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Effect of bio-stimulants on growth and chemical composition of mahogany seedlings grown under treated wastewater (TWW)

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***Abstract*---**A pot experiment was carried out to study the effect of treated waste water (TWW) on mahogany seedlings and the role of bio-stimulants in alleviating the harmful impacts, using –6- months' old seedlings. The TWW was used in 3 types coded as 75%WW/25%PW, 50%WW/50%PW and 25%WW/75%PW, according the ratio between treated wastewater (WW) and potable water (PW). All seedlings grown under the different types of TWW treatments were sprayed with some

bio stimulants. The results revealed that irrigating mahogany seedlings with 25WW/ 75PW, significantly increased plant height, improved the formation of leaves, fresh weight leaves, 25WW/ 75PW increased total indoles whereas using 75WW/25PW or 50WW/50PW, for irrigation produced thicker and heavier FW of stems. FW of leaves with 50WW/50PW gave the heaviest fresh and dry weights of leave and roots diameter, total phenols, free proline and total soluble sugars. The treatment of 75WW/25PW formed significantly longer roots, both 75WW/25PW 0r 50WW/50PW, insignificantly increased fresh weight of roots. All bio-stimulants significantly increased plant height and seaweed extract, salicylic acid and Pot. silicate were the most effective. PS treatment exhibited the thickest stems. The number of leaves was the highest with PS, SE and FA. Spraying PS, GABA, SE, were the most effective in increasing weights of leaves. GABA significantly increased the root length followed by FA and Salicylic acid. In both seasons, PS and SE treatments increased the root diameter .The interaction between putrescine and 25WW/75PW or SE – PS and (50%%/50PW) gave the tallest plant height. The treatment of PS + 50WW/50PW or 75WW/25PW irrigation treatments increased stems thickness. Irrigation with 25WW/ 75PW with PS, Pt or FA, increased the number and weight of leaves. Irrigation with of 75WW/25PW+ GABA increased stem fresh weight, whereas 50WW/50PW + GABA produced the highest dry weight of leaves.75WW/25PW or 50WW/50PW with SE or PS improved root diameter. The fresh weight of roots responded well to GABA, SE and FA. Spraying seedling with FA and SaA increased total phenols, whereas GABA and SaA gave the highest indoles content. Putrescence and GABA gave the highest proline content and Pot. Silicate and GABA gave the highest sugar content. The contents of lead and cadmium were highest with 75WW/25PW and 50WW/50PW. Salicylic acid and fulvic acid increased the accumulation of Pb, whereas salicylic acid and seaweed extract increased the accumulation of Cd. The highest content of lead recorded under 75WW/25PW +SaA, but the highest Cd-content was recorded under 50WW/50 PW+FA and 75WW/25 PW + SA.

***Keywords*---**Waste water, bio-stimulants, fulvic acid, GABA, seaweed, lead.

1. Introduction

Mahogany is a large, semi-evergreen hardwood tropical tree (Fam.: *Meliaceae*) it has a rounded beautiful canopy, used as a popular landscape and street trees, it is one of the most valuable timber trees, with a great color ranges of wood, so it is used for making fine jewelries, decorative veneers and fine boat interiors.[1]. In arid and semi-arid regions, water is becoming scarce resource, with a critical problems in many regions of the world. As, the rapid increases in population and industrial projects have led to use low quality water (drainage, saline and treated or not –treated wastewater).So, it is necessary to find out new sources of water for

irrigation. Wastewater, is considered one of the best solutions [2] and it should be well managed, to save fresh water resources.In many countries, TWW has been successfully used for irrigation, but using it incessantly increases the TSS, CEC and heavy metals values in soil, creating harmful impacts on plants. The conjunctive use of TWW and potable one (tap, potable water, PW) or groundwater could be employed. In some studies usage of TWW in irrigation could enhance growth and improved productivity of plants, due to good supply of different nutrients. In this regard, [3,4] reported the beneficial effects on some plants and trees. The approach of using TWW in irrigation of timber-forest tree species helps to overcome health hazards of TWW. It is successfully implemented in the green belts and help in recovering the ecological balance and improving the environmental conditions.

The TWW resources(primary and secondary effluent), can improve soil quality and plant growth owing to their nutrient elements and organic matter(as natural conditioners), but there are limits, due to heavy metals and pathogens, as mentioned by Abulroos et al., [5,6,7,8]. A study was carried out by [9] indicated significant increases in plant height, shoot diameter and biomass of the saplings with TWW. The use of TWW was successfully applied for irrigating, crops, fruits, forest and timber trees. [10] carried out a study to maximize the usage of TWW in conjunction with any available water resource, using three types of crops, which irrigated by A: 50: 50% TWW : groundwater(GW), 100% (GW) or 75% TWW :25% (GW) 100% TWW, ,they indicated that properties of soil were almost similar, without significant differences with these waters, but N, P, K were significantly increased with TWW treatments, with a significant increase in plant productivity and insignificant changes in heavy metals of crops. Irrigation with TWW had a high influence on soil parameters (OM,N, P, Ca, Al, Fe, Pb and Zn)and plants growth [11] and harvest was significantly higher in TWW-irrigated plots. [8] used TWW on mahogany seedlings and found that the growth and chemical constituents of seedlings in primary TWW was superior, followed by secondary one. On 2012, [12], on *Tipuanaspeciosa* seedlings, revealed that primary TWW exhibited the highest values for vegetative growth and biomass of tree seedlings and recommended the use of sewage effluent in irrigating trees seedlings in calcareous soil to the improveproperties and production of timber trees. [13] on *Eucalyptus camaldulensis, E. citriodora, Pinushalepensis, P. brutia, P. pinea and Cupressus sempervirens,* indicated that irrigation with sewage water had elevated the level of metals compared to well water, but within the permissible limits for irrigation and lead, cobalt and cadmium were the most abundant.

