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EFFECT OF HUMIC AND SALICYLIC ACIDS ON DIFFERENT TREE TYPES GROWTH PLANTED IN OIL-CONTAMINATED SOIL

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SUMMARY

The recent study, carried out in 2022 in Bismayah City, Baghdad, Iraq, aimed to investigate the influence of humic and salicylic acids on the growth traits of different trees. The first factor was humic acid (H) with three levels - water spray (H_0 control), 5 g L^{-1} (H_1), and 10 g L^{-1} (H_2). The second factor was salicylic acid with three levels, i.e., water spray (S_0 control), 250 mg L^{-1} (S_1), and 500 mg L^{-1} (S_2). The third factor was four tree types, namely, *Eucalyptus camaldulensis* L. (T_1), *Albizia lebbeck* L. (T_2), *Ficus carica* L. (T_3), and *Morus nigra* L. (T_4). The study comprised a randomized complete block design with three replications. The results showed that humic acid, 10 g L^{-1} (H_2), significantly increased the stem diameter (28.20 mm), plant height (36.50 cm), shoot length (37.64 cm), and leaf chlorophyll content (29.45 mg g^{-1}). The salicylic acid spray of 500 mg L^{-1} (S_2) significantly enhanced the plant height (32.59 cm), shoot length (36.82 cm), and leaf chlorophyll content (28.22 mg g^{-1}). *Albizia lebbeck* L. (T_2) excelled with an enhanced stem diameter (34.54 mm) and plant height (46.77 cm). *Eucalyptus camaldulensis* L. (T_1) was superior with an extended shoot length (43.64 cm). However, *Ficus carica* L. (T_3) was superb in the leaf chlorophyll content (29.51 mg g^{-1}). The interactions among the study factors significantly affected all the studied vegetative growth traits.

Keywords: Humic acid, salicylic acid, phytoremediation, transplants, plant height, foliar application

Key findings: The studied traits significantly increased especially when sprayed with humic acid at 10 g L^{-1} and salicylic acid at 500 mg L^{-1}. Moreover, the transplants varied among themselves in vegetative growth characteristics.

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INTRODUCTION

Phytoremediation represents the plants' ability to treat pollutants. Some plants have developed their capabilities to remove some toxins selectively from the soil because of their adaptation to living in soils contaminated with heavy elements and oil residues, which includes the ability to absorb and regulate metal distribution within plant tissues (Gaur et al., 2021; Kafle et al., 2022). The phytoremediation process is a cheap and environmentally friendly method to reduce heavy metals' effects. Specific types of plants can accumulate large quantities of pollutants in their tissues without showing signs of toxicity. These plants can remove heavy elements polluting the soil if they contain biomass and sufficient requirements to complete the vital processing. Heavy metals exist in low concentrations within natural ecosystems in soil, indicating no problem with their presence. However, the problem appears when their concentrations in the soil increase to reach a degree of toxicity to humans, plants, or animals.

Excessive accumulation of heavy metals in the soil comes from natural or unnatural activities, such as wastewater and industrially polluted water usage, or as a result of air precipitation from vehicle exhausts, factories, or chemicals, such as dyes, medicines, and agricultural chemicals, production and packaging waste of factories, detergents, petrochemicals, hospital waste, homes, slaughterhouses, and farms. All these factors cause an increase in heavy metal concentrations, especially in the soil surface layer (Kanwal et al., 2019; Gaur et al., 2021; Kafle et al., 2022). An accumulation phenomenon is a natural process in some plants for amassing heavy metals without toxicity symptoms appearance. Nearly 400 plant species belonging to 45 families are distinct as accumulating plants, characterized by gathering heavy metals in the vegetative system to the root system better than one. Meanwhile, the percentage for non-accumulating plants is less than one; the absorption process of heavy elements occurs within the plant root system through unique mechanisms prepared for this purpose, representing a large part of processes and biomechanics that reduce toxic readiness of these elements in soils contaminated with them.

