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Effect of Crude Oil on Seeds Germination, Some Morphological Parameters and Accumulation of *Gossypium hirsutum* and *Zea mays*

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ABSTRACT:

Background: This study investigated the effect of crude oil (six different concentrations) on seed germination of two plants (Zea mays and Gossipium hirsutum) and the growth of these plants when planted in oil-polluted soil. Total hydrocarbons were measured also.

Materials: Six concentrations of crude oil were prepared (2.5, 5, 7.5, 10, 12.5, and 15)%, 10 seeds of both plants were put in petri dishes of each concentration with three replicates. 75 gm of petroleum were mixed with 1 kg of soil; 5 kg of polluted soil were put in pots underlined with aluminum foil; the pots were watered and left for 72 hrs. Then seeds are put in, and plants are harvested after two months. One gram of ground parts of the plant is taken, adding 10 ml of 1:1 solvent (ethanol/ chlorophorm) with shaking for 30 min. Optical density read on (520 nm) .

Results: Results showed Z. mays seeds were less tolerant than those of G. hirsutum , the highest significant germination value was of 2.5 % for each plant while the lowest was for the concentration achieved when treated with 15 % for G. hirsutum and 12.5 % for Zea mays . that stem radius, leaf area, and height were significantly reduced as compared with the control. The results showed an accumulation of TH in the plants.

Keywords: Total hydrocarbons, *Gossypium hirsutum*, *Zea mays*, seed germination, and morphological parameters.

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INTRODUCTION:

The continuous development of the global economy and petroleum exploration leads to soil pollution with petroleum compounds, which has become a global concern, as petroleum products have been used as a major source of energy, resulting in significant environmental pollutants worldwide [1]. This results in pollution of groundwater underlying this soil with organic compounds that affect the agricultural crops' productivity and economy and leads to many problems because oil-polluted soil will lose its natural properties; it may require many years to recover [2].

Crude oil and its derivatives consist of oil hydrocarbons that affect soil by changing the physical and chemical nature of the polluted soil as a result of unsuitable conditions by changing the natural values of nutrients and minerals or toxicants [3]. The toxic effects are attributed to the oil's role in dissolving cell membrane lipids, resulting in the passage of cell internal components to the outside [4].

Economy affected in areas that depend on agricultural crops causes high rates of poverty and unemployment [5]. Oil-polluted soil will not be suitable for plant cultivation, according to the type of pollution, acute or chronic [6,7]. Most crops can't tolerate petroleum pollution, resulting in growth parameter reduction, especially in high total hydrocarbon concentrations [8]. It was reported that the plant growth parameters (leaf area, stem radius and height) all were reduced, especially at higher pollution concentrations [9]. *Zea mays* seed germination was reduced with crude oil pollution [7], also plants can concentrate hydrocarbons in their tissues which indicates that these soil pollutants may enter the food chain causing problems for human health [10].

MATERIALS AND METHODS:

Addition of crude oil to unpolluted soil:

75 gm of petroleum were mixed with 1 kg of soil, 5 kg of polluted soil were put in pots underlined with aluminum foil, pots were watered and left for 72 hrs. until they achieved the stable soil-oil state [11].

Seed germination

Six concentrations of crude oil were prepared (2.5, 5, 7.5, 10, 12.5, and 15)%, 10 seeds of both plants were put in petri dishes of each concentration with three replicates. Then two ml of distilled water and (Tween 80) were added also, in addition to a control group (seeds and distilled water). Then incubated in 30^{o} c for twenty days [11].

Morphological measurements:

Measurements were done after four weeks. Length was taken with measuring metric tape, while the radius of the stem was measured with a digital caliper and the area of the leaf was taken by a digital planimeter.

Total hydrocarbons extraction

One gram of ground parts of the plant taken, adding 10 ml of 1:1 solvent (ethanol / chlorophorm) with shaking for 30 min. Optical density read on (520 nm) [12].