[14] on olive tree reported that TWW was a sustainable solution to face water scarcity, it increased soil pH, EC, OM, major elements,but chlorophyll and total phenols decreased. On *Acacia ampliceps* and*A. origena,*[2] stated that primary TWW plus *Rhizobium* inoculum increased growth,improved nodulation and nutrient content of the seedlings.[15] mentioned that TWW significantly increased soil organic matter. [16] Stated that TWW increased soil salinity and the conc. of Pb, Zn, Cu and Cd.[17] mentioned that the world's supply of clean freshwater is steadily decreasing and the use of non-traditional water (TWW),should be increased, as an alternative option ,with caring about the potential problems associated with its use.

The use of bio stimulants as salicylic acid , potassium silicate, putrescence, fulvic and humic acids, seaweed extract ,GABA(gamma amino butyric acid) and others substances in alleviating harmful impacts of abiotic stress on plants was studied by many authors. The role of potassium silicate (PS) due to its positive impact on plant growth, increasing the rate of photosynthesis and chlorophyll and decreasing evapo-transpiration, inducing metabolic increases in citric acid and malic acid levels, and decreases in fructose, glucose, sucrose, and myo-inositol contents.[18,19, 20 ,[21] and [22]. Whereas, [23] found that it lessened the activity of enzymatic antioxidants (CAT, POD, and SOD), which in turn improved growth and roots, but increased the concentrations of soluble assimilates (total soluble proteins and total soluble sugars), which enriched the plant growth. Salicylic acid (SA) is a plant phenol, it is use as regulator hormone, due to its role in the defensive mechanism against abiotic stresses [24]. The foliar spray of SA counteracted significantly the harmful effects of salinity stress and increased chlorophyll, soluble sugars, proline and free amino acids contents [25]. GABA is a non-protein amino acid, has multiple functions under stressed conditions [26], it is an important intermediate of N-metabolism and amino acid biosynthesis, which indirectly affect plant growth and development. The exogenously applied GABA improved the overall vigor of plants under environmental stresses [27].

This study aimed to maximize and optimize the use of TWW in conjunction with potable water (PW) at various ratios and bio-stimulates treatments as an alternative source of water and to assess their effects on mahogany seedling growth under a long-term use and emphasis the role of bio stimulation treatments in reducing the unfavorable effects.

2. Experimental (materials and methods)

The present study was carried out at Ornamental Horticulture Department, Fac., of Agric., Cairo Univ., and National Research Center (NRC),Egypt during two successive seasons (2019/2020) and (2020/2021),aiming to find out the response of mahogany (*Swietenia mahagoni*) to some bio-stimulants and growth regulators and their effects on vegetative growth and chemical constituents of *Swietenia mahagoni* under irrigation with different levels of treated waste water conjucted with fresh water (potable). The study lasted for 12 months in the first and second seasons.

Plant materials:
6-months old seedlings of *Swietenia mahogany* (50 cm height, 7-8mm diameter) were obtained from Ornamental Horticulture Institute, Giza, Egypt.

Wastewater:
It was obtained from sewage station of Zenin, Bolak Al Dakrou rregion, Giza governorate, Egypt. (The average some composition is shown in Table-a).

Bio-stimulants and growth regulators:
Six commercial bio-stimulants and growth regulators (potassium silicate at 250ppm, seaweed extract 3g/l, putrescence at 50 ppm, fulvic acid at 3g/l, salicylic acid at 100ppm an gamma amino butyric acid (GABA)at 100ppm) were

used in addition to control treatment. The different Bio-stimulants and growth regulators were obtained from Bio-world Chemical Santa Cruz Company, Egypt.

Layout of the experiment:
The layout of the experiments was a complete randomized split block design including three concentrations (75%, 50% and 25% treated wastewater) and 6 treatments of bio-stimulants and growth regulators, in addition to control treatment were used in combination with irrigation treatments, including 21treatment, each treatment (9seedlings) contained 3 replicates**.**

Seedling cultivation:
It was carried out at shaded area at the nursery of Hort. Depart., Fac. of Agric, Cairo Univ. All seedlings were transplanted on 10 thMay, during the two successive seasons, in long plastic pots (35–cm) filled with sandy: clay (1:1,v/v, 10kg/ pot). All plants (pots) were fertilized with compost (El-Ahram) as a basic dressing, at a rate of 500g / pot. Irrigation with the 3 types of TWW was conducted after a month from transplanting at the rate of 2.4 liter/ pot according to field capacity. Using the types of TWW as main plots were used as follows that designated (coded) as A; (75W25P):75% treated waste water + 25% potable water. B: (50W50P):50% treated wastewater + 50% potable water. C; (25W75P):25% treated wastewater +75% potable water. All seedlings grown under the 3 types of TWW, were subjected to the foliar spray with potassium silicate (PS, at 250 ppm).Seaweeds extract (SE, at 3000ppm), Putrescine (Pt,at50 ppm), fluvic acid (FA, at 3000 ppm) salicylic acid (SaA, at100 ppm), gamma amino butyric acid (GABA, at 100 ppm) and control (as subplots.). The physical and chemical properties of used soil were: Sandy loam texture, EC dsm-1(1.26), pH (7.9 at 1:2.5),CaCO3 3.57%,O.M. 0.23%, The average composition of TWW and PW used in irrigation are shown in Table(a).

