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Use of fenugreek plant Trigonella *foenum-graecum* L. and spraying with melatonin and glutathione in treating soils contaminated with petroleum hydrocarbons

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Abstract

The experiment was conducted during the 2023-2024 growing season in the College of Education - Al-Qurna / University of Basrah fields. To treat soils contaminated with crude oil in Basrah Governorate and to study the effect of oxidative stress of crude oil and antioxidants melatonin and glutathione on some vegetative growth traits. The study examined the effects of two factors: soil contamination with crude oil at concentrations of 0, 2, 4, and 6 g/kg, and three spray treatments – water (control), melatonin, and glutathione at concentrations of 100 and 200 mg/L. The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replicates, and the averages were compared at a significance level of 0.05. Following the analysis of experimental measurements taken after plant growth, the results indicated that plants exhibited tolerance and resistance to varying crude oil concentrations. However, as crude oil levels increased, vegetative growth indicators, including plant height, fresh weight, and dry weight, showed a noticeable decline. This highlights the negative impact of higher crude oil concentrations on plant growth and development. The results of external treatment with melatonin and glutathione were an increase in the above characteristics, as the control plants were sprayed with melatonin and glutathione at a concentration of 200 mg. L-1 gave the highest plant height and fresh and dry weights, reaching 46.00 and 50.33 cm, 7.39 and 6.63 g, and 1.08 and 1.62 g, respectively. As for the content of total hydrocarbons TPHs in the root system and remaining in the soil after planting for plants, it increased with increasing concentrations of crude oil in the soil. The percentage of total treatment with spraying plants with melatonin at a concentration of 200 mg. L-1 reached 95.24, 95.33, 87.78, and 79.09% respectively, and sprayed with glutathione at a concentration of 200 mg. L-1 reached 95.52, 96.13, 86.95 and 80.10%.

Keyword: Fenugreek; crude oil; melatonin; Glutathione.

I. Introduction

The Phytoremediation is a green technology that utilizes plants to eliminate organic and inorganic contaminants from soil and water. Petroleum hydrocarbons are among the most significant organic pollutants, posing serious risks to various life forms (Abha and Singh, 2012; Al-Ali *et al.*, 2016). These pollutants are introduced into the environment through exploration, transportation, industrial processes, spills, and gas emissions (Al-Atbee, 2018).

Soil pollution, particularly in agricultural areas, has become a growing concern due to the risk of pollutant transfer to crops and subsequent entry into the food chain, threatening human health (Huang *et al.*, 2007). Abbasian *et al.*, (2016) found that oil pollutants negatively impact soil permeability and water quality, hinder microbial activity, and alter the soil's chemical composition and nutrient availability.

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Environmental contaminants can disrupt plant metabolic processes, such as photosynthesis, carbohydrate synthesis, gas exchange, and respiration. This occurs by damaging mitochondrial membranes and impairing the natural function of biological systems (Ghalachyan *et al.*, 2014). Scientists have shown that there are many plant species used in phytoremediation and environmental restoration (Saum *et al.*, 2018), which are characterized by dense vegetative growth, deep roots, large biomass, and the ability to accumulate pollutants in their tissues without harm, including local herbaceous plants, fenugreek *Trigonella foenum-graecum* L., which is a local plant belonging to the legume family Fabaceae. It is one of the widely spread herbaceous plants in nature. The fenugreek plant is described as having a branched, highly branched stem and tripartite leaves, its roots are taproots containing root nodules, and it can fix nitrogen. Toledo Ramos *et al.*, (2009) conducted a study on the phytoremediation of petroleum hydrocarbons using *Sebastiania commersoniana*. The plant was tested at three crude oil concentrations – 25, 50, and 75 g/kg of soil – achieving a removal rate of up to 60%.

