vegetative and root fraction is due to the role of *G. mosseae* in the promotion of soil microbes around the roots, in particular the presence of hydrocarbons in them. Plants have different effects on micro-organisms in the rhizosphere and their degradation of soil hydrocarbons (Merkl et al. 2005). As for the treatment of the addition of oil, it has been shown that soil occurs in the soil. This process promotes the activity of microorganisms analyzing hydrocarbons, such as bacteria and fungi through the secretions of the root evoked by the presence of oil. So the plant and microbial interactions have significant importance because of the ability of the microbial bioaccumulation of pollutants (Borukhova 2014), A significant difference in the amount of hydrocarbons for the treatment of root interference is due to the fact that *G. mosseae* enhances enzymatic activity and non-enzymatic antioxidants in plants that grow in soils contaminated with hydrocarbons and petroleum products (Rajtor and Piotrowska-Seget, 2016 Giving *G. mosseae* a greater chance of removing as much of the hydrocarbons in the soil as possible with the host plant, (Gao *et al*., 2010).

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