Table (a): The average composition of the water used in irrigation

Parameter of treated water	Unit	TWW	Tap water	Code No501	Parameter of treated water	Unit	TWW	Tap water	Code No501
PH	-	6.9	6.79	-	Pd	mg/L	0.18	<0.001	5.0
Total D.S.	mg/l	614	34	2000	Co.	mg/L	<0.01	<0.01	0.2
Total PO4	mgPO4/l	5.7	ND	30	Iron	mg/L	0.8	<0.01	5.0
Na	mg Na/l	189	52	230	Mn	mg/L	0.32	<0.01	0.2
Sulfate	mgSO4/l	311	ND	500	Ni.	mg/L	<0.001	<0.001	0.2
Ca.	mg/L	<0.001	<0.001	0.01	Zinc	mg/L	<0.01	<0.01	5.0

Soil physical and chemical analyses were carried out using the procedures described by [28], and by [29].

Data recorded:
The following data were recorded during the two seasons: 1. Plant growth parameters: Plant height (cm), stem diameter (mm), No. of leaves, root length (cm), root collar diameters (mm), shoots and roots fresh weights (g).

Chemical analyses:
were carried out at laboratories of National Research Center (NRC) and Fac. of Agric., Cairo Univ., to determine the leaf contents of total sugars (mg/g FW), total phenols (mg/g FW).total indoles (mg/100 g FW)and proline content (mg/g FW)and leaves contents of lead (Pd) and cadmium(Cd).

Chemical determinations:
were done as follows:1-The total phenolic contents of the alcoholic (GAE)were estimated using the FolinCiocalteau reagent.2-Proline content: it was determined briefly as 0.5 g FW finely ground with liquid nitrogen in a mortar. 10 mL of sulfo-salicylic acid 3% were added to the well-ground extract, and the extracts were centrifuged for 5 min at 6000 rpm. After centrifugation, 2 mL of supernatant were separated and placed in falcons, and 2 mL dimenhydrinate and 2 mL of glacial acetic acid were added to the supernatant. Next, 2 mL of toluene were added, then gently shaken for 30 s, finally the red supernatant (proline) was separated and spectrophotometrically measured at 520 nm, [30]. 3- Total indoles(mg/g fresh weight) were determined in ethanolic extract using 4-dimethylaminobenzaldehyde reagent as described by [31].4-Sugar content was estimated according to [32]).5-The contents of cadmium (Cd)and lead (Pb) were determined by Perkin Elmer, 3300 Atomic Absorption Spectrophotometer.

Statistical analysis

The data were recorded after 12 months from transplanting and statistically analyzed according to [33]. The L.S.D, test at 5% was used to compare between means of treatments.

3. Results

3.1. Vegetative growth

The plant height of mahogany seedlings in response to the following treated waste water (TWW): (75% wastewater + 25% Potable water).,designated as (75WW/25PW), 50% treated wastewater(50% wastewater + 50% Potable water).designated as (50WW/50PW)and 25% treated wastewater (25%) + 75% Potable water),designated as (25WW/75PW) and bio stimulants (Table.1) indicated that, in the first season, irrigating seedlings with municipal wastewater at (25WW/ 75PW) significantly increased plant height compared with the treatment of (75WW/25PW).

On the other hand, spraying plant with all bio-stimulants significantly increased it, and Putrescine (Pt) as well Seaweeds extract (SE) gave the tallest plants. The interaction between putrescine and (25WW/75PW) followed by Seaweeds SE and (50WW/50PW) gave the tallest plant height. In the second one, growing seedlings under 50WW/50PW,gave the tallest plants followed by25WW/75PW,but no significant differences between them. Regarding the response to bio stimulants and their interactions with TWW, indicated that Pot.silicate (PS) was the most effective, compared with the other bio-stimulants. The interaction revealed that irrigating at (50WW/50PW) combined with zero treatment followed by PS gave the tallest plants.

In both seasons, Irrigating seedlings with (75WW/25PW) or (50WW/50PW) produced thicker stems than those treated with (25WW/75PW), without significant differences between them, moreover, spraying seedlings with PS followed by control plants exhibited the thickest stems, regardless, irrigation treatments. In the second season, the combined treatment between (PS)and irrigation at 50WW/50PW or 75WW/25PW gave the thickest stems (1.86 and 1.73cm ,respectively).

From Table.1, it can be seen that the formation of leaves of mahogany seedling was significantly increased in response to (25WW/75PW) treatment, giving the greatest number of leaves.(41.56 and 55.46,in the first and second seasons, respectively). On the other side, the response of leaves formation to the application of different bio stimulants, differed between the two experimental seasons, as the great responses in the first one were attributed to PS,SE and Fulvic acid(FA), but in the second one, the treatments of FA and Pt were the most effective in this respect. Regarding the interaction, growing seedlings under 25WW/ 75PW with spraying PS (first season) and growing them under all irrigation types with the application of FA, formed the highest number of leaves.