Qasim (2019) conducted a study on the phytoremediation of petroleum hydrocarbons using three plant species: barley (Hordeum vulgare), Medicago sativa, and Portulaca grandiflora. The plants were exposed to crude oil concentrations of 20, 50, 75, and 100 g/kg of soil. The results indicated varying levels of efficiency in crude oil removal, with barley proving to be the most effective. Athfua and Hamid (2023) examined the phytoremediation potential of three native plants adapted to the Basra environment for treating crude oil-contaminated soil. The study focused on tamarisk (*Tamarix aphylla*), queen of the night (*Cestrum nocturnum*), and ain al-bazun (*Catharanthus roseus*), evaluating their capacity to mitigate soil contamination. The study showed the superiority of tamarisk in treating soil contaminated with crude oil. To stimulate the vegetative growth of plants and increase their efficiency in absorbing pollutants, researchers turned to using biostimulants such as amino acids and hormones such as Melatonin and glutathione. (Al-Tamimi, 2021) observed that when treating the soil of two leguminous plants, namely Jerusalem thistle Parkinsonia aculeata L. and Damascus thistle Acacia farnesiana L., with crude oil at concentrations of 20, 40 and 60 g. kg-1 soil and spraying them with glutathione at concentrations of 100 and 200 mg. L-1, the growth characteristics were improved and the ability of the two plants to remove pollutants from the soil was increased. Ibrahim (2024) showed that treating hot pepper plants with melatonin and glutathione under conditions of heavy element pollution was superior to plants sprayed with the two compounds in their vegetative characteristics compared to the control treatment.

II. . MAJOR FORMAT GUIDELINES

The study was conducted in the fields of the College of Education - Al-Qurna, University of Basra-Al-Qurna District, north of Basra Governorate, in the winter season 2023-2024, in an agricultural medium consisting of soil brought from agricultural fields north of Basra Governorate, and peat moss produced by the German company at a ratio of 1:3. Its physical and chemical properties and texture were estimated in the laboratories of the Department of Soil and Water Sciences. Marine Sciences Center. University of Basra, as shown in Table (1). The content of petroleum hydrocarbons was estimated in the Nahran Omar laboratory as shown in Table (2). 180 pots with a diameter of 22 cm and a height of 24 cm were prepared. Fenugreek seeds were obtained from local markets. After a test was conducted to determine the germination rate by taking a random sample of 100 seeds, every 25 seeds were placed in a Petri dish containing a filter paper saturated with distilled water in the laboratory at room temperature for three days. After that, the germinated seeds were calculated and the germination rate was 100%. The agricultural medium was treated with crude oil brought from the West Qurna 2 field and an analysis of its components was conducted in the Nahran Omar laboratory as shown in Table (2). 54 kg were weighed for each treatment (9 pots for each treatment x soil weight in the pot 6 kg). Crude oil was added to the agricultural medium at the required concentrations, which are 0, 2, 4, 6 g. kg $soil^{-1}$. Then the pots were filled with the agricultural medium at a rate of 6 kg for each pot. The seeds were planted directly in the pots were planted two days after treatment with crude oil on 11/10/2023, by placing ten seeds in each pot.

Irrigation was carried out immediately after planting and thinning was carried out 20 days after planting by leaving six plants in the pot. All agricultural operations followed in producing this crop were

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continued, including irrigation, weeding, and hoeing. Irrigation was carried out according to the plant's needs. Weeding was also carried out for the bushes by pulling them out by hand whenever necessary. The plants were sprayed preventively to combat biting insects, aphids, and whiteflies using the pesticide Avaunt at a concentration of 25 ml. 100 L^{-1}. To prevent fungal diseases, the pesticide Dithene was used at a concentration of 2 g. L^{-1}.

The experiment involved twenty factorial treatments, combining five factors: a control (using only RO water), glutathione at concentrations of 100 and 200 mg/L, melatonin at 100 and 200 mg/L, and four levels of crude oil contamination (0, 2, 4, and 6 g/kg of soil). Aqueous solutions of the specified chemicals were prepared at the required concentrations. To enhance the spray distribution, a few drops of Tween 20 were added as a spreading agent. The plants were sprayed in the early morning, targeting the vegetative parts until fully saturated, using a 2-liter hand sprayer.