Trees are economical plant species for bioremediation because they have a deeper root system and have rapid growth and high transpiration rates, allowing them to remediate higher quantities of contaminants (Thawale et al., 2006; Yan, 2012). Phytoremediation of crude petroleum oil pollution primarily depended on plants' interaction with contaminated soils. Plants reduce the level of soil pollution by several mechanisms, including phytodegradation, phytostabilization, phytovolatilization, evapotranspiration, and phytoaccumulation, then soil reclamation happens (Nedjimi, 2021; Hussein et al., 2022). Plant roots can alter the soil's physical and chemical properties by secreting exudates that enhance microbial transformation and degradation of soil pollutants (Suman et al., 2018). The cheapest plant species for bioremediation are trees because of a deeper root system, ideal for sites with low fertility and poor structure, and have rapid growth and high transpiration rates, allowing them to remediate higher quantities of contaminants (Yan, 2012).

Humic acids are organic substances of complex chemical composition that are more stable than their parent substance; they result from the biochemical decomposition of animal and plant wastes by microorganisms, where hydrophilic aromatic compounds predominate. Humic acid application to soil or foliar spraying leads to an increase in plant growth by enhancing root and vegetative growth, enriching macro and micronutrients in the soil, and reducing soil pollution with toxic elements (Ali et al., 2014; Al-Marsoumi and Al-Hadethi, 2020). Numerous research studies indicated the positive effect of humic acid on many vegetative growth characteristics. Abdullah and Khalaf (2020) conducted an experiment that included adding three levels of humic acid spray (0, 5, and 10 g L^{-1}) on mulberry transplants (Morus nigra L.), showing the superiority of spraying humic acid versus the control treatment in the leaf dry weight, leaves' chlorophyll content, shoot length, and stem diameter. Mahmood et al. (2022) studied the effects of Powhumus at two concentrations (0 and 100 mg L^{-1}) on the growth of Albizia lebbeck L. seedlings. Results showed the Powhumus superiority in vegetative growth. Alghanim et al. (2023) studied the effect of humic acid application at three concentrations (0, 5, and 10 g $Tree^{-1}$) on the growth of apple trees, with results showing that adding humic acid gives better shoot length and leaf chlorophyll content than the control treatment.

Salicylic acid and its chemical composition, orthro-hydroxybenzoic acid, is one of the phytohormones produced by the plant. Its isolation for the first time materialized in 1828 in Munich City, Germany, from a willow tree bark calling it Salicin. Ten years later, a scientist, Raffaele Piria, named it Salicylic acid. Cinnamic acid is the main material for manufacturing salicylic acid in plants, and it plays an influential role in regulating most biological activities, such as plant growth, photosynthesis, and flowering (Al-Khafaji, 2014). Many studies indicated that spraying of salicylic acid to trees positively affected growth. Mazzuchelli et al. (2014), in their studies to investigate the effect of the salicylic acid application on the growth of eucalyptus (E. urophylla × E. grandis hybrid) seedlings when applied with four concentrations (without addition, 100 mg L^{-1}, 200 mg L^{-1}, and 300 mg L^{-1}), showed salicylic acid had a notable effect on increasing vegetative growth characteristics. Ibrahim et al. (2019) conducted an experiment that included spraying with salicylic acid (SA) at 50, 100, and 200 ppm once, twice, and thrice on the growth and fruiting of Sultani fig trees under Minia Governorate conditions. The control treatment, spraying SA at 50 to 200 ppm once, twice, or thrice, considerably improved all vegetative growth aspects and leaf chlorophyll contents compared with the control treatment. This study aimed to investigate the influence of spraying humic and salicylic acids in the growth of four types of transplants planted in soils contaminated with oil residues.

