



Effect of irrigation water quality and foliar-sprayed glycine betaine on vegetative, anatomical traits and antioxidant activity of kapok seedlings



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ABSTRACT

The present experiment was performed throughout two successive seasons of 2020/2021 and 2021/2022 to intended to response of kapok (*Ceiba pentandra* L.) seedlings to irrigated with secondary treated sewage water (TSW), agricultural drainage water (ADW) and Nile irrigation water (NIW) either direct (100%) or mixed TSW or ADW with NIW (25, 50, and 75%) in addition to using foliar glycine betaine (GB) application at (0.0 mM and 50 mM) on vegetative growth parameters, Anatomical structures of leaf and antioxidant activity. Results showed that irrigation with directly (100%) of TSW and ADW decreased plant height, number of leaves on plant, stem diameter/plant, fresh weight of stem, leaves and roots, root diameter, dry weight of stem, leaves and roots, leaf area/ plant, root length, leaf thickness, leaf mid-vein; however, increased free proline content, total phenolic content and antioxidant activity. As well as, GB significantly increased all vegetative growth parameters, free proline content, total phenolic content, leaf thickness, leaf mid-vein and antioxidant activity. But also with irrigation kapok seedlings at TSW or ADW and NIW at 25 and 50% give better results likewise irrigation with NIW for all parameters. Combination with GB and water treatments give better results at 50 mM GB and TSW or ADW at 25 and 50% mixing with NIW. Concluded that from irrigation kapok seedlings with TSW or ADW preferably was mixed with NIW percentage 50:50 gave better results on vegetative growth characters of kapok leaves seedlings like control seedlings irrigated with NIW, and spraying of GB principally at the concentration of 50 mM combined together has the possibility enhancing and progressing the characters of kapok.

Keywords: Woody trees, toxic heavy metals, treated waste water, agriculture drainage water, water stress, irrigation water.

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1. INTRODUCTION

Ceiba pentandra L. is a promising woody tree, known as kapok tree or silk cotton tree which belongs to Family Malvaceae. It is an emergent, fast growing tree species. It can grow to 30m tall or more, with a straight, largely branchless trunk that culminates in huge spreading and has been found to possess many medicinal and nutritional properties (Burkill, 1995). The kapok fibers are utilized locally as fiberfill in pillows, quilts, and some soft toys and also manufacturing of pulp and paper (Hori *et al.*, 2000; Walia *et al.*, 2009). *Ceiba pentandra* L. is a tree up to 50 m high which occurs in areas ranging from tropical America to Asia through Africa (Aubreville and Leroy, 1975). The plant is well reputed in the African traditional medicine for treating many illnesses, such as, headache, dizziness, constipation, mental troubles and fever. It is also used as diuretic (Busson *et al.*, 1965; Bouquet and Debray, 1989).

Egypt is one of the countries affected by climate change impacts, within its borders and outside its borders, it is strongly predicted that the River Nile levels will experience major decrease. The water of the Nile is becoming increasingly insufficient for the requirement in Egypt due to population growth, surface irrigation systems, water contamination, irregular distribution of water resources, and frequent droughts caused by extreme global weather patterns. Since 2000, Egypt is below the water poverty limit and new water resources are sought to cover the shortage by the year 2050 (MWRI, 2010). The Nile River provides nearly 97% of Egypt's freshwater supply. Egypt's share of Nile waters is fixed at 55.5 billion cubic meters annually. As a result, Egypt will

not be able to meet increasing water demand using freshwater from the Nile and has been developing non-conventional sewage water reuse strategies to meet future demands. The USAID mission in Cairo began promoting strategies for water reuse in 2004, and guidelines for safe and direct reuse of treated sewage water for agricultural purposes were approved in 2005 (Egyptian Code 501/2005) (Ronald *et al.*, 2011).

Glycine betaine (N,N,N-trimethyl glycine) is extremely soluble in water but includes a non-polar hydrocarbon moiety that consists of three methyl groups. The molecular features of glycine betaine GB allow it to interact with both hydrophilic and hydrophobic domains of macromolecules, such as enzymes and protein complexes. Studies in vitro have indicated that GB is not merely a non-toxic, cellular osmolyte that raises intracellular osmolarity when a cell is exposed to stress-induced hyperosmotic conditions: it has been well documented that, in vitro, GB stabilizes the structures and activities of enzymes and protein complexes and maintains the integrity of membranes against the damaging effects of excessive salt, cold, heat and freezing (Gorham, 1995).

The aim of this study is to investigate the effect of using TSW and agriculture drainage water (ADW) for irrigating seedlings compared with those irrigated by Nile irrigation water on the biomass growth of trees, anatomical characteristics and antioxidant activity to raise the amount of water income from the available resources in Egypt.

2. MATERIAL AND METHODS

This study was carried out at private farm in Beni-Suef Governorate, under directorate of botanical garden during the

two successive seasons of (2020/2021 and 2021/2022). Seeds of *Ceiba pentandra* L. were obtained from the local trees, Fayoum, Egypt, during the season (2019/2020). Firstly, the seeds were sown in plastic pot (10 cm top diameter) filled with 2 kg of sand and clay soil (3:1v/v) at the first week of April for both seasons, then the seedlings were transplanted after 90 days from sowing date into plastic pot (20 cm top diameter) in the same soil. Secondly, after one year from sowing for both seasons the seedlings were cultivated in plastic pots (40 cm top diameter) filled with 20 kg sandy soil each pot containing one seedling. Physical and chemical properties of the experimental soil were conducted according to the methods and procedures outlined and described by Klute (1986) and Page *et al.* (1982), respectively. The obtained results were tabulated in Table 1.

2.1. Water treatments

Types of different water (ADW, TSW and Nile irrigation water (NIW) were obtained from different resources: TSW was obtained from (Bilateral sewage

treatment plant in the new city of Beni-Suef) water analyses are shown in Table 2, ADW was obtained from (agricultural irrigation water canal in the village of Naim Beni-Suef water) analyses are shown in Table 2, NIW obtained from (irrigation canal in the village of Naim Beni-Suef). The different experiments were carried out in combination with ADW and NIW at (0:100, 25:75, 50:50, 75:25 and 100:0%), respectively. Also combination with TSW and Nile irrigation water at (0:100, 25:75, 50:50, 75:25 and 100:0%), respectively, with concentration of antioxidant foliar GB (0 and 50 mM).