The vegetative group underwent two spray treatments – the first was applied 30 days after planting, and the second followed 15 days later. The experiment was designed as a split-plot layout, with crude oil concentrations serving as the main plot factor, while glutathione and melatonin concentrations were treated as sub-plot factors. A randomized complete block design (R.C.B.D.) was employed, with three replicates and an average of three pots per replicate. Data were analyzed using analysis of variance (ANOVA), and the Revised Least Significant Differences Test (R.L.S.D.) was conducted to compare means at a significance level of 0.05, following the methodology of Al-Rawi and Abdul-Aziz (1980).

TABLE (1): SOME PHYSICAL AND CHEMICAL PROPERTIES OF AGRICULTURAL SOIL

Attributes	Value
Electrical conductivity (E.C) deciSiemens.m-1	6.12
(pH)	7.66
Total nitrogen (g.kg-1)	1.30
Ready phosphorus (mg.kg-1)	21.20
Ready potassium (mg.kg-1)	67.94
Organic matter(%)	1.30
Petroleum hydrocarbons TPH (mg.kg-1)	32.6
Soil separations(%)	
Sand	20.3
Silt	67.2
Clay	39.9
soil texture	Silty Clay

A. Experimental Measurements

Field readings were taken for plants at the end of the experiment from each experimental unit, then the average was calculated for each plant, and they included the following: - Plant height (cm), fresh weight

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of the plant (g), dry weight (g), total content of hydrocarbons in the soil TPHs (mg. kg^{-1}), and the percentage of total treatment of the soil %.

III. . RESULTS AND DISCUSSION

Table 2 demonstrates that crude oil levels, melatonin, and glutathione spray treatments, along with their interactions, significantly influenced plant height. The results indicate that increasing crude oil concentrations led to a notable reduction in plant height compared to the control (plants not exposed to crude oil). The lowest average plant height (25.33 cm) was observed in plants subjected to 6 g/kg of crude oil, whereas the tallest plants (34.20 cm) were grown in uncontaminated soil. Notably, plants exposed to 2 g/kg of crude oil exhibited a similar height (34.20 cm) to the control, indicating no significant difference between these treatments.

The table further highlights the positive impact of melatonin and glutathione spray treatments on plant height. Plants treated with glutathione at 200 mg/L achieved the greatest average height of 44.83 cm, outperforming all other treatments, except for those sprayed with melatonin at 200 mg/L, which resulted in an average height of 42.75 cm – a difference that was not statistically significant.

The interaction between the two factors (oil levels and spray treatments) also had a pronounced effect. Plants sprayed with glutathione at 200 mg/L and grown in uncontaminated soil achieved the highest recorded height of 50.33 cm. In contrast, the shortest plants (18.00 cm) were those that received no glutathione or melatonin and were cultivated in soil containing 6 g/kg of crude oil.

TABLE (2): EFFECT OF OXIDATIVE STRESS OF CRUDE OIL AND TREATMENT WITH MELATONIN AND GLUTATHIONE ON PLANT HEIGHT (CM)

Antioxidants (mg/L^{-1})	Crude oil concentrations (g.kg^{-1})				Average antioxidant effect
	0	2	4	6	
Zero (comparison factor)	23.33	22.33	21.33	18.00	21.25
100 Melatonin	24.00	26.00	23.67	20.00	23.42
200 Melatonin	46.00	46.67	43.33	35.00	42.75
100 Glutathione	27.33	27.00	24.00	19.00	24.33
200 Glutathione	50.33	49.00	45.33	34.67	44.83
Average effect of crude oil	34.20	34.20	31.53	25.33	
L.S.D. 0.05	Antioxidants		Crude oil		Antioxidants × Crude Oil
	2.42		2.17		4.85

A. Fresh weight of the green part of fenugreek plant (g)

Table 3 illustrates the main effects of crude oil, melatonin, glutathione spray treatments, and their interactions on the fresh weight of the vegetative group. Plants exposed to crude oil exhibited significantly lower fresh weight compared to the control group (plants not exposed to crude oil). The lowest fresh weight (4.90 g) was recorded in plants grown in soil contaminated with 6 g/kg of crude oil.