MATERIALS AND METHODS

This study began in Bismayah City, 10 km southeast of Baghdad, from 20 February to 31 December 2022, to investigate the influence of humic and salicylic acid sprays on four transplant types' growth and leaf chlorophyll content. The transplants were about two years old, healthy, and approximately the same vigor and size. The first factor is humic acid spray (H) at three levels comprising water-only spray (H_0), spraying with 5 g L^{-1} (H_1), and spraying with 10 g L^{-1} (H_2). The second factor is salicylic acid spray with three concentrations - water spray (S_0), spraying with 250 mg L^{-1} (S_1), and spraying with 500 mg L^{-1} (S_2). The third factor consisted of four tree types, Eucalyptus camaldulensis L. (T_1), Albizia lebbeck L. (T_2), Ficus carica L. (T_3), and Morus nigra L. (T_4). Spraying operations continued three times (twice in spring and once in October).

Traits measurement

The study measured the stem diameter (mm) using a Vernier at the start (20 February) and end of the experiment (31 December), calculating the difference between them. Using a metric tape, measuring plant height (cm) from each experimental unit occurred at the beginning and end of the experiment, with the difference between them calculated for an increase in plant height. For shoot length (cm), four branches' measurements from each experimental unit used a metric tape at the beginning and end of the experiment and then computed the difference between them for shoot length. For leaf chlorophyll contents (mg g-1 fresh weight), representative fresh leaf samples at the middle part of shoots taken in the first week of November for analysis of chlorophyll employed the calorimetric method, according to Mackinney (1941).

Statistical analysis

Treatments had three replications (three transplants in each experimental unit) at split blocks design in an RCBD. The number of transplants used was 324 (81 transplants for each plant type). The obtained results underwent an analysis of variance according to Elsahookie and Wuhaib (1990) using LSD 0.05 for comparing differences between various treatment means.

RESULTS AND DISCUSSION

Stem diameter

Data concerning the effect of treatments on increasing stem diameter are in Table 1. The data revealed that humic acid at 10 g L^{-1} (H_2) significantly increased stem diameter (28.20 mm), while lower values of increases in stem diameter were in the H_0 treatment (21.47 mm). The results showed that spraying of salicylic acid had no significant effect in increasing stem diameter. Transplants varied among them in stem diameter increases, as Albizia transplants excelled in stem diameter rise at 34.54 mm, while Fig transplants were the minimum for stem diameter (15.74 mm). The interactions between humic acid spray and transplant types significantly affected an increase in stem diameter, especially the interaction between Albizia transplants and humic acid spray at 10 g L^{-1} (H_2) at 40.35 mm, while the interaction between Fig transplants unsprayed gave the minimum stem diameter (14.98 mm).

Table 1. Effect of humic and salicylic acid spraying on increase in stem diameter (mm) of four transplants planted in soil contaminated with oil residues.

Tree type (T)	Humic acid (H)	Salicylic acid (S)			T × H
		S_0	S_1	S_2	
	H_0	22.75	23.57	24.92	23.75
Eucalyptus (T_1)	H_1	26.46	28.34	20.99	25.26
	H_2	32.96	33.81	35.84	34.20
	H_0	27.77	29.81	30.15	29.24
Albizia (T_2)	H_1	33.18	34.09	34.80	34.03
	H_2	39.01	40.78	41.26	40.35
	H_0	14.68	15.07	15.18	14.98
Fig (T_3)	H_1	15.31	15.50	15.90	15.57
	H_2	16.03	16.38	17.64	16.68
	H_0	17.58	18.02	18.16	17.92
Mulberry (T_4)	H_1	19.26	20.08	20.84	20.06
	H_2	21.03	21.38	22.24	21.55
$LSD_{0.05}$		4.46			2.56
T × S					T
Eucalyptus (T_1)		27.39	28.57	27.25	27.74
Albizia (T_2)		33.32	34.89	35.40	34.54
Fig (T_3)		15.34	15.65	16.24	15.74
Mulberry (T_4)		19.29	19.83	20.41	19.84
$LSD_{0.05}$		2.56			1.86
H × S					H
H_0		20.70	21.62	22.10	21.47
H_1		23.55	24.50	23.13	23.73
H_2		27.26	28.09	29.25	28.20
$LSD_{0.05}$		2.13			1.23
S		23.84	24.74	24.83	
$LSD_{0.05}$		N.S			

Table 2. Effect of humic and salicylic acid spraying on increase of plant height (cm) of four transplants planted in soil contaminated with oil residues.