2.2. Glycine betaine application

After stability of seedlings, the seedlings were transplanted after 45 days from transferred seedlings were irrigated with concentrations of different water mixing ratios (water treatments), seedlings were sprayed with GB at the different concentrations (0.0 and 50 mM), after 75, 105 and 135 days from transferred seedlings, respectively. GB was obtained from (sigma company).

Table 1. Physical and chemical properties of the experimental soil in the two seasons of 2021 (SI) and 2022 (SII)

Physical properties								
	Clay (%)		Silt (%)		Sand (%)		Soil texture	
SI	5.1		5.6		89.3		sandy	
SII	4.9		5.1		90.61		sandy	
Chemical properties								
	ECe (dS/m at 25 °C)					pH		
SI	2.39					7.12		
SII	2.37					7.06		
	Soluble cations (meq/l)					Soluble anions (meq/l)		
	Na ⁺	Mg ²⁺	Ca ²⁺	K ⁺	HCO ₃	CO ₃ ²⁻	Cl ⁻	SO ₄ ²⁻
SI	13.2	7.34	3	0.36	6.86	0.00	10.8	6.24
SII	13.9	6.8	2.8	0.29	6.03	0.00	10.75	6.92
	Available macronutrients (mg/kg soil)				Available micronutrients (mg/kg soil)			
	N	P	K		Fe	Zn	Cu	Mn
SI	4.25	4.45	108		0.285	0.07	0.385	0.208
SII	4.25	4.01	110		0.271	0.06	0.368	0.194

Table 2. Analysis of treated sewage water (TSW) and agricultural drainage water (ADW) used in this study during the two seasons of 2021 (SI) and 2022 (SII)

Properties	TSW	ADW	TSW	ADW
	SI		SII	
ECe (dS/m at 25 °C)	4.19	3.15	4.15	3.11
pH (at 25 °C)	7.19	7.34	7.14	7.41
Na (meq/L)	54.1	5.5	53.9	5.62
Ca ⁺ :Mg	14	6	14.2	6.11
Sodium adsorption ratio	20.5	3.18	20.3	3.22

2.3. Experimental layout

The experimental design used was a split-plot in randomized complete blocks with three replications. Main plots consisted of application water quality (9 treatments) while sub-plots were allocated to GB concentrations (2 treatments). All treatments (18 treatments) with three replications, each replication containing ten plastic-pot; each treatment consisted of thirteen plastic-pot.

2.4. Data recorded

2.4.1. Vegetative growth characters

In both seasons vegetative growth characters were evaluated as follows: Plant height (cm) was measured starting from the ground level to the apical meristem of the main stem. A number of leaves on plant is mathematically calculated by estimating the net number of leave surround main stem of kapok plants. Stem diameter/plant (mm) was measured by using sealy So707-Digital Electronic Vernier Caliper 0-150 mm/0-6" above ground by 5 cm. Fresh weight of stem, leaves and roots (g) mathematically calculated using fresh weight of each plant organ stem weighing. Root diameter (mm) was measured by using Sealy So707-Digital Electronic Vernier Caliper 0-150 mm/0-6" under ground by 2 cm. Dry weights of stem, leaves and roots (g) was gained by drying at 70°C in a forced-air oven till the weight became constant. Leaf area/plant (m²) mathematically calculated using leaf area-leaf weight relationship as illustrated by Nassar (1986). Root length (cm) was measured starting from the ground level to the apical meristem of the main root.

2.4.1.1. Leaf osmoprotectants compounds and antioxidant activity

Total phenolic content in plant (mg g⁻¹ dry weight) was extracted and determined by using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965) and modified by Hashemi *et al.* (2011). Free proline (mg g⁻¹ dry weight) was colorimetrically determined using ninhydrin reagent as outlined by Bates *et al.* (1973). Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity was measured by the DPPH assay (Lee *et al.*, 2003) was utilised with some modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20°C until use. The working solution (6×10^{-5} M) was prepared by mixing 6 mL of the stock solution with 100 mL of methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, as measured using spectrophotometer. Extract solutions of different concentrations (0.1 mL of each) were vortexed for 30 s with 3.9 mL of DPPH solution and left to react for 30 min, after which the absorbance at 515 nm was recorded. A control with no added extract was also analysed. Scavenging activity was calculated as follows: DPPH radical-scavenging activity (%) = $[(A_{\text{control}} - A_{\text{sample}})] / (A_{\text{control}})] \times 100$, where A is the absorbance at 515 nm.

2.4.2. Anatomical study

For anatomical study, ADW and NIW at (0:100, 25: 75, 50:50, 75:25 and 100:0) respectively also combination with TSW and NIW at (0:100% , 25: 75% , 50:50%, 75:25% and 100: 0%) respectively, with

concentrations of antioxidant foliar GB (0 and 50 mM) were taken with length (1cm) at the age of 65 days from transplanting of kapok pepper plant. Samples were taken from the fully expanded leaf (4th leaf from the growing tip of the plants including the mid-vein). Samples were killed and fixed in formalin acetic acid solution (10 ml formalin + 5 ml glacial acetic acid + 50 ml ethyl alcohol 95% + 35 ml distilled water) for 72 hours, then washed in 50% ethyl alcohol, dehydrated, cleared in n-butyl alcohol series and embedded in paraffin wax of 56-58 °C melting point. Cross sections of 20-25µ thick were cut, using a rotary microtome, adhered to slides by "Haupt's adhesive" then stained with the crystal violet–erythrosin combination, cleared in carbol-xylene and mounted in Canada balsam. Slides were microscopically analyzed and sections were microphotographed according to Nassar and El-Sahhar (1998).

2.4.3. Statistical analysis

Appropriate analysis of variance was performed on results of each experiment. Comparisons among the treatment means were performed using the Revised Least Significant Difference procedure at $p \leq 0.05$ level as illustrated by Waller and Duncan (1969).

3. RESULTS AND DISCUSSION

3.1. Growth parameters

It was noticed that irrigation TSW and ADW caused a remarkable decrease and damage in all vegetative growth parameters (Tables 3-5). There was a significant decrease in plant height, number of leaves on plant, stem diameter/plant, fresh weight of stem, leaves and roots, root diameter, dry weight of stem, leaves and roots, leaf area/plant and root length were negatively affected as well by irrigated at 100%. On the other side, under mixing TSW or ADW with NIW at percentage 50:50 there was positive results for all growth. Likewise control seedlings which irrigated with NIW.