Table 1. Effect of TWW and bio stimulants on plant height, stem diameter, and No. of leaves, during the two seasons: 2019/2020 and 2020 /2021

Treatments	2019/2020				2020 /2021			
	Plant height (cm)							
TWW	75 %	50%	25%	Mean	75 %	50%	25%	mean
Control	95.040	132	145.596	124.212	147.762	158.462	147.462	151.22
Pot. silicate	111.996	144.804	148.800	135.2	148.631	154.662	157.500	153.59
Seaweed Ext.	129.204	150.396	147.996	142.532	151.354	134.231	144.931	143.50
Putrescence	138.00	105.996	161.604	135.200	150.131	144.131	148.862	147.70
Fulvic acid	102.12	139.596	135.204	125.640	143.331	137.731	143.850	141.63
Salicylic acid	126.804	141.996	145.596	138.132	146.762	144.662	157.262	149.56
GABA	115.596	147.6	129.204	130.800	146.3	153.062	142.369	147.24
Mean (A)	116.965	137.484	144.857	-----	147.753	146.705	148.890	-----
LSD 5%	A:7.67	B: 9.45	AxB:12.8	------	A:6.44	B:8.95	AxB:12.53	----
	Stem diameter (cm)							
Control	1.53	1.58	1.43	1.51	1.70	1.66	1.42	1.593
Pot. silicate	1.57	1.63	1.53	1.58	1.73	1.86	1.50	1.696
Seaweed Ext.	1.53	1.37	1.36	1.42	1.67	1.46	1.53	1.553
Putrescence	1.47	1.39	1.38	1.41	1.54	1.41	1.37	1.440
Fulvic acid	1.48	1.57	1.30	1.45	1.64	1.63	1.41	1.580
Salicylic acid	1.54	1.47	1.43	1.48	1.49	1.30	1.42	1.403
GABA	1.51	1.53	1.40	1.49	1.63	1.59	1.42	1.550
Mean (A)	1.52	1.51	1.40		1.63	1.56	1.44	--
LSD 5%	A:NS	B:0.13	AxB:0.16	------	A:0.16	B:0.19	AxB:0.22	
	Number of leaves/plant							
Control	30.66	39.24	40.00	36.63	50.80	49.64	49.72	50.53
Pot. silicate	34.41	46.80	51.24	44.15	35.84	57.88	57.38	50.36
Seaweed Ext.	41.24	37.59	46.44	41.74	40.64	56.73	54.17	50.51
Putrescence	27.02	30.39	37.25	31.55	55.52	50.27	60.36	55.38
Fulvic acid	44.19	42.24	37.00	41.14	73.52	69.71	62.16	68.46
Salicylic acid	36.39	29.64	42.00	36.01	43.92	46.44	50.76	47.04
GABA	38.66	37.27	44.00	36.63	45.16	43.96	51.48	46.87
Mean (A)	34.50	37.45	41.56	----	49.64	53.81	55.46	----
LSD 5%	A:2.55	B:4.00	AxB:6.88	------	A:2.78	B:5.23	AxB:8.22	

As shown in Table (2) concerning the effect of TWW and bio-stimulants on fresh weight leaves (g), the recorded results revealed that irrigating seedlings with 25WW/75PW, in the first season and 50WW/50PW in the second on, gave the heaviest fresh weights, with significant differences compared with 75WW/25PW.

Treating seedlings with foliar sprays of PS and GABA followed by SE, regardless irrigation treatments were the most effective in this respect in the first season, whereas, in the second one, there was another trend, as the application of FA followed by Pt then SE exhibited heavier weights than the other treatments. The combined treatment of 25WW/75 PW and 50WW/50PW with the application of PS gave the greatest effect in in increasing fresh weight of leaves, in the first season, bur in the second one, it is clear that all irrigation treatments when combined with FA was the most effective in this regard.

Also, results (Table2) showed that irrigating with 50WW/50PW, in the first season and 25WW/75PW in the second one, produced the heaviest dry weight of leaves. Spraying seedlings with GABA and PS as foliar sprays, gave the highest values of leaves dry weight, in the first season, whereas, in the second one, the most effective treatments were GABA, Pt and SE. The combined treatment of 50WW/50PW + GABA showed the greatest effect on dry weight of leaves, in both seasons.

On the other hand, irrigating mahogany seedlings with 75WW/25PW and 50WW/50PW, in both seasons, increased stem fresh weight in comparison with 25WW/75PW.The effect of bio-stimulants on this parameter, indicated that GABA and SE were the most effective in both seasons, followed by PS and SaA, in the first and second seasons, respectively. Under the irrigation of 75WW/25PW, spraying with GABA, in the both seasons, increased stem fresh weight to the maximum values(138.89and 149.28 g) , followed by SE treatment, in the first season(**132.69g**), and PS in the second one(**143.37g**),combined with irrigation at 75WW/25PW.

Table 2. Effect of TWW and bio stimulants on fresh and dry weights of leaves and fresh weight of stem (g), during the two seasons: 2019/2020 and 2020 /2021

Treatments	2019/2020				2020 /2021			
	Fresh weight of leaves (g)							
TWW	75 %	50%	25%	Mean	75 %	50%	25%	Mean
Control	29.59	43.16	43.56	38.77	45.72	54.64	47.72	49.36
Pot. silicate	30.96	51.48	54.24	45.56	32.25	63.66	54.38	50.10
Seaweed Ext.	37.07	41.34	48.55	42.32	39.58	62.43	55.77	52.59
Putrescence	24.31	33.43	39.33	32.36	49.96	55.29	63.35	56.20
Fulvic acid	37.07	43.16	34.99	38.41	67.96	71.88	64.16	68.33
Salicylic acid	32.75	32.60	46.85	37.40	39.52	51.08	59.88	50.16
GABA	37.54	49.16	46.35	44.35	40.64	48.35	51.48	46.82
Mean (A)	32.76	42.05	44.84	------	44.66	58.19	56.68	-----
LSD 5%	A:2.55	B:4.00	AxB:6.88	------	A:2.78	B:5.23	AxB:8.22	-----
	Dry weight of leaves (g)							
Control	8.39	11.65	10.58	10.21	9.97	10.68	10.77	10.47
Pot. silicate	9.24	13.89	12.93	12.02	10.74	13.41	12.23	12.13
Seaweed Ext.	10.26	12.16	11.68	11.36	13.49	12.17	12.54	12.73