Tree type (T)	Humic acid (H)	Salicylic acid (S)			T × H
		S_0	S_1	S_2	
	H_0	28.14	28.73	29.76	28.88
Eucalyptus (T_1)	H_1	30.04	30.81	31.81	30.88
	H_2	34.71	40.16	41.46	38.78
	H_0	34.43	36.36	38.22	36.33
Albizia (T_2)	H_1	41.70	45.07	47.87	44.88
	H_2	52.80	60.18	64.32	59.10
	H_0	16.90	17.55	18.23	17.56
Fig (T_3)	H_1	19.11	19.55	20.17	19.61
	H_2	19.98	20.40	22.07	20.81
	H_0	22.31	22.81	23.24	22.79
Mulberry (T_4)	H_1	23.73	23.94	24.56	24.08
	H_2	25.53	27.12	29.32	27.32
$LSD_{0.05}$		1.09			0.59
T × S					T
Eucalyptus (T_1)		30.96	33.23	34.34	32.85
Albizia (T_2)		42.97	47.20	50.14	46.77
Fig (T_3)		18.66	19.17	20.16	19.33
Mulberry (T_4)		23.86	24.62	25.71	24.73
$LSD_{0.05}$		0.59			0.39
H × S					H
H_0		25.44	26.36	27.36	26.39
H_1		28.64	29.84	31.10	29.86
H_2		33.25	36.96	39.29	36.50
$LSD_{0.05}$		0.56			0.32
S		29.11	31.06	32.59	
$LSD_{0.05}$		0.32			

The interactions between salicylic acid spray and transplants substantially affected the stem diameter, especially with the interaction treatment T_2S_2 giving 35.40 mm, while the interaction treatment T_3S_0 gave the minimum stem diameter of 15.34 mm. As for foliar spraying of humic acid and their interaction with salicylic acid spray, they showed a notable effect in stem diameter growth, especially the interaction treatment H_2S_2 at 29.25 mm compared to the control treatment (H_0S_0), which gave the lowest value for increase in stem diameter at 20.70 mm. Triple interactions between study factors significantly affected stem diameter, particularly the $T_2H_2S_2$ treatment, with the highest increase of 41.26 mm compared with the interaction treatment $T_3H_0S_0$ that gave the lowest values for this trait at 14.68 mm.

Plant height

The results indicated a significant increase in plant height with the spraying with humic acid, where the treatment H_2 gave the highest averages for this trait, amounting to 36.50 cm, significantly superior to the other two treatments (Table 2). The results further confirmed that an increase in spray concentrations of salicylic acid also boosts plant height, as the third treatment, 500 mg Transplants^{-1} (S_2), was significantly superior to treatments S_0 and S_1 at 32.59 cm. The Albizia transplants (T_2) gave the utmost plant height increase of 46.77 cm, while a lower value in plant height rise was in Fig transplants (T_3) (19.33 cm). Interactions between humic acid spray and transplant types markedly affected an elevation of plant height, especially the interaction treatment T_2H_2, which gave a maximum plant height of 59.10 cm. Meanwhile, a lower value of plant height was 17.56 cm in T_3H_0 interaction. As for spraying salicylic acid and its interaction with transplant types, the T_2S_2 treatment had the foremost plant height of 50.14 cm. A lower value of this trait of 18.66 cm resulted in the T_3S_0 interaction. The interaction between humic acid and salicylic acid spray notably boosted plant height, especially at the H_2S_2 treatment at the highest value of 39.29 cm compared with the control treatment (H_0S_0); giving the lowest value for this trait of 25.44 cm. Triple interactions between study factors remarkably caused an escalation of plant height, especially the $T_2H_2S_2$ treatment. It gave the maximum plant height of 64.32 cm versus the interaction treatment ($T_3H_0S_0$), with the lowest value for this trait of 16.90 cm.