In a study conducted by Houda *et al.* (2016) on *Populus nigra* and *Populus alba* reported that TSW decreases leaves number, Ali *et al.* (2011) on *Swietenia mahagoni* found TSW reduced root length, Banon *et al.* (2011) on polygala and lantana plants discovered that TSW reduce plant dry weight Belhaj *et al.* (2016) on three vegetable crop plants (tomato, radish and lettuce) reported that TSW decreases all vegetative growth, The study attributed such negative effect to the toxic levels of heavy metals in waste water. Similar results were obtained in Tables 3-5. In contrast Ali *et al.* (2011) on *Swietenia mahagoni* seedlings and Ali *et al.* (2012) on *Tipuana speciosa*, and Amin (2021) on *Myrtus comunis*, *Eucalyptus camaldulensis* and *Cupressus sempervirens*, they indicated secondary TSW increased vegetative growth. Akhkha *et al.* (2019) on *Calotropis procera* plants found that secondary TSW had no significant effect on vegetative growth. Vegetative growth decreased with TSW might be found heavy metals, Abdel-Shafy (1994) indicated that heavy metals problem may be accompanied by a reduction in the nutrient uptake plant, disorders in their metabolism and a reduction in the ability to fix phosphorous or nitrogen in the plant.

The vegetative growth characters (plant height, number of leaves on plant, stem diameter/plant, fresh weight of stem, leaves and roots, root diameter, dry weight of stem, leaves and roots, leaf area/ plant and root length) were positive significantly affected by foliar application of glycine betaine at 50 mM comparing to seedlings unsparing (Tables 3-5). Similar results were obtained by Amin and Al-Atrash (2020) on *Populus nigra* seedlings, Shehzad *et al.* (2019) on oat (*Avena sativa*), Cisse *et al.* (2021) on *Dalbergia odorifera* seedlings, Zhang *et al.* (2021) on *Dalbergia odorifera* seedlings. Hu and Hu (2012) on perennial ryegrass indicated GB attributed to its

protective effect on peroxidation-linked membrane deterioration and scavenging free radicals, also **Kanu *et al.* (2017)** indicated that GB enhanced plant growth under stress condition, may be due to its osmotic protection.

The interaction of GB at concentration of 50 mM under water treatments TSW and ADW Tables 3-5, GB enhanced the above-mentioned vegetative growth characters (plant height, number of leaves on plant, stem diameter/plant, fresh weight of stem, leaves and roots, root diameter, dry weight of stem, leaves and roots, leaf area/ plant and root length) of kapok plants under irrigation TSW and ADW in both seasons of study.

3.1.1. Leaf osmoprotectants compounds and antioxidant activity

By irrigation with TSW and ADW, the highest contents of total phenolic, proline and antioxidant activity were obtained. The highest content was produced by irrigation at 100% (Table 6). Results indicated that TSW and ADW enhanced total phenolic content and proline content, this agreement with **Akkawi *et al.* (2018)** on *Origanum syriacum*, *Micromeria fruticose*, *Mentha Spicata*, *Rosmarinus officinalis*, and *Salvia Fruticose* found TSW increased total phenolic content in plants than well water. As well as, **Zagorchev *et al.* (2013)** and **Varela *et al.* (2016)** indicated the plant can accumulate the phenolic compounds which can play a major role to relieve the damage caused by environmental stresses. But also, **Caliskan *et al.* (2017)** reported that phenols own an antioxidant activity which can scavenge the reactive oxygen species (ROS) under abiotic stress in *Hypericum pruinatum*, **Saima Kausar *et al.* (2017)** on fenugreek plants they indicated that TSW increased proline content in plants, the main cause was due to high levels of heavy metals that sewage water may contain. As well **Chandrasekhar and SandhyaRani (1996)** on *Crotalaria striata* reported that

increased accumulation of proline in stressed plants may be an adaptation to compensate for the energy for growth and survival and thereby help the plant tolerate stress. **Ahmed *et al.* (2000)** discovered accumulation of proline is considered to be a protective adaptation and that the survival of plants under stress conditions depends upon the regulation of metabolic processes and quantities ratio between the protective and the toxic intermediates. **Ibrahim (2008)** indicated that proline is considered as a cytoplasm protective osmolyte necessary for adaptation to stress and the increased proline concentration could be a good parameter for stress tolerance plant. In contrast **Akhkha *et al.* (2019)** on *Calotropis procera* seedlings indicated that irrigated with TSW showed any not significant changes in proline content of leaves. In this study we found secondary TSW increased antioxidant activity in Kapok seedlings (Table 6 and Fig. 2). This study agreement with **Bernstein *et al.* (2009)** on *Origanum vulgare*, and **Ali-Shtayeh *et al.* (2018)** on *Origanum syriacum* var. *syriacum* they indicated secondary TSW increased antioxidant activity in seedlings, **Ashraf (2009)** reported that there is an antioxidant defense mechanism in plants exposed to stress. This defense mechanism includes enzymatic and non-enzymatic antioxidants, **Abd El-Baky *et al.* (2003)** indicated the mechanisms that decrease ROS and enhance antioxidant enzyme system in plants have important roles in imparting tolerance in plants under environmental stress conditions, **Kahkonen *et al.* (1999)**, reported that to decrease the elevated levels of ROS during oxidative stress conditions plants encouraged production of antioxidants indicates the exposure to less than optimal conditions. **Munné-Bosch and Alegre (2000)** indicated that chloroplasts are protected from oxidative stress by regulated compartmentation of oxidation products.

Mittler (2002) reported that heavy metals contained in TSW may increase antioxidant activity and reactive oxygen production in plants, as well as increased antioxidant content and antioxidant activity were demonstrated in many plants in response to environmental stresses.