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THE RESULTS AND DISCUSSION:

Seed germination:

The experiment involved six concentrations (2.5, 5, 7.5, 10, 12.5, and 15)% in addition to a control group (seeds with distilled water). Results showed *Z. mays* seeds were less tolerant than those of *G. hirsutum* , the highest significant germination value was of 2.5 % for each plant while the lowest was for the concentration achieved when treated with 15 % for *G. hirsutum* and 12.5 % for *Zea mays* Figure (1).

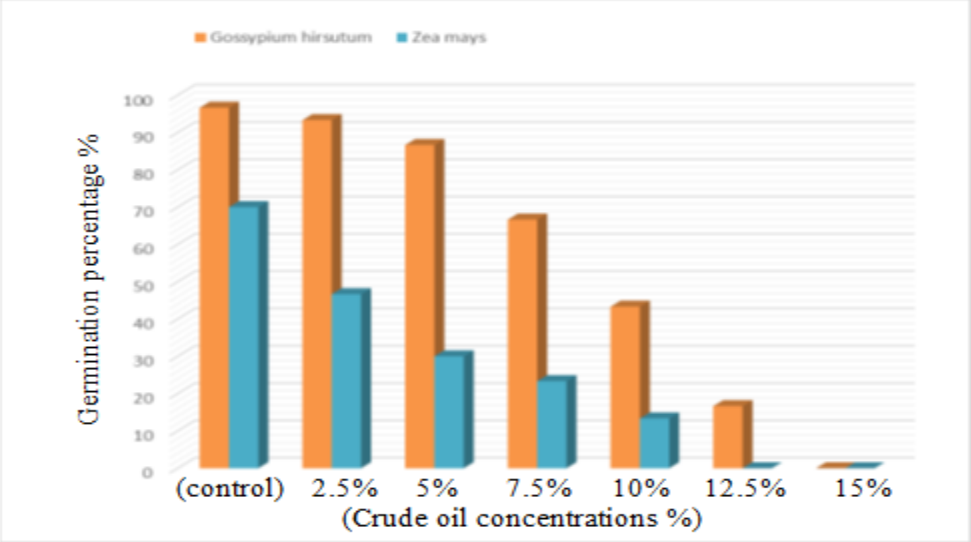

Figure 1: Percentage of seed germination in crude oil

Inhibition of germination is related to the degree of pollution and the existence of polar compounds because these compounds are dissolving in the water surrounding the seed then entering through the coat of the seed causing polar narcosis [13]. The treatment of seeds with crude oil leads to embryo injury because of the capacity of volatile compounds to penetrate the seed coat which stop germination [14].

Results of [15] reported that seed germination effected by crude oil negatively as recorded by many studies; four plans (*Z. mays*, *Vigna radiate*, *Sorghum vulgare* and *Pennisetum glaucum*) were treated with four concentrations of diesel (2.5, 5, 7.5 and 10)%, all of them tolerated the lower concentrations (2.5 and 5)%, total germination rates were (43.7 - 86.7)%, in the concentration (7.5) % there was a significant reduction in *Z. mays* seed germination (74) % , and *Pennisetum glaucum* (67) %. [16] also recorded that germination of *Z. mays* stopped at the concentration (10)% while *Sorghum bicolor* stopped at (5)%.

Morphological parameters

Morphological parameters of both plants were taken after two months of cultivating in polluted soil, results showed that stem radius, leaf area, and height were significantly reduced as compared with control tables (1) and (2).

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Table (1): Height, leaf area and stem radius of *G. hirsutum*

No	Treatments	Stem radius mm	Leaf area Cm^2	Hieght cm
1	Unpolluted soil + plant (control)	3.32±0.2 c	25.6±0.49 c	32±1.5 c
2	Polluted soil + plant	2.23±0.01 a	7.7±0.1 a	16.9±0.35 a
3	Polluted soil + plant + bacteria	2.43±0.03 b	9.5±0.2 b	20.03±0.45 b
4	Polluted soil + plant + fungi	2.32±0.15 ab	8.1±0.15 a	18.2±0.2 b
5	Polluted soil + plant+ bacteria+ fungi	2.44±0.04	9.5±0.2 b	20.2±0.25 b

* Each value represents mean ± standard deviation.
* The similar letters of each parameter refer to a non-significant difference $p > 0.05$.