Shoot length

The outcomes showed a significant increase in shoot length with the spraying with humic acid, where the treatment H_2 gave the highest averages for this trait, amounting to 37.64 cm, significantly superior to the two treatments (Table 3). Results also confirmed that an increase in spray concentrations of salicylic acid boosts shoot length, with the S_2 treatment remarkably superior to treatments S_0 and $S_{1,}$ which gave 36.82 cm. The Eucalyptus transplants (T_1) provided the highest increase in shoot length of 43.64 cm, while a lower increase in value for shoot length was in Fig transplants (T_3) (23.64 cm).

Table 3. Effect of humic and salicylic acid spraying on increase in shoot length (cm) of four transplants planted in soil contaminated with oil residues.

Tree type (T)	Humic acid (H)	Salicylic acid (S)			T × H
		S_0	S_1	S_2	
Eucalyptus (T_1)	H_0	40.69	41.49	43.05	41.74
	H_1	42.16	42.93	44.36	43.14
	H_2	44.50	45.24	48.39	46.04
Albizia (T_2)	H_0	36.86	37.52	38.27	37.54
	H_1	37.95	38.28	39.48	38.57
	H_2	39.70	42.21	47.19	43.03
Fig (T_3)	H_0	20.83	21.51	23.09	21.80
	H_1	22.00	22.89	24.26	23.05
	H_2	24.35	25.50	28.36	26.07
Mulberry (T_4)	H_0	30.81	31.49	33.03	31.77
	H_1	32.30	32.80	34.29	33.13
	H_2	34.39	35.47	38.19	36.01
$LSD_{0.05}$		0.58			0.37
T × S					T
Eucalyptus (T_1)		42.45	43.22	45.26	43.64
Albizia (T_2)		38.17	39.33	41.64	39.71
Fig (T_3)		22.39	23.30	25.23	23.64
Mulberry (T_4)		32.50	33.25	35.17	33.64
$LSD_{0.05}$		0.37			0.30
H × S					H
H_0		32.29	33.00	34.35	33.22
H_1		33.60	34.22	35.59	34.47
H_2		35.73	37.10	40.53	37.79
$LSD_{0.05}$		0.27			0.16
S		33.87	34.77	36.82	
$LSD_{0.05}$		0.16			

Table 4. Effect of humic and salicylic acid spraying on the leaf chlorophyll content (mg.g^{-1} fresh weight) of four transplants planted in soil contaminated with oil residues.

Tree type (T)	Humic acid (H)	Salicylic acid (S)			T × H
		S_0	S_1	S_2	
	H_0	24.32	24.57	24.98	24.62
Eucalyptus (T_1)	H_1	24.93	25.26	25.88	25.35
	H_2	25.33	26.14	27.31	26.26
	H_0	25.30	25.60	26.01	25.63
Albizia (T_2)	H_1	26.96	27.33	27.96	27.41
	H_2	28.41	29.28	30.21	29.30
	H_0	26.20	26.74	27.15	26.69
Fig (T_3)	H_1	28.89	29.46	29.98	29.44
	H_2	31.40	32.46	33.36	32.40
	H_0	25.84	26.16	26.56	26.18
Mulberry (T_4)	H_1	27.47	27.85	28.53	27.95
	H_2	28.95	29.83	30.78	29.85
$LSD_{0.05}$		0.20			0.10
T × S					T
Eucalyptus (T_1)		24.86	25.33	26.06	25.41
Albizia (T_2)		26.89	27.40	28.06	27.45
Fig (T_3)		28.83	29.55	30.16	29.51
Mulberry (T_4)		27.42	27.95	28.62	27.99
$LSD_{0.05}$		0.10			0.02
H × S					H
H_0		25.41	25.77	26.17	25.78
H_1		27.06	27.48	28.09	27.54
H_2		28.52	29.43	30.41	29.45
$LSD_{0.05}$		0.10			0.06
S		26.99	27.55	28.22	
$LSD_{0.05}$		0.06			