Foliar application of GB caused increased contents of total phenolic, proline and antioxidant activity than without foliar GB (Table 6). Similar results indicated that **Shehzad *et al.* (2019)** on *Avena sativa*, **Alamer and Ali (2022)** on *Tagetes erecta* plants, **Safwat and Abdel Salam (2022)** on *Ocimum basilicum*, they found GB increased total phenolic in plants. In contrast results **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings, **Cisse *et al.* (2022)** on *Dalbergia odorifera* plants, they found GB decreased total phenolic. **Amin and Al-Atrash (2020)** on *Populus nigra* seedling, **Alasvandyari and Mahdavi (2017)** on *Carthamus tinctorius* **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings and **Shehzad *et al.* (2019)** on *Avena sativa* they found GB increased proline content in plants. In contrast, **Hu and Hu (2012)** on perennial ryegrass *Lolium perenne* and **Zhang *et al.* (2021)** on *Dalbergia odorifera*, they found GB no significant on proline content in plants. In Table 6 and Fig. 2 data showed that GB enhanced antioxidant activities; this study confirmed with **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings and **Zhang *et al.* (2021)** on *Dalbergia odorifera* they indicated GB increased antioxidant activity in leaf plant. **Malekzadeh (2015)** reported that during abiotic stresses, GB can protect plant cells from oxidative stress by enhancing the antioxidant system. **Shan *et al.* (2016)** indicated that exogenous GB provides chilling tolerance in *Prunus persica* by improving the antioxidant activities. **Farooq *et al.* (2009)** found that exogenous GB application in various plants ameliorates negative effects of stress on

photosynthesis by scavenging the ROS and via activation of antioxidant activities. It was reported that an increase of antioxidant activities in *Oryza sativa* under stress.

Effect between water treatments and GB on increasing antioxidant activity content in kapok dry leave was noticed (Table 6). The highest of total phenolic, proline and antioxidant activity content was recorded from plants irrigated with ADW and TSW at 100% and spraying GB at 50 mM in the first and second seasons, respectively, with significant difference in both seasons GB.

3.1.2. Leaf anatomical study

Data in Table 7 and Fig. 1 comparisons with seedlings irrigated with NIW, leaf thickness and leaf mid-vein were responding differently due to irrigation with TSW and ADW. Irrigation with TSW or ADW at 100% caused a decreased almost all anatomical features of kapok plant leaves including (height of mid-vein and height, width of vascular bundle and diameter of xylem vessel, leaf blade thickness, palisade tissue thickness, spongy tissue thickness and upper epidermis layer) as compared to NIW. As well as, irrigation with TSW and ADW at 25 and 50% observed no significantly than irrigation seedlings at NIW. **Aldesuquy (2014)** indicated sewage water at concentration 100% caused decrease significant in leaf thickness and ground tissue thickness. But also, at concentration 25 and 50% Increases ground leaf thickness and ground tissue thickness. As well, at all concentrations decreased phloem area and opened stomata number on both upper and lower epidermis in flag leaves of wheat plants. Also, **Aldesuquy *et al.* (2013)** indicated that water stress caused considerable decreases in leaf thickness, ground tissue thickness, metaxylem vessel area, xylem vessel area, vascular bundle area in wheat plants.

Spraying with GB at 50 mM had the highest values of leaf thickness (height of mid-vein and height, width of vascular

bundle and diameter of xylem vessel) and leaf mid-vein (leaf blade thickness, palisade tissue thickness, spongy tissue thickness, upper epidermis layer) as compared to no spraying seedlings (Table 7 and Fig. 1). In previous studies, applying GB improved leaf midrib thickness and leaf thickness, **Shehzad *et al.* (2019)** on oat (*Avena sativa* L.) and **Aldesuquy *et al.* (2013)** on two wheat cultivars indicated foliar-applied GB

improved the leaf midrib thickness and leaf thickness.

The interaction between water treatment and foliar application (Table 7 and Fig. 1) was significant. It can be inferred that spraying kapok seedlings with GB along with irrigation TSW and ADW added a further increase leaf thickness and leaf mid-vein compared to water treatment or GB alone in both seasons.

Table 3. Main and interaction effects of water treatments (WT) and glycine betaine (GB) foliar application on plant height, leaves number/plant, root length and stem diameter of kapok seedlings during 2021 (SI) and 2022 (SII) seasons:

WT	GB	Plant height (cm)		leaves number/plant		Root length (cm)		Stem diameter (mm)	
		SI	SII	SI	SII	SI	SII	SI	SII
NIW		90.33 A	91.00 A	20.33 A	20.50 A	65.00 A	64.67 A	20.33 A	20.50 A
ADW 1		86.50 A	86.33 AB	20.17 A	19.83 AB	62.50 A	62.66 A	20.17 A	21.00 A
ADW 2		80.50 B	82.33 BC	18.19 A	18.32 AB	59.83 A	61.17 A	19.67 A	19.67 A
ADW 3		67.50 C	71.83 D	12.35 B	13.83 C	50.50 B	48.83 B	13.83 B	15.00 B
ADW 4		64.00 C	58.50 E	10.00 C	10.84 D	46.83 B	38.68 B	11.67 CD	11.50 CD
TSW 1		85.33 AB	82.17 BC	19.32 A	19.00 AB	65.17 A	64.17 A	20.17 A	19.33 A
TSW 2		80.00 B	78.00 CD	18.15 A	17.50 B	62.50 A	61.50 A	19.33 A	19.50 A
TSW 3		66.00 C	63.50 E	9.83 C	10.66 D	45.17 BC	41.67 A	13.50 BC	13.00 C
TSW 4		47.83 D	46.17 F	8.19 C	9.67 D	40.00 C	40.18 B	11.33 D	10.50 D
	0 mM	71.11 B	69.00 B	14.75 B	14.32 B	53.00 B	50.00 B	15.44 B	15.37 B
	50 mM	79.00 A	77.63 A	16.22 A	16.81 A	58.00 A	57.40 A	17.89 A	17.96 A
NIW	0 mM	88.33 ab	88.67 a-c	19.29 ab	19.68 ab	61.33 a-d	60.00 a	19.00 a-c	19.00 a-c
	50 mM	92.33 a	93.33 a	21.34 a	21.29 a	68.67 ab	69.32 a	21.67 a	22.00 a
ADW 1	0 mM	83.00 bc	82.67 b-d	20.04 a	19.34 ab	60.33 a-d	60.68 a	19.33 abc	20.00 ab
	50 mM	90.00 ab	90.00 ab	20.09 a	20.31 ab	64.67 a-c	64.65 a	21.00 ab	22.00 a
ADW 2	0 mM	75.67 cd	79.33 cde	16.28 bc	17.00 bc	55.00 c-f	57.33 ab	18.33 bc	18.67 bc
	50 mM	85.33 ab	85.33 a-d	20.11 a	19.65 ab	64.67 a-c	65.00 a	21.00 ab	20.66 ab
ADW 3	0 mM	62.67 f	71.33 e	10.05 de	12.00 de	49.33 e-g	41.32 c	12.33 fg	13.34 ef
	50 mM	72.33 de	72.33 e	14.67 c	15.61 cd	51.67 d-g	56.34 ab	15.33 de	16.68 cd
ADW 4	0 mM	62.33 f	56.67 f	9.00 de	10.00 ef	46.33 f-h	36.68 c	11.00g	10.33 fg
	50 mM	65.67 ef	60.33 f	11.31 d	11.58 ef	47.33 f-h	40.64 c	12.33 fg	12.69 ef
TSW 1	0 mM	82.00 bc	78.00 de	18.00 ab	18.33 a-c	61.00 a-d	59.69 a	19.00 a-c	18.32 bc
	50 mM	88.67 ab	86.33 a-d	21.34 a	19.62 ab	69.33 a	68.67 a	21.33 ab	20.31 ab
TSW 2	0 mM	74.00 d	71.00 e	16.29 bc	15.43 cd	58.33 b-e	57.34 ab	17.67 cd	18.00 bc
	50 mM	86.00 ab	85.00 a-d	20.00 a	19.57 ab	66.67 ab	65.69 a	21.00 ab	21.00 ab
TSW 3	0 mM	58.33 f	54.67 f	10.05 de	8.77 f	45.67 f-h	39.67 c	12.33 fg	11.33 fg
	50 mM	73.67 d	72.33 e	10.02 de	12.57 de	44.67 gh	43.66 bc	14.67 ef	14.67 de
TSW 4	0 mM	35.00 g	38.67 g	7.28 e	8.58 f	38.00 h	37.00 c	10.00 g	9.31 g
	50 mM	60.67 f	53.67 f	9.64 de	10.69 ef	42.00 gh	43.00 bc	12.67 e-g	11.69 e-g