Putrescence	8.07	9.02	8.65	8.58	11.99	12.75	13.25	12.66
Fulvic acid	9.26	11.65	8.69	9.87	13.31	12.01	12.46	12.59
Salicylic acid	8.18	9.82	10.37	9.46	9.48	9.96	12.47	10.65
GABA	10.38	14.27	12.19	12.28	13.75	14.42	13.58	13.92
Mean (A)	9.11	11.78	10.73	------	11.82	12.20	12.47	------
LSD 5%	A:0.75	B:0.82	Ax :1.2 8	------	A:0.70	B:0.82	AxB:1.22	------
	Fresh weight of stems (g)							
Control	86.56	112.20	83.62	94.13	123.70	130.33	118.13	124.05
Pot. silicate	111.19	110.10	114.21	111.83	143.37	120.63	124.25	129.42
Seaweed Ext.	132.69	116.19	105.79	118.22	135.46	138.70	120.77	131.64
Putrescence	127.33	83.67	96.10	102.37	112.10	116.54	112.31	113.65
Fulvic acid	93.39	87.79	101.65	94.28	129.67	124.88	129.48	128.01
Salicylic acid	104.27	123.85	86.15	104.76	136.16	133.41	123.18	130.92
GABA	138.89	113.54	92.64	115.02	149.28	126.90	135.53	137.24
Mean (A)	113.47	106.76	97.17	------	132.82	127.34	123.38	------
LSD 5%	A:12.52	B:14.66	AxB:16.8	------	A:10.78	B:9.23	AxB:13.22	------

Root growth

Table (3) exhibited the response of root length (cm), roots fresh weights (g) and root diameter (cm) to TWW and bio stimulants treatment and their interactions. The obtained data revealed that in the first season there was no significant differences among all types of TWW, but in the second one it can be noticed that seedlings that irrigated with 75WW/25PW formed significantly longer roots than the two other treatments. On the other side, spraying mahogany seedlings with GABA in the first season, significantly increased the root length as compared to the control pants. In the second one, most bio stimulants treatments (FA, Pt and Salicylic acid and GABA) improved the root length, regardless irrigation types, with no significant differences among them. By comparing the interaction effects, it can be indicated that, GABA with all irrigation treatments in the first season was the most effective in this respect. But, in the second one, treating plants with GABA or Pt with using irrigation treatment at 75WW/25PW were the most effective, then use of SA when the irrigation at the ration 25WW/75PW was used. Also, Table(3) exhibited that roots diameter were the highest at 50WW/50PW in the first season and at 25WW/75PW,in the second one. Spraying PS and SE in both seasons, increased the root diameter (with no significant differences between them), as compared to the control and other bio stimulant treatments.

In the first season, the interaction between irrigation at 75WW/25PW or 50WW/50PW and bio stimulant application of SE gave the highest values of root diameter, whereas, in the second one, PS and SE were the most effective, under these irrigation treatments, respectively. Irrigating mahogany seedlings with 75WW/25PW and 50WW/50PW, insignificantly increased the fresh weight of roots in the first season, but significantly increased it in the second one, as compared with 25WW/75PW treatment. The effect of bio-stimulants on fresh weight of roots indicated that GABA and SE in the first season as well as FA, GABA and SE in the second one, were the most effective in increasing the fresh weight of roots .Under the irrigation of 75WW/ 25PW, spraying plants with GABA, in the both seasons, increased root fresh weights to the maximum values (**64.48**and 65.76g). The treatments of SE and GABA (first season) and SE, Pt and

PS (second one), improved the fresh weight of roots of plants grown with 50WW/50%PW.

Table 3. Effect of TWW and bio stimulants on root length (cm), root diameter (cm) and fresh weight of roots (g), during the two seasons: 2019/2020 and 2020 /2021