Interactions between humic acid spray and transplant types significantly influence boosting shoot length, especially the interaction treatment (T_1H_2), with the highest shoot length rise of 46.04 cm. Meanwhile, the lower value of shoot length was 21.80 cm in the T_3H_0 interaction. As for spraying salicylic acid and its interaction with transplant types, the T_1S_2 treatment gave the highest shoot length surge of 45.26 cm, while a lower value of this trait was 22.39 cm in the T_3S_0 interaction. Interactions between humic acid and salicylic acid spray meaningfully affected elevating shoot length, especially at the H_2S_2 treatment, with a maximum value of 40.53 cm compared with the control treatment (H_0S_0) with the lowest value for this trait of 32.29 cm. Triple interactions between study factors notably influenced shoot length buildup, especially the $T_1H_2S_2$ treatment. It gave the most shoot length extension of 48.39 cm versus the interaction treatment ($T_3H_0S_0$), with the minimum value for this trait of 20.83 cm.

Leaf chlorophyll content

The results indicated a significant increase in leaf chlorophyll content with the spraying with humic acid, where the H_2 treatment exhibited the highest averages for this trait, amounting to 29.45 mg g^{-1}, which was considerably superior to the other two treatments (Table 4). Results also confirmed that an upsurge in spray concentrations of salicylic acid boosts leaf chlorophyll content, as the S_2 treatment proved superior to treatments S_0 and S_1, which gave 28.22 mg g^{-1}. The Fig transplants (T_3) delivered the foremost leaf chlorophyll content of 29.51 mg g^{-1}, while the leaf chlorophyll content is lowest in Eucalyptus transplants (T_1) (25.41 mg g^{-1}). Interactions between the humic acid spray and transplant types significantly affected leaf chlorophyll content, especially the interaction treatment T_3H_2, which provided the maximum leaf chlorophyll content of 32.40 mg g^{-1}. A lower value of leaf chlorophyll content was 24.62 mg g^{-1} in the T_1H_0 interaction. As for spraying salicylic acid and its interaction with transplant types, the T_3S_2 treatment displayed the best leaf chlorophyll content of 30.16 mg g^{-1}, while a lower value of this trait was 24.86 mg g^{-1} in the T_1S_0 interaction. Interactions between humic acid and salicylic acid spray significantly influenced leaf chlorophyll content, especially at the H_2S_2 treatment, with the maximum value of 30.41 mg g^{-1} compared with the control treatment (H_0S_0), with the minimum value for this trait of 25.41 mg g^{-1}. Triple interactions between study factors remarkably affected leaf chlorophyll content, especially the $T_3H_2S_2$ treatment. It gave the foremost leaf chlorophyll content of 33.36 mg g^{-1} versus the interaction treatment $T_1H_0S_0$, which gave the lowest value for this trait of 24.32 mg g^{-1}.

These results can be due to the role of humic acid in physiological processes. This role encourages enzymes to transfer photosynthesis products and cell division and elongation (Al-Hayani and Al-Hadethi, 2023), increasing growth. Another reason for the increase in most studied vegetative traits may be due to the role of humic acid, which has a physiological action on plants similar to auxin, affecting plant growth (Jindo *et al*., 2012). As indicated by Pizzeghello *et al*. (2013), humic acid can play a similar role to cytokinin and gibberellin in cell division and elongation, as reflected in an increase in plants' vegetative growth. Salicylic acid's role in increasing vegetative traits is due to it boosting auxin and cytokinin contents (Shakirova *et al*., 2003; Popov *et al*., 2022; Dukenov *et al*., 2023). Auxins refer to one of the chief factors in the cambium activity within higher plants and increase cell division of meristematic cells, which leads to an upsurge in plant vegetative characteristics.

CONCLUSION

Foliar spraying of four transplant species with humic and salicylic acids at a concentration of 10 g L^{-1} and 500 mg L^{-1}, respectively, positively affected improving their vegetative growth characteristics. Moreover, transplants differed significantly among them in most studied traits, as Albizia transplants excelled in stem diameter and plant height increase, while Fig transplants were superior in leaves' chlorophyll content.

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