Table (2): Height, leaf area and stem radius of *Z. mays*

No	Treatments	Stem radius mm	Leaf area Cm^2	Hieght cm
1	Unpolluted soil + plant (control)	3.74±0.31 b	82.1±3.7 c	35.3±1.9 c
2	Polluted soil + plant	2.1±0.02 a	31.4±2.98 a	23.7±7 a
3	Polluted soil + plant + bacteria	2.28±0.02 a	36.6±1.2 b	28±1.3 b
4	Polluted soil + plant + fungi	2.08±0.07 a	33±1.35 ab	23.8±0.7 a
5	Polluted soil + plant+ bacteria+ fungi	2.54±0.48 a	36.3±1.11 b	27.8±1.6 b

* Each value represents mean ± standard deviation.
* The similar letters of each parameter refer to a non-significant difference ($p > 0.05$.

Crude oil pollution is changing soil biological, chemical, and physical properties, which is reducing productivity and growth of crops [5]. Because of decreasing the availability of many nutrients that are necessary for growth [3]. [17] found that Maize could be formed form cob yield at a pollution percentage (21) % but it is less than untreated soil by (60)%.

Pollutants are passing inside the cell, reducing the selectivity of the cell membrane, resulting in cell death [18]. Low soil moisture is the other factor causing the reduction of plant biomass [19].

Results revealed a non-significant growth in the treatments with bacteria and the treatment of combination (bacteria and fungi) because of the activity of microorganisms in altering the soil pH, producing compounds against pathogens, and increasing the uptake of nutrients and water [20].

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Accumulation of total hydrocarbons (TH) in plants

The results showed an accumulation of TH in the plants. A significant accumulation decrease was recorded for plants that were treated with microorganisms when compared with the control. *Z. mays* accumulated hydrocarbons less than *G. hirsutum*.

Table 3: Total hydrocarbons concentrations (mg/gm) dry weight in *G. hirsutum* and *Z. mays* plants cultivated in crude oil-polluted soil after two months

No	Treatments	*Z. mays*	*G. hirsutum*
1	Unpolluted soil + plant (control)	N.D a	N.D a
2	Polluted soil + plant	0.0024±0.0003 c	0.0033±0.0006 c
3	Polluted soil + plant + bacteria	0.0015±0.0003 b	0.0023±0.0001 b
4	Polluted soil + plant + fungi	0.0018±0.0003 b	0.0025±0.0001 b
5	Polluted soil + plant+ bacteria+ fungi	0.0015±0.0002 b	0.0022±0.0001 b

* N.D = not detected
* Each value represents mean ± standard deviation.
* The similar litters of each parameter refer to non-significant difference $p > 0.05$.

The plants represent the first ring in the terrestrial food chain and web; they act as driving pump of solar radiation and pollutants purifying systems because they are uptaking water soluble pollutants by their roots and transporting or translocating them in their different tissues, then volatizing or sequestering them [21]. Bio-accumulation of pollutants depends on their concentrations in the ambient medium [20].

Author [12] used *Z. mays* was used by to remediate crude oil polluted soil, results revealed that the accumulation percentage was decreased when the concentration increased, this was explained by the activity of plant metabolism. The accumulation in *G. hirsutum* was higher than in *Z. mays* because plants can show different tolerance patterns to pollutants [22].

Z. mays is a c4 plant which has an increased photosynthesis rate compared to *G. hirsutum* which is belongs to c3 plant, c4 plants are growing with rapid rates and high biomass which reflects the high economic income in comparison with c3 plant [23]. C3 plants are less resistance for pollutants than c4 plants [24]. *Z. mayz* represents a typical c4 plant which needs less water than c3 plant that means less pollutant absorbance [25].

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Conclusions:

We concluded that *Zea mays* seeds were more sensitive to crude oil than *Gossypium hirsutum* seeds, while *Gossypium hirsutum* morphological parameters were reduced more than those of *Zea mays*. Also the accumulations of total hydrocarbons in *Zea mays* were less than in *Gossypium Hirsutum* which means that *Zea mayz* was most efficient in bioremediation of crude oil than *Gossypium Hirsutum*.

Conflict of interest.

There are non-conflicts of interest.

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