Treatments	2019/2020				2020 /2021			
	Root length (cm)							Mean
TWW	75 %	50%	25%	Mean	75 %	50%	25%	
Control	28.86	26.00	32.43	29.09	36.77	32.33	39.66	36.25
Pot. silicate	28.44	29.19	26.40	28.01	37.33	37.50	29.66	34.83
Seaweed Ext.	30.39	30.84	32.00	31.07	45.33	42.33	39.55	42.40
Putrescence	32.44	30.33	31.20	31.32	57.33	44.66	37.33	46.44
Fulvic acid	30.81	26.45	31.59	29.61	49.77	42.55	47.33	46.55
Salicylic acid	26.00	28.89	30.85	28.58	35.33	42.33	54.66	44.11
GABA	34.40	32.55	32.84	33.23	59.66	34.33	35.66	43.21
Mean (A)	30.19	29.18	31.05	------	45.93	39.43	40.55	------
LSD 5%	A:NS	B: 2.44	AxB:5.82	------	A:2.44	B:3.85	AxB:6 .50	------
	Root diameter (cm)							
Control	1.12	1.52	1.45	1.36	1.47	1.52	1.47	1.49
Pot. silicate	1.67	1.53	1.43	1.54	1.88	1.73	1.64	1.72
Seaweed Ext.	1.73	1.87	1.59	1.73	1.57	1.77	1.53	1.62
Putrescence	1.37	1.58	1.47	1.47	1.49	1.57	1.63	1.56
Fulvic acid	1.20	1.48	1.23	1.30	1.41	1.53	1.77	1.57
Salicylic acid	1.13	1.53	1.43	1.36	1.50	1.54	1.65	1.56
GABA	1.27	1.56	1.63	1.49	1.39	1.37	1.55	1.44
Mean (A)	1.36	1.58	1.47	-------	1.53	1.58	1.61	-----
LSD 5%	A: 0.19	B: 0.25	Ax B: 0.37	---------	A: 0.23	B: 0. N.S	Ax B: 0.40	------
	Fresh weight of roots (g)							
Control	46.46	45.74	42.14	44.78	57.55	49.46	43.54	50.19
Pot. silicate	46.76	49.35	50.70	48.94	62.34	57.35	40.41	53.37
Seaweed Ext.	54.51	59.22	53.66	55.80	50.42	60.69	59.68	56.93
Putrescence	54.83	52.31	49.15	52.10	56.54	61.99	42.81	53.78
Fulvic acid	43.50	36.50	43.59	41.20	59.74	50.06	65.21	58.34
Salicylic acid	42.75	50.83	40.08	44.55	39.92	47.76	58.16	48.61
GABA	64.48	56.14	49.96	56.86	65.76	54.49	50.18	56.81
Mean (A)	50.47	50.02	47.04		56.04	54.54	51.43	
LSD 5%	A: N.S	B: 4.15	AxB:6.28	------	A: 3.44	B:4.65	Ax B: 7.53	

3.2. Chemical composition

As presented in Table (4), concerning the effect of TWW and bio stimulants on the content of total phenols of leaves (mg / g F.W), indoles leaves (mg/100g F.W), total proline content of leaves (mg/g F.W), soluble sugar(mg/g F.W), as well as lead (Pd)and cadmium(Cd) contents, the results indicated the content of total phenols of leaves was higher in seedlings that irrigated with 50WW/50 PW) than the other two types. Spraying seedlings with FA and SaA increased the content of total phenols, regardless the irrigation treatments. Seedling grown under 50WW/50 PW without bio-stimulant treatments showed the highest increase in content of total phenols of leaves, followed by PS and GABA.

Also, data revealed that growing seedling of mahogany under 25WW/ 75PW treatment increased the content of total indoles to 8.13 mg/100g FW, whereas plants grown under 75WW/25PW and 50WW/50PW contained 7.2 and 7.95 mg, respectively. Spraying SaA and GABA gave the highest values of indoles (9.73 and 7.91 mg/100g FW), there was a great reduction in indoles content with the application of PS. Regarding the interactions, the highest contents of indoles in seedlings grown under all the irrigation treatments (75WW/25PW, 50WW/50PW and 25WW/75PW) were recorded in combined with control, Pt , followed by SaA, respectively. Spraying plants with GABA and SE growing under 50WW/50PW and 25%WW/75PO, respectively, exhibited marked increases. The content of free proline in response to irrigation with TWW, revealed that growing seedling under 50WW/50PW treatment, showed the highest content (**0.369 mg/g F.W)**. On the other hand, spraying putrescen (Pt) followed by GABA gave the highest content (0.413 and 0.327, respectively).The lowest value (0.270) was recorded with control. Regarding the interactions, the highest contents of proline (0.513) was estimated in seedlings grown under irrigation treatment of 50WW/50PW.The content of total soluble sugars in seedling under 50WW/50PW treatment, showed the highest content(13.41**mg)** .Spraying pot. silicate followed by GABA gave the highest content(13.76and **12.95** ,respectively). Regarding the interactions, the highest content (**18.66mg**) was recorded in seedlings grown under irrigation treatment of 50WW/50PW and treated with PS followed by irrigation with (75WW/25PW) combined with GABA treatment.

Table 4. Effect of TWW and bio stimulants on the contents of total phenols, total indoles, total proline and soluble sugars, lead content and cadmium content in leaves of mahogany seedlings, during the second seasons (2020 /2021)

Treatments	Total phenols of leaves (mg / g F.W)				Indoles leaves (mg/100g F.W)			
TWW	75 %	50%	25%	MeanB	75 %	50%	25%	MeanB
Control	15.49	16.52	10.29	14.10	8.96	7.85	6.74	7.85
Pot. silicate	13.46	16.44	11.11	13.67	4.76	6.36	6.43	5.85
Seaweed Ext.	15.47	12.1	9.72	12.43	6.76	7.73	8.33	7.62
Putrescence	16.19	16.11	8.67	13.65	6.19	9.1	7.62	7.64
Fulvic acid	13.78	15.82	13.91	14.50	8.41	7.14	7.67	7.74
Salicylic acid	14.17	14.10	14.91	14.39	8.37	8.59	12.24	9.73
GABA	13.81	16.24	8.96	13.00	6.94	8.91	7.89	7.91
Mean (A)	14.627	15.33	11.08		7.20	7.95	8.13	
	Total free proline content of leaves (mg/g F.W)				Total soluble sugars (mg/g F.W)			
Control	0.313	0.263	0.233	0.270	14.35	14.75	8.85	12.65
Pot. silicate	0.216	0.383	0.310	0.303	12.69	18.66	9.95	13.76
Seaweed Ext.	0.316	0.303	0.313	0.311	13.11	13.01	10.18	12.10
Putrescence	0.343	0.513	0.383	0.413	9.24	11.78	9.83	10.28
Fulvic acid	0.313	0.373	0.266	0.317	9.96	12.00	10.98	10.98
Salicylic acid	0.320	0.333	0.310	0.321	10.57	11.68	11.21	11.15
GABA	0.303	0.413	0.266	0.327	15.86	12.00	11.00	12.95
Mean (A)	0.303	0.369	0.297		12.25	13.41	10.29	
	Lead content (Pd, ppm)				Cadmium content (Cd,ppm)			
Control	3.6	5.2	2.1	3.63	0.7	3.4	1.2	1.73