Values marked with the same letter(s) within the main and interaction impacts are statistically similar using Revised Least Significant Difference test. Uppercase letter(s) refers to differences within the main effects and lowercase letter(s) refers to differences within the interaction effects. NIW= Nile irrigation water 100%, ADW1= agricultural drainage water 25%:Nile irrigation water 75%, ADW2=agricultural drainage water 50%: Nile irrigation water 50%, ADW3=agricultural drainage water 75%:Nile irrigation water 25%, ADW4=agricultural drainage water100%, TSW1= treated sewage water 25%:Nile irrigation water 75%, TSW2= treated sewage water 50%:Nile irrigation water 50%, TSW3= treated sewage water 25%: Nile irrigation water 75%, TSW4= treated sewage water 100%.

Table 4. Main and interaction effects of water treatments (WT) and glycine betaine (GB) foliar application on leaf area/plant, root diameter, stem fresh weight and Leaves fresh weight of kapok seedlings during 2021 (SI) and 2022 (SII) seasons

WT	GB	Leaf area/plant (m ²)		Root diameter (mm)		Stem fresh weight (g)		Leaves fresh weight (g)	
		SI	SII	SI	SII	SI	SII	SI	SII
NIW		0.0884 A	0.0887 A	19.67 A	19.67 A	94.11 A	92.18 A	23.04 A	22.97 A
ADW 1		0.0879 A	0.0877 A	19.33 AB	19.50 A	88.99 AB	87.42 A	21.88 A	22.16 A
ADW 2		0.0877 A	0.0879 A	17.33 AB	18.17 A	83.62 B	86.18 A	20.93 A	21.76 A
ADW 3		0.0831 B	0.0827 B	14.33 C	13.83 B	50.33 C	50.46 B	16.47 B	15.03 B
ADW 4		0.0821 B	0.0817 BC	14.33 C	13.83 B	39.13 D	35.08 C	12.90 BC	13.48 B
TSW 1		0.0879 A	0.0883 A	19.33 AB	18.83 A	89.84 AB	88.24 A	21.71 A	23.31 A
TSW 2		0.0871 A	0.0876 A	17.83 AB	18.00 A	85.75 AB	85.17 A	22.61 A	21.75 A
TSW 3		0.0817 BC	0.0823 B	13.67 CD	13.83 B	37.64 D	37.85 C	14.78 BC	14.56 B
TSW 4		0.0803 C	0.0805 C	11.67 D	11.50 B	27.53 E	25.22 D	11.80 C	11.73 B
	0 mM	0.0844 B	0.0843 B	15.4 B	15.40 B	60.31 B	59.43 B	16.44 B	16.44 B
	50 mM	0.0859 A	0.0862 A	17.4 A	17.30 A	72.35 A	71.19 A	20.47 A	20.61 A
NIW	0 mM	0.0877 a	0.0888 a	19.33 ab	19.00 a-c	92.02 ab	88.57 a-c	20.51 a-c	20.37 bc
	50 mM	0.0890 a	0.0886 a	20.00 a	20.33 a	96.21 a	95.78 a	25.58 a	25.57 a
ADW 1	0 mM	0.0871 a	0.0870 a	18.33 a-c	18.33 a-d	82.85 bc	80.53 c	19.82 a-d	20.31 bc
	50 mM	0.0887 a	0.0884 a	20.33 a	20.67 a	95.14 ab	94.31 ab	23.93 ab	24.01 ab
ADW 2	0 mM	0.0875 a	0.0872 a	16.33 b-e	17.67 a-d	73.18 c	79.87 c	19.26 b-d	19.39 b-d
	50 mM	0.0878 a	0.0886 a	18.33 a-d	18.67 a-d	94.06 ab	92.50 ab	22.60 a-c	24.13 ab
ADW 3	0 mM	0.0827 bc	0.0819 bc	13.00 e-i	14.00 e-h	41.96 e-g	42.98 e	14.57 d-g	12.80 e-g
	50 mM	0.0835 b	0.0834 b	15.67 c-g	13.67 e-h	58.70 d	57.94 d	18.38 b-e	17.26 c-e
ADW 4	0 mM	0.0809 c-e	0.0805 c	13.33 e-i	12.33 f-h	34.52 f-h	28.91 f	12.29 fg	12.11 eg
	50 mM	0.0833 b	0.0829 bc	15.33 c-h	15.33 d-g	43.75 ef	41.24 e	13.51 e-g	14.85 d-g
TSW 1	0 mM	0.0877 a	0.0880 a	18.33 a-c	18.33 a-d	86.18 ab	83.59 bc	18.30 b-e	20.17 bc
	50 mM	0.0880 a	0.0886 a	20.33 a	19.33 ab	93.51 ab	92.89 ab	25.13 a	26.45 a
TSW 2	0 mM	0.0870 a	0.0874 a	16.33 b-e	16.34 b-e	83.27 bc	80.98 c	19.98 a-d	19.71 b-d
	50 mM	0.0872 a	0.0878 a	19.33 ab	19.68 ab	88.24 ab	89.36 a-c	25.25 a	23.79 ab
TSW 3	0 mM	0.0795 de	0.0809 c	12.33 hi	12.00 gh	25.28 h	25.29 f	12.49 fg	11.94 g
	50 mM	0.0838 b	0.0837 b	15.00 c-h	15.66 c-f	50.00 de	50.41 de	17.07 c-f	17.18 c-f
TSW 4	0 mM	0.0790 e	0.0772 d	11.00 i	10.68 h	23.52 h	24.17 f	10.77 g	11.19 g
	50 mM	0.0816 b-d	0.0837 b	12.33 hi	12.34 f-h	31.53 gh	26.26 f	12.82 e-g	12.26 e-g