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In contrast, the highest fresh weight (5.96 g) was observed in plants not exposed to crude oil. This value did not differ significantly from plants treated with 2 g/kg (5.61 g) and 4 g/kg (5.38 g) of crude oil.

The table also indicates that spraying plants with melatonin and glutathione at both concentrations enhanced fresh weight compared to untreated plants. The highest fresh weight (6.77 g) was recorded in plants sprayed with 200 mg/L of melatonin. This was not significantly different from plants sprayed with 200 mg/L of glutathione, which achieved a fresh weight of 6.50 g.

The interaction between oil contamination and spray treatments produced a significant effect on plant fresh weight. Plants sprayed with 200 mg/L of melatonin and grown in uncontaminated soil had the highest fresh weight, reaching 7.39 g. Conversely, the lowest fresh weight (3.42 g) was recorded in plants grown in soil contaminated with 6 g/kg of crude oil and not subjected to any spray treatments.

TABLE (3): EFFECT OF OXIDATIVE STRESS OF CRUDE OIL AND TREATMENT WITH MELATONIN AND GLUTATHIONE ON FRESH WEIGHT OF PLANT (G)

Antioxidants (mg/L^{-1})	**Crude oil concentrations (g.kg^{-1})**				**Average antioxidant effect**
	0	2	4	6	
Zero (comparison factor)	4.91	4.36	3.86	3.42	4.14
100 Melatonin	5.46	4.84	4.58	4.25	4.78
200 Melatonin	7.39	6.98	6.53	6.19	6.77
100 Glutathione	5.42	4.80	5.42	4.87	5.13
200 Glutathione	6.63	7.08	6.53	5.77	6.50
Average effect of crude oil	5.96	5.61	5.38	4.90	
L.S.D. 0.05	Antioxidants		Crude oil		Antioxidants × Crude Oil
	0.48		0.43		0.97

B. Dry weight of the vegetative group of fenugreek plant (g)

The results presented in Table 4 indicate that crude oil contamination, melatonin, glutathione treatments, and their interactions significantly affected the dry weight of the vegetative parts of fenugreek plants. As crude oil concentration increased, the dry weight of plants decreased. The lowest dry weight (0.92 g) was observed in plants grown in soil with 6 g/kg of crude oil, which did not differ significantly from plants treated with 4 g/kg (0.97 g). Conversely, the highest dry weight (1.11 g) was recorded in plants grown in uncontaminated soil, showing no significant difference from those grown in soil treated with 2 g/kg of crude oil (1.10 g).

The table further reveals that plants sprayed with 200 mg/L of glutathione or melatonin had significantly higher dry weights compared to other spray treatments. The highest dry weights were 1.43 g and 1.32 g for glutathione and melatonin treatments, respectively, with no significant difference between the two.

The interaction between crude oil levels and spray treatments also showed a notable effect. Plants sprayed with 200 mg/L of glutathione and grown in uncontaminated soil exhibited the highest dry weight (1.62 g). In contrast, the lowest dry weight (0.31 g) was recorded in plants grown in soil contaminated with 2

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g/kg of crude oil and not treated with melatonin or glutathione. This was not significantly different from plants grown in soil contaminated with 6 g/kg of crude oil, which had a dry weight of 0.44 g.