Pot. silicate	6.4	1.6	1.0	3.00	1.0	3.8	1.4	2.07
Seaweed Ext.	6.3	5.2	1.9	4.46	3.9	3.8	1.3	3.00
Putrescence	3.8	4.0	1.6	3.13	1.1	3.0	1.2	1.77
Fulvic acid	4.1	7.2	3.1	4.80	1.8	5.4	1.4	2.87
Salicylic acid	7.8	5.3	4.4	5.83	4.1	3.4	2.1	3.20
GABA	3.6	1.9	2.4	2.63	2.6	1.8	1.4	1.93
Mean (A)	5.1	4.3	2.4	------	2.17	3.5	1.4	------

As for the content of lead (Pd), it can be noticed (Table4), that seedlings irrigated with 75WW/25PW contained the highest Pd than the other two irrigation treatments. Spraying seedlings with salicylic acid followed by fulvic acid increased the accumulation of Pd than the other treatments and control. Results of the interaction treatments, revealed that the highest content (7.8 ppm) was recorded in seedlings grown under irrigation treatment of 75WW/25PW and treated with **Salicylic acid**. Moreover, spraying plants growing under 50WW/50PW with fulvic acid exhibited marked accumulation of lead content (7.20 ppm).

From Table (4), as for the content of cadmium (Cd), it can be noticed that seedlings irrigated with 75WW/25PW accumulated the highest Cd content. Spraying seedlings with salicylic acid, followed by seaweed extract increased the accumulation of Cd than the other bio-stimulants, and control. Results of interaction, indicated that the highest Cd-content (5.4 ppm) was recorded in seedlings grown under irrigation treatment of 50WW/50 PW and treated with fulvic **acid**, followed by spraying plants growing under 75WW/25 PW with salicylic acid (4.1ppm).

4. Discussion

The plant height of mahogany seedlings in response to treated waste water (TWW), indicated, irrigating at (25WW/ 75PW) significantly increased it. Spraying plant with all bio-stimulants significantly increased PH (plant height) and seaweed extract & salicylic acid (SaA) and Pot. Silicate (PS) was the most effective, in the first and second seasons respectively. The interaction between putrescine and (25WW/75PW) followed by SE and (50WW/50PW) (in first season) gave the tallest plant height and (50WW/50PW) + zero bio. Followed by (50WW/50PW) + PS gave the tallest plants (in second season).

Irrigating seedlings with (75WW/25PW) or (50WW/50PW) produced thicker stems than (25WW/75PW). Spraying PS followed by control plants exhibited the thickest stems, regardless, irrigation treatments. In this regard, [4],[34], [35],[36] and [37] used TWW to irrigate forestry, olive trees, grapevines and vegetables, due to its great benefits for plants, soil and environment, also it can be used for fruit trees cultivated in under drought environments like olive [38], mandarin [39], lemon trees [40] and grapevines [41]. [42] promote the use of TWW to irrigate forests and several landscape trees projects in many countries, if it was not done properly, it can increase soil salinity and cause pollution of ground and surface water. [42], put many strategies for managing TWW to overcome salinity and toxicity hazards, including selection methods and application of TWW in the irrigation of forestry trees. TWW can be incorporated for crop irrigation. [43] Assessed the possibility of reusing TWW for nursery of ornamental trees using the tertiary effluent, and

stated the nutrient content was able to maintain good plant growth. [44] Successfully irrigated *Pinus brutia* using TWW with low mortality. Also, [13] irrigated some woody trees grown at Egyptian-Chinese Friendship Forest (Sadat city) with TWW and reported that N, P and K at 30-60 cm soil depths were increased as well as Pb, Cd and Ni.

Many studies have not reported any negative impacts of TWW irrigation on tree health, but reported increases in biomass [45,46].Moreover, [2] stated that TWW irrigation promoted the growth and nodulation of *Acacia*spp. On the other hand, [47] stated that TWW produced excessive heavy elements accumulations within the plant and soil, but negatively affected yield and quality.

In our study, most of the bio stimulants showed great benefits in alleviating the impacts of TWW irrigation. In general, regardless TWW. All bio-stimulants significantly increased plant height and seaweed extract, salicylic acid and potassium silicate were the most effective. Potassium silicate treatment exhibited the thickest stems, whereas No. of leaves was the highest with PS, SE and FA treatments. Spraying seedlings with PS, GABA and SE, effectively increased the weights of leaves. GABA treatment significantly increased the root length, followed by FA and Salicylic acid. In both seasons, PS and SE treatments increased the root diameter.