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Table 5. Main and interaction effects of water treatments (WT) and glycine betaine (GB) foliar application on fresh weight of roots, stem dry weight, leaves dry weight and roots dry weight of kapok seedlings during 2021 (SI) and 2022 (SII) seasons

WT	GB	Roots fresh weight (g)		Stem dry weight (g)		Leaves dry weight (g)		Roots dry weight (g)	
		SI	SII	SI	SII	SI	SII	SI	SII
NIW		87.19 A	87.96 A	30.31 A	28.99 A	6.703 A	6.712 A	28.90 A	28.10 A
ADW 1		83.31 A	82.45 A	28.25 AB	27.92 AB	6.503 A	6.535 AB	27.63 A	27.28 A
ADW 2		83.77 A	84.12 A	25.91 BC	25.19 BC	6.040 AB	6.280 AB	26.10 A	25.73 A
ADW 3		55.02 B	53.10 B	13.24 D	12.89 DE	4.150 C	4.210 C	18.89 B	15.86 BC
ADW 4		34.18 D	32.85 C	11.17 D	10.03 EF	2.757 D	2.677 D	14.25 C	12.67 C
TSW 1		84.79 A	84.47 A	25.85 BC	25.99 A-C	6.210 AB	6.273 AB	27.16 A	27.07 A
TSW 2		81.76 A	85.23 A	24.11 C	23.56 C	5.538 B	5.805 B	26.21 A	25.99 A
TSW 3		42.86 C	34.80 C	13.03 D	13.65 D	2.785 D	2.958 D	16.55 BC	16.53 B
TSW 4		26.85 E	24.48 D	10.19 D	9.09 F	2.337 D	2.588 D	14.78 C	13.94 BC
	0 mM	59.80 B	57.59 B	17.94 B	17.47 B	4.259 B	4.387 B	20.58 B	19.77 A
	50 mM	69.03 A	68.95 A	22.51 A	21.93 A	5.301 A	5.399 A	23.97 A	14.45 B
NIW	0 mM	84.92 ab	85.22 a-c	29.00 a	27.44 ab	5.837 b-d	5.827 c-f	27.97 a-c	27.63 ab
	50 mM	89.47 a	90.71 a	31.62 a	30.54 a	7.570 a	7.597 a	29.83 a	28.57 ab
ADW 1	0 mM	78.03 bc	75.29 d	26.74 ab	25.06 bc	6.140 bc	5.893 c-e	26.92 a-c	26.34 ab
	50 mM	88.60 a	89.61 ab	29.76 a	30.77 a	6.867 ab	7.177 ab	28.34 a-c	28.21 ab
ADW 2	0 mM	78.46 bc	79.33 cd	22.34 bc	22.38 cd	5.277 cd	5.667 d-f	24.33 b-d	23.16 bc
	50 mM	89.09 a	88.91 ab	29.47 a	28.01 ab	6.803 ab	6.893 a-c	27.88 a-c	28.31 ab
ADW 3	0 mM	50.42 e	44.55 fg	10.57 e-g	11.03 f-h	3.503 e	3.610 g	17.84 f-h	13.69 ef
	50 mM	59.62 d	61.66 e	15.90 de	14.75 ef	4.797 d	4.810 f	19.93 d-f	18.02 de
ADW 4	0 mM	30.79 fg	27.68 h	10.25 fg	9.12 gh	2.903 e	2.643 gh	12.40 i	10.89 f
	50 mM	37.57 f	38.01 g	12.08 e-g	10.94 f-h	2.610 e	2.710 gh	16.11 f-i	14.45 d-f
TSW 1	0 mM	80.66 a-c	79.19 cd	22.86 bc	22.88 cd	5.517 cd	5.837 c-f	25.09 a-c	24.74 ab
	50 mM	88.91 a	89.75 ab	28.84 a	29.10 ab	6.903 ab	6.710 a-d	29.24 ab	29.41 a
TSW 2	0 mM	75.03 c	81.72 b-d	19.83 cd	18.89 de	4.640 d	5.077 ef	23.45 c-e	23.33 bc
	50 mM	88.49 a	88.73 ab	28.39 a	28.22 ab	6.437 a-c	6.533 b-d	28.97 ab	28.65 ab
TSW 3	0 mM	36.11 f	23.80 h	11.32 e-g	12.92 fg	2.247 e	2.497 h	14.22 g-i	13.68 ef
	50 mM	49.61 e	45.80 f	14.73 d-f	14.38 f	3.323 e	3.420 gh	18.88 e-g	19.39 cd
TSW 4	0 mM	23.80 g	21.65 h	8.57 g	7.50 h	2.270 e	2.433 h	13.02 hi	14.53 d-f
	50 mM	29.91 fg	27.39 h	11.81 e-g	10.68 f-h	2.403 e	2.743 gh	16.55 f-i	13.36 ef

Values marked with the same letter(s) within the main and interaction impacts are statistically similar using Revised Least Significant Difference test. Uppercase letter(s) refers to differences within the main effects and lowercase letter(s) refers to differences within the interaction effects. NIW= Nile irrigation water 100%, ADW1= agricultural drainage water 25%:Nile irrigation water 75%, ADW2=agricultural drainage water 50%: Nile irrigation water 50%, ADW3=agricultural drainage water 75%:Nile irrigation water 25%, ADW4=agricultural drainage water100%, TSW1= treated sewage water 25%:Nile irrigation water 75%, TSW2= treated sewage water 50%:Nile irrigation water 50%, TSW3= treated sewage water 25%: Nile irrigation water 75%, TSW4= treated sewage water 100%.