TABLE (4): EFFECT OF OXIDATIVE STRESS OF CRUDE OIL AND TREATMENT WITH MELATONIN AND GLUTATHIONE ON DRY WEIGHT OF PLANT (G)

Antioxidants (mg/L^{-1})	**Crude oil concentrations (g.kg^{-1})**				**Average antioxidant effect**
	0	2	4	6	
Zero (comparison factor)	0.75	0.31	0.60	0.44	0.52
100 Melatonin	1.01	0.95	0.90	0.83	0.92
200 Melatonin	1.08	1.41	1.31	1.47	1.32
100 Glutathione	1.11	0.95	0.89	0.82	0.94
200 Glutathione	1.62	1.91	1.17	1.02	1.43
Average effect of crude oil	1.11	1.10	0.97	0.92	
L.S.D. 0.05	Antioxidants		Crude oil		Antioxidants × Crude Oil
	0.08		0.07		0.16

The results from Tables 2, 3, and 4 showed a decrease in vegetative growth indicators when treating the soil with crude oil, and the effect increased with increasing oil concentration in the soil. The reason is due to the effect of petroleum hydrocarbons in reducing the content of chlorophyll pigments in the leaves Table (5), which led to inhibiting the photosynthesis process and affected vegetative growth, which reduced plant height and fresh and dry weights, as petroleum hydrocarbons hinder the absorption of water by plants, which causes a decrease in the turgor pressure of the cells and reduces their elongation and reduces plant height (Lisar *et al.*, 2012). It may lead to suffocation of the roots due to the oil filling the pores, as it reduces their absorption, which causes a decrease in plant growth (Odjegba and Atebe, 2007). It also affects the permeability and chemical composition of the soil and reduces its readiness for nutrients, in addition to its negative effects on the activities of microorganisms (Abbasian *et al.*, 2016). All of these factors may be the reason for reducing plant height and weight. Fresh and dry. The reason for the increase in vegetative growth indicators when spraying with glutathione and melatonin is due to their role in increasing the plant's ability to confront both biotic and abiotic stresses, as they are antioxidants (Rouhier *et al.*, 2008). Glutathione plays a role in the formation of salicylic acid (Rouhier *et al.*, 2008). This acid increases the photosynthesis process, which leads to an increase in vegetative characteristics and works to increase cytokinin, which causes rapid cell division and works to increase the efficiency of the roots in absorbing nutrients. This is consistent with Al-Hayani (2015) on the mung bean plant and consistent with Al-Tamimi (2021) on the Sham thistle and Jerusalem thistle plants. Melatonin also has effects similar to auxin and works to regulate plant growth and development under stress conditions.

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C. Total Content of Petroleum Hydrocarbons in Soil After Planting

Table (5) reveals that crude oil levels, melatonin, glutathione spray treatments, and their interactions significantly influenced the percentage of petroleum hydrocarbons in the soil. Higher crude oil concentrations corresponded with increased petroleum hydrocarbon levels. The highest concentration (31.41 µg/g) was observed in soil treated with 6 g/kg of crude oil, while the lowest concentration (3.09 µg/g) was recorded in soil without crude oil contamination.

The table also highlights the notable effect of melatonin and glutathione in reducing petroleum hydrocarbon concentrations across all treatment levels. Among the spray treatments, plants sprayed with 200 mg/L of glutathione demonstrated the most substantial reduction in hydrocarbons, with soil concentrations measuring 10.89 µg/g. This was not significantly different from the melatonin spray treatment at 200 mg/L, which resulted in a hydrocarbon concentration of 11.22 µg/g.

The interaction between crude oil and spray treatments further underscored this trend. The lowest hydrocarbon concentration (1.48 µg/g) was found in the soil of plants sprayed with 200 mg/L of glutathione and grown in uncontaminated soil. This was closely followed by soil from plants sprayed with melatonin at the same concentration (1.58 µg/g). Conversely, the highest petroleum hydrocarbon concentration (41.52 µg/g) was observed in soil contaminated with 6 g/kg of crude oil and left untreated with spray solutions.

The results suggest that antioxidants, such as glutathione and melatonin, enhance vegetative growth, contributing to lower petroleum hydrocarbon levels in the soil, as evidenced by data from Tables 2, 3, and 4.