The interaction between putrescine and 25WW/75PW or the treatments of SE and PS under 50%%/50PW irrigation type, gave the tallest plant height. The treatment of PS for seedlings irrigated with 50WW/50PW or 75WW/25PW increased stems thickness. Irrigating seedlings with 25WW/ 75PW with spraying PS or FA increased the number and weights of leaves. Whereas, irrigation with 75WW/25PW combined with GABA treatment increased stem fresh weigh. Also, under 50WW/50PW treatment, treating with GABA produced the highest dry weight of leaves. Under irrigation of 75WW/25PW or 50WW/50PW plants treated with SE or PS exhibited better root diameter. The fresh weight of roots responded well to GABA, SE and FA treatments. Spraying seedling with FA and SaA increased total phenols, whereas GABA and SaA gave the highest indoles content. Putrescene (Pt) and GABA increased the proline content to the highest value. Spraying seedlings with PS and GABA gave the highest sugar content.

In a brief, the most effectives bio-stimulants in our study were PS, GABA, SE followed by FA .It was reported in many studies that potassium silicate (PS) could use to alleviate abiotic stresses, due to its positive impact on plant growth and productivity, silicon as a key component improves plant growth, increases photosynthesis, decreases respiration, increases the chlorophyll, leaf area and improves the quality of treated plants. [19], [20], [21] and [22]. [48] stated that foliar spray with K silicate induced metabolic changes, it increases in citric acid and malic acid levels, and decreases in fructose, glucose , and sucrose. Whereas,. [23] Used K-silicate under salinity stress and recorded a reduction in the activity level of enzymatic antioxidants (CAT, POD, and SOD), which in turn improved the vegetative and roots growth, also, it reduced the osmolyte levels and increased soluble assimilates (total soluble proteins and total soluble sugars), which enriched the plant growth.

Several functions of GABA in plants have been described in detail [49], [26]. GABA acts as an endogenous signaling molecule in plant growth regulation and plant development [50],it is important component in the regulation of carbon: nitrogen metabolism [51]. It promotes plant growth and mitigates stress [52] and [53]. The different roles of GABA in plants might be functionally linked and difficult to be separated from each other [54].The foliar application of GABA acts in the same way as endogenous GABA could induce changes of the physiological and biochemical mechanisms under stress conditions [55]. [56] Stated that net photosynthesis rate, chlorophyll, anti-oxidant enzymes and nitrogen metabolism were positively influenced as well as the morphological growth of the plant.

Studies revealed that FA and SE could alleviate the harmful effect of TWW, lead and cadmium and reduced lead toxicity via reducing Pb uptake [57] and [58]. [48] Reported that foliar FA enhanced plant growth under Cd stress and .0.5 g/L had best ameliorative effects to Cd toxicity and improved photosynthesis, inhibit Cd accumulation and increased Fe-uptake. Whereas, [59] on *Paeoniaostii* showed that FA treatment significantly increased the leaf water content as well as antioxidant enzyme activities, proline and improved photosynthetic parameters and chlorophyll. It could induce antioxidant enzymes to eliminate ROS, reduce membrane lipid peroxidation and decrease damage to photosynthesis. The contents of lead and cadmium were highest with 75WW/25PW.Salicylic acid and fulvic acid increased the accumulation of Pd, whereas salicylic acid and seaweed extract increased the accumulation of Cd. The highest content of lead recorded under 75WW/25PW +SaA, but the highest Cd-content was recorded under 50WW/50 PW+FA and 75WW/25 PW + SA. [60] stated that humic and fulvic acids, owing to it carboxyl and phenolic hydroxyl groups, they form strong bonds with heavy metals which makes them stabilizing agents,decrease the adverse effects of the Cd and increased the growth and root parameters, chlorophyll values, compared to the control. Salicylic acid (SA) is a plant phenol ,used as internal regulator hormone due to its role against a biotic stresses [24].The foliar spray of SA counteracted significantly the harmful effects of salinity stress and increased chlorophyll content, soluble sugars, proline and free amino acids [25].

5. Conclusion

This study aimed to maximize and optimize the use of TWW in conjunction with potable water (PW) at various ratios and bio-stimulates treatments as an alternative source of water and to assess their effects on mahogany seedling growth under a long-term use and emphasis the role of bio stimulation treatments in reducing the unfavorable effects.

In our study, most of the bio stimulants showed great benefits in alleviating the impacts of TWW irrigation. In general, regardless TWW. All bio-stimulants significantly increased plant height and seaweed extract, salicylic acid and potassium silicate were the most effective. Potassium silicate treatment exhibited the thickest stems, whereas No. of leaves was the highest with PS, SE and FA treatments. Spraying seedlings with PS, GABA and SE, effectively increased the weights of leaves. GABA treatment significantly increased the root length, followed by FA and Salicylic acid. In both seasons, PS and SE treatments increased the root diameter.

The interaction between putrescine and 25WW/75PW or the treatments of SE and PS under 50%%/50PW irrigation type, gave the tallest plant height. The treatment of PS for seedlings irrigated with 50WW/50PW or 75WW/25PW increased stems thickness. Irrigating seedlings with 25WW/ 75PW with spraying PS or FA increased the number and weights of leaves. Whereas, irrigation with 75WW/25PW combined with GABA treatment increased stem fresh weigh. Also, under 50WW/50PW treatment, treating with GABA produced the highest dry weight of leaves. Under irrigation of 75WW/25PW or 50WW/50PW plants treated with SE or PS exhibited better root diameter. The fresh weight of roots responded well to GABA, SE and FA treatments. Spraying seedling with FA and SaA increased total phenols, whereas GABA and SaA gave the highest indoles content. Putrescene (Pt) and GABA increased the proline content to the highest value. Spraying seedlings with PS and GABA gave the highest sugar content.

6. Conflicts of interest: "There are no conflicts to declare"

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