Table 6. Main and interaction effects of water treatments (WT) and glycine betaine (GB) foliar application on Leaf elemental contents (Antioxidant activity, Proline and phenolic content) of kapok seedlings during 2021 (SI) and 2022 (SII) seasons

WT	GB	Antioxidant activity (%)		Proline content (mg g ⁻¹)		Phenolic (mg g ⁻¹)	
		SI	SII	SI	SII	SI	SII
NIW		53.24 F	53.17 G	0.553 C	0.721 BC	0.463 E	0.497 D
ADW 1		58.99 EF	59.44 FG	0.611 BC	0.618 D	0.519 DE	0.541 CD
ADW 2		63.62 DE	63.07DEF	0.752 A	0.742 BC	0.549 D	0.577 C
ADW 3		68.82 CD	68.87CD	0.792 A	0.798 AB	0.654 C	0.686 B
ADW 4		78.09 AB	78.74 AB	0.811 A	0.819 A	0.759 A	0.787 A
TSW 1		60.56 E	61.00 EF	0.575 C	0.579 DE	0.505 DE	0.511 D
TSW 2		67.46 D	67.33 CDE	0.665 B	0.708 C	0.570 D	0.581 C
TSW 3		74.26 BC	74.39 BC	0.765 A	0.76 ABC	0.680 BC	0.720 B
TSW 4		83.39 A	83.536 A	0.811 A	0.821 A	0.724 AB	0.732 AB
	0 mM	65.59 B	65.597 B	0.702 B	0.704 B	0.510 B	0.531 B
	50 mM	69.62 A	69.860 A	0.728 A	0.732A	0.695 A	0.720 A
NIW	0mM	51.70 i	55.71 gh	0.534 g	0.517 g	0.372 h	0.399 g
	50mM	54.79 hi	50.63 h	0.572 fg	0.566 fg	0.555 c-e	0.594 c-e
ADW 1	0mM	57.21 g-i	57.80 f-h	0.619 e-g	0.616 ef	0.436 f-h	0.467 fg
	50mM	60.77 f-h	61.08 d-h	0.602 fg	0.619 ef	0.602 cd	0.615 cd
ADW 2	0mM	62.97 f-h	44.50 e	0.760 a-d	0.750 a-d	0.479 e-g	0.499 ef
	50mM	64.27 f-h	62.08 d-h	0.743 a-d	0.735 b-d	0.619 c	0.655 c
ADW 3	0mM	66.49 e-g	67.80 c-f	0.795 ab	0.807 a-c	0.553 c-e	0.598 cd
	50mM	71.16 b-f	69.94 b-e	0.789 a-c	0.788 a-c	0.756 b	0.774 b
ADW 4	0mM	76.37a-e	80.34 ab	0.811 ab	0.822 ab	0.630 c	0.655 c
	50mM	79.80 a-c	77.15 a-c	0.8102 ab	0.816 a-c	0.888 a	0.918 a
TSW 1	0mM	58.13 g-i	58.50 e-h	0.572 fg	0.583 e-g	0.420 gh	0.433 fg
	50mM	63.00 f-h	63.50 d-g	0.578 fg	0.575 e-g	0.589 cd	0.588 c-e
TSW 2	0mM	65.71 fg	65.41 d-g	0.653 d-f	0.750 a-d	0.515 d-f	0.520 d-f
	50mM	69.20 d-f	69.26 b-f	0.677 c-f	0.666 de	0.625 c	0.642 c
TSW 3	0mM	70.75 c-f	70.82 a-c	0.729 b-e	0.7220 cd	0.577 cd	0.600 cd
	50mM	77.78 a-d	46.92 a-d	0.802 ab	0.808 a-c	0.783 b	0.839 ab
TSW 4	0mM	80.96 ab	80.19 ab	0.848 a	0.843 a	0.608 cd	0.606 cd
	50mM	85.82 a	86.88 a	0.801 ab	0.799 a-c	0.839 ab	0.859 ab

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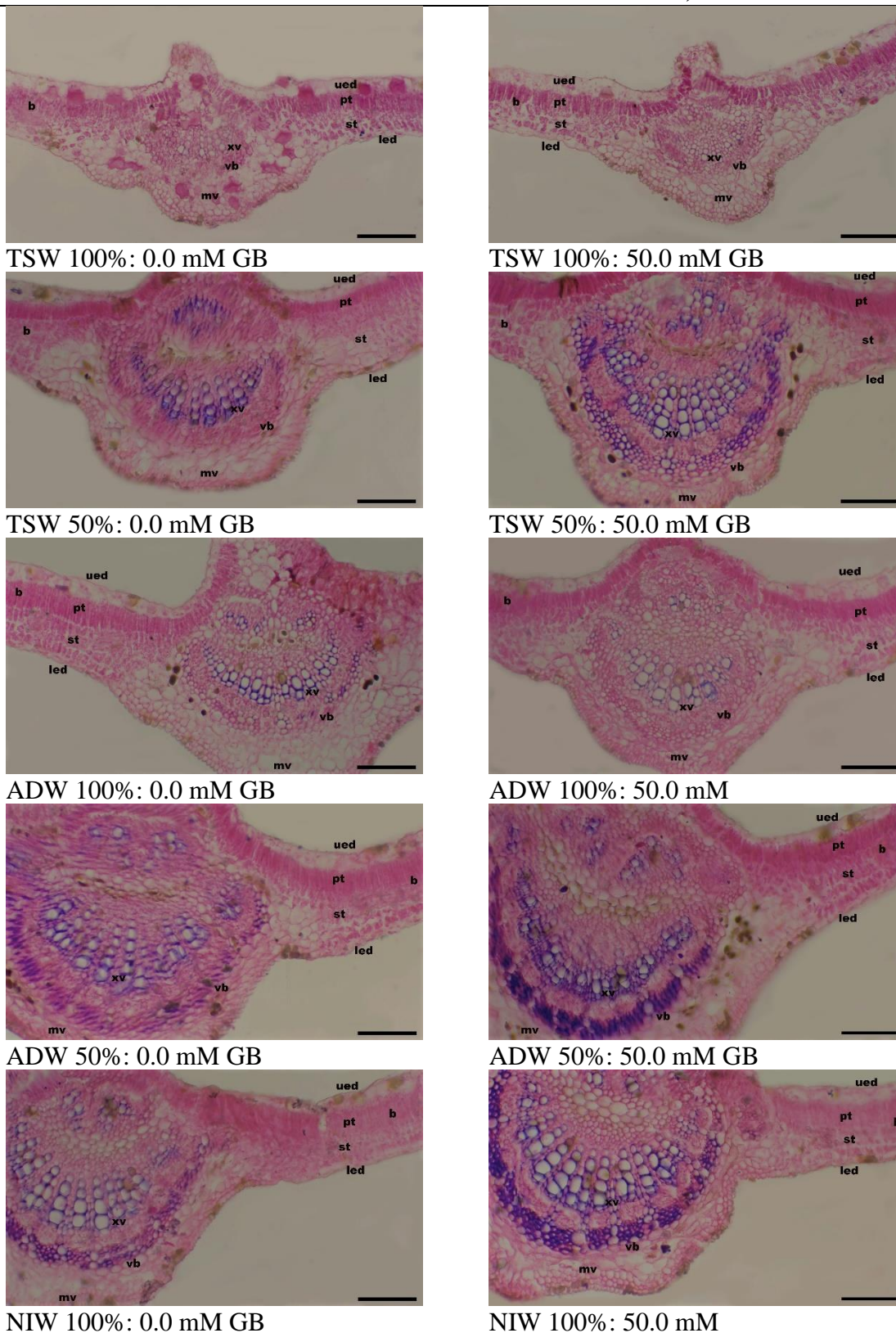