TABLE (5): EFFECT OF OXIDATIVE STRESS OF CRUDE OIL AND TREATMENT WITH MELATONIN AND GLUTATHIONE ON TPH REMAINING IN SOIL

Antioxidants (mg/L^{-1})	Crude oil concentrations (g.kg^{-1})				Average antioxidant effect
	0	2	4	6	
Zero (comparison factor)	4.90	14.16	22.22	41.52	20.70
100 Melatonin	3.95	11.15	19.43	35.14	17.42
200 Melatonin	1.58	5.16	14.12	24.03	11.22
100 Glutathione	3.56	10.03	20.15	33.48	16.80
200 Glutathione	1.48	4.22	15.01	22.87	10.89
Average effect of crude oil	3.09	8.94	18.19	31.41	
L.S.D. 0.05	Antioxidants		Crude oil		Antioxidants × Crude Oil
	0.16		0.14		0.32

D. Total Soil Treatment Percentage %

Table (6) shows the effect of crude oil and spraying with antioxidants melatonin and glutathione and their interactions on the percentage of total soil treatment, as the rate of the effect of crude oil at a concentration of 2 g. kg^{-1} soil in the total treatment of crude oil in the soil was significantly higher, reaching 91.47%. Compared to the rest of the oil concentrations 4 and 6 g. kg-1, which reached 84.16% and 72.67%, respectively. Also, the control soil treatment, not treated with crude oil, and the treatment with spray treatments were superior, as it gave a rate of 90.61%. The table shows the significant effect of the spray treatments in this characteristic, as the spray treatments with melatonin and glutathione were superior in the total treatment of crude oil in the soil at a concentration of 200 mg. L^{-1}, for each of them,

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as it gave a rate of (89.36)% and (89.67) % respectively. The interaction between the two study factors had a significant effect on this characteristic. Where the percentage of total treatment in the soil not treated with crude oil and which was sprayed with melatonin and glutathione at a concentration of 200 mg. L-1 exceeded (95.24) % and (95.52) % respectively. While the total treatment of crude oil decreased in the soil treated with crude oil at a concentration of 6 g. kg-1 soil and not treated with spray treatments, reaching (63.88) %.

TABLE (6): EFFECT OF OXIDATIVE STRESS OF CRUDE OIL AND TREATMENT WITH MELATONIN AND GLUTATHIONE ON TOTAL SOIL TREATMENT

Antioxidants (mg/L^{-1})	Crude oil concentrations (g.kg^{-1})				Average antioxidant effect
	0	2	4	6	
Zero (comparison factor)	85.10	86.87	80.55	63.88	79.10
100 Melatonin	88.19	89.28	83.08	69.44	82.49
200 Melatonin	95.24	95.33	87.78	79.09	89.36
100 Glutathione	89.01	89.76	82.47	70.86	83.02
200 Glutathione	95.52	96.13	86.95	80.10	89.67
Average effect of crude oil	90.61	91.47	84.16	72.67	
L.S.D. 0.05	Antioxidants		Crude oil		Antioxidants × Crude Oil
	0.22		0.19		0.44

The rates of plant treatment vary according to the concentrations due to the root secretions of different organic compounds that stimulate microorganisms to break down pollutants. Lu *et al.*, (2010) showed that there are two main factors that affect the analysis of oil, which are root secretions and aeration. Secretions are chemical compounds that act as biological factors that excrete metabolic enzymes for microorganisms, and the microbial enzyme resulting from the metabolic process is the basic substance that breaks down hydrocarbons. It is known that the legume family, including fenugreek, is characterized by a symbiotic relationship with nitrogen-fixing bacteria in the soil in the root nodules. During this process, an exchange of signals occurs between the bacteria and the host plant, after which the plant is allowed to penetrate its roots and the plant is stimulated to form new root nodules to fix nitrogen (Oldroyd *et al.*, 2011).

IV. . CONCLUSION

The study concludes that fenugreek is efficient in treating soil contaminated with crude oil and that treatment with glutathione and melatonin increases the activity of vegetative and root growth, which stimulates microorganisms in the root zone, leading to the breakdown of hydrocarbons by microorganisms. The study recommends the use of fenugreek in treating soil contaminated with oil, as well as the use of antioxidants. The study also recommends the use of other leguminous plants.

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