Fig. 1. Transverse sections in kapok leaves treated with treated sewage water (TSW) and agricultural drainage water (ADW) as well as GB. vb: vascular bundle, mv: mid vein, b: blade, xv: xylem vessel, ued: upper epidermis, pt: palisade tissue, st: sponge tissue, led: lower epidermis. Bares represent 200 μ m.

Table 7. Main and interaction effects of water treatments (WT) and glycine betaine (GB) foliar application on leaf anatomical structure of kapok seedlings during 2022 season

WT	GB	Average height of mid-vein (μm)	Average height of vascular bundle (μm)	Average width of vascular bundle (μm)	Average diameter of xylem vessel (μm)	Average thickness of leaf blade (μm)	Average the upper epidermis layer (μm)	Average thickness of palisade tissue (μm)	Average thickness of spongy tissue (μm)
NIW		1278.82 A	359.72 A	853.02 A	42.83 A	278.79 A	45.12 A	82.46 A	91.80 A
ADW 2		1132.69 AB	338.77 A	762.25 AB	41.17 AB	259.82 AB	42.28 AB	76.47 AB	84.41 AB
ADW 4		834.71 C	193.09 C	430.12 CD	26.50 D	207.05 BC	38.29 BC	60.25 BC	67.08 C
TSW 2		1055.17 BC	319.87 A	735.94 BC	40.17 AB	235.57 AB	39.68 BC	75.15 AB	84.31 AB
TSW 4		597.83 D	177.49 C	378.59 D	30.17 C	178.75 D	34.39 DE	54.76 CD	62.28 CD
	0 mM	949.341B	269.36B	641.09B	35.444B	226.555B	39.68A	69.123B	80.595B
	50 mM	1058.73A	311.79A	686.80A	37.889A	237.956A	40.625A	72.301A	85.617A
NIW	0 mM	1299.57 ab	339.46 a-c	850.11 a	42.00 ab	275.26 ab	38.36 a-c	76.76 a-c	98.60 ab
	50 mM	1358.08 a	379.98 ab	849.93 a	43.67 a	282.33 a	40.06 ab	88.16 a	105.00 a
ADW 2	0 mM	1102.21 bc	314.85 a-c	740.73 b	39.67 bc	242.09 a-c	39.66 a-c	75.66 bc	80.15 de
	50 mM	1263.18 ab	362.70 ab	743.77 b	41.67 ab	239.54 a-c	42.89 ab	77.29 a-c	95.67 a-c
ADW 4	0 mM	725.08 d	180.67 de	362.75 d	24.00 d	197.49 ef	38.87 bc	58.35 de	64.11 fg
	50 mM	944.32 cd	205.51 cd	497.48 cd	29.00 cd	216.60 de	37.70 b-d	62.14 cd	70.04 e-g
TSW 2	0 mM	1014.19 bc	285.66 b-d	731.99 bc	38.00 bc	216.66 cd	39.12 a-c	74.33 bc	81.33 de
	50 mM	1276.14 ab	354.09 a-c	739.88 ab	40.33 ab	241.49 a-c	40.24 ab	76.97 a-c	87.30 cd
TSW 4	0 mM	585.27 e	160.72 ef	330.33 de	28.67 cd	172.21 fg	34.74 cd	52.23 ef	59.47 g
	50 mM	610.39 de	194.28 c-e	426.86 c-e	31.67 c	185.29 ef	34.03 c-e	57.29 de	65.08 fg

Values marked with the same letter(s) within the main and interaction impacts are statistically similar using Duncan's multiple range test. Uppercase letter(s) refers to differences within the main effects and lowercase letter(s) refers to differences within the interaction effects. NIW= Nile irrigation water 100%, ADW1= agricultural drainage water 25%:Nile irrigation water 75%, ADW2=agricultural drainage water 50%: Nile irrigation water 50%, ADW3=agricultural drainage water 75%:Nile irrigation water 25%, ADW4=agricultural drainage water100%, TSW1= treated sewage water 25%:Nile irrigation water 75%, TSW2= treated sewage water 50%:Nile irrigation water 50%, TSW3= treated sewage water 25%: Nile irrigation water 75%, TSW4= treated sewage water 100%.

4. CONCLUSION

Our results showed that the vegetative growth characters of Kapok leaves seedlings effective negatively to irrigation by TSW and ADW 100%. But also, mixing TSW or ADW with NIW percentage 25:75 and 50:50 give better results on vegetative growth characters of Kapok leaves seedlings like control seedlings which irrigated with NIW. As glycine betaine spraying 50 mM proved to have remarkably positive effects on vegetative growth of leaves Kapok seedlings. As well as, the interactive between the two studied factors, the irrigation with TSW, ADW at 25, 50 % with spraying GB at 50 mM significantly improved the vegetative growth of Kapok seedlings than other treatments. Concluded that from irrigation kapok seedlings with TSW or ADW preferably was mixing with

NIW percentage 50:50 give better results on vegetative growth characters of kapok leaves seedlings like control seedlings which irrigated with NIW, and spraying of GB principally at the concentration of 50 mM combined together has the possibility enhancing and progressing the characters of kapok.

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