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Effect of irrigation water quality and foliar-sprayed glycine betaine on photosynthetic pigments, water status, and element content of kapok seedlings

Abd-Allah H. A. Hassan[*], Feisal M. A. Matter, Mahmoud A. Hassanain, Alaa I. B. Abou-Sreea

Horticulture Department, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt

ABSTRACT

This study aims to examine the main and interaction effects of irrigation with treated sewage water (TSW), agricultural drainage water (ADW) and foliar spraying with glycine betaine (GB) on physiological parameters and chemical composition of kapok (*Ceiba pentandra* L.). Therefore, a pot experiment was conducted during the summer seasons of 2021 and 2022 in a private farm in Beni-Suef Governorate, Egypt. The experimental layout was a split plot in a randomized complete blocks design with three replications. Kapok seedlings were irrigated with Nile irrigation water (NIW) either directly (100%) or mixed TSW or ADW with NIW (25, 50, and 75 %) in addition to glycine betaine (GB) was applied at (0.0 mM and 50 mM). Results showed that irrigation with TSW (100%) and ADW (100%) decreased pigments content (Chl *a*, Chl *b*, and carotenoids), membrane stability index (MSI), relative water content (RWC), relative chlorophyll content (SPAD value), leaf thickness, N, P, K and Ca content, but increased Na, Cu, Ni, Mn, Zn, and Pb content. Moreover, GB significantly increased Chl *a*, Chl *b*, carotenoids, MSI, SPAD, RWC, N, P, K, and Ca content and antioxidant activity, as well as reduced Na, Cu, Ni, Mn, Zn and Pb content in leaf. Irrigating kapok seedlings with TSW or ADW and NIW at 25, 50% give better results similar to those irrigated with NIW for all parameters. GB and water treatments interaction give better results at 50 mM GB, and TSW or ADW mixed with NIW at rates of 25%, and 50%, respectively. Therefore, spraying of GB (50 mM) and irrigation with TSW or ADW mixed with NIW at the rate of 50% improved physiological parameters, chemical constituents, and nutrient content compared to control conditions.

Keywords: Kapok, woody trees, water stress, heavy metals, agriculture drainage water, water stress.

[*]Corresponding author’s e-mail address: aha13@fayoum.edu.eg

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1. INTRODUCTION

Kapok (*Ceiba pentandra* L.) commonly locally known as silk-cotton or Kapok tree, belongs to the Bombacaceae family. It is cultivated in low and rainforest zone i.e., Guinea Savanna zone, and Southeast Asia, Kapok tree are widely used in herbal medicine in South America, West and South India, Sri-Lanka and other South east Asian countries and parts of Africa **(Menant, 1980)**. The fibers are lustrous, yellowish-brown in color, light, inelastic and brittle, mainly used as a stuffing material for mattresses and pillows. Furthermore, fibers can be used in upholstery, life preservers, and other water-safety equipment because of their excellent buoyancy, and for insulation against sound and heat due to their air-filled lumen (**Kobayashi *et al*. (1977).** Seed oil can be used as oil sorbent for diesel, hydraulic oil, engine oil, and diesel water mixture in batch and continuous system **(Lim and Huang, 2007).**

The use and reuse of treated sewage water (TSW) and agricultural drainage water (ADW) can augment the available water in many countries where fresh water is in short supply. In the present time, climate change is inevitable **(Koutsoyiannis, 2013)** where water sector is the most vulnerable to this change **(Rosegran *et al.*, 2002; Elba *et al.*, 2017).** The unfavorable balance between Egypt's water demand and supply necessitates the use of unconventional water sources, such as groundwater and recycled water, to combat water scarcity. In particular, recycling agricultural drainage water is nearing the maximum; future increases would be difficult to obtain **(Rassoul, 2006)**. Egypt's water quality concerns depend on usage pattern and population density among different water bodies. In addition, severe droughts resulting from climate change and increased protected farming make it difficult for water to be provided stably. Therefore, wastewater reuse for agriculture gained international interest as an alternative water supply. Wastewater treatment is suitable for irrigation in many ways and is already widely employed worldwide **(El-Sayed, 2020)**.

Various approaches have been utilized to cut down the heavy metal stress in plants. One such approach is the application of osmoprotectants such as glycine betaine (GB) to reduce the heavy metal stress in plants. GB (N,N,N-trimethylglycine) is a synthesized in chloroplast from serine through betaine aldehyde, choline and ethanolamine **(Hanson and Scott, 1980).** It acts as an osmoprotectants inhibiting the production of ROS and free radicals **(Fariduddin *et al.*, 2013)**, regulates all the required gene expressions that create additional antioxidants enzymes SOD, POD, and CAT, and successfully scavenges the unwarranted ROS under Cd stress **(Ali *et al.*, 2013).**

Therefore, this study aims to investigate the effect of treated sewage water and agriculture drainage water used for irrigating compared with Nile irrigation water on kapok's physiological parameters and chemical constituents, to raise the amount of water income from the available resources in Egypt.

2. MATERIAL AND METHODS

2.1. Experimental location and plant material

Pot experiments were conducted during two successive seasons (2020/2021 and 2021/2022) at a private farm in Beni-Suef, Egypt. Seeds of *Ceiba pentandra* L. were obtained from kapok trees located at Fayoum, Egypt, during (2019/2020 and

2020/2021) seasons. The seeds were sown in a plastic pot (10 cm top diameter) filled with 2 kg of sand and clay soil (3:1, *v/v*) in the first week of April for both seasons, The seedlings were transplanted to a plastic pot (20 cm diameter) after 90 days from sowing after 1 year from sowing the seedlings were transplanted to in a plastic pot (40 cm diameter) filled with 20 kg sandy soil with one seedling in each pot. Soil physical and chemical properties **(Table 1)** were determined using methods described by **Klute (1986)** and **Page *et al.* (1982)**, respectively.

2.2. Treatments and the experimental design

Water used in the experiment (ADW, TSW, and NIW) was obtained from different sources; TSW was obtained from (a bilateral sewage treatment station located at Beni-Suef Egypt), ADW was obtained from (the agricultural irrigation water canal in Naim village Beni-Suef) and NIW was obtained from (irrigation canal in Naim village Beni-suef. The ADW mixed with NIW at a rate of (0:100%, 25:75%, 50:50%, 75:25% and 100:0 %), respectively. Also , the TSW mixed with NIW at a rate of (0:100%, 25:75%, 50:50%, 75:25% and 100:0%), respectively, The analysis of water used in the experiment during two seasons (2020/2021 and 2021/2022) is shown in **Table 2**. Glycine betaine (GB) was obtained from (Sigma company®) and was applied at a rate of (0 and 50 mM).

After the stability of the 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 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. The physical and chemical properties of soil used in the experiment during two seasons (2020/2021 SI and 2021/2022 SI)

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 ºC)				pH		
SI	2.39				7.12		
SII	2.37				7.06		
	Soluble cations (meq l^{-1})				**Soluble anions (meq l^{-1})**		
	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. The analysis of water used in the experiment during two seasons (2020/2021 and 2021/2022)

Properties	2020/2021		2021/2022	
	TSW	ADW	TSW	ADW
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
Fe (mg/L)	18.86	11.08	18.69	11.23
Zn (mg/L)	4.10	6.32	4.13	6.39
Cu (mg/L)	0.39	0.42	0.36	0.47
Mn (mg/L)	0.13	0.59	0.14	0.62
Pb (mg/L)	6.54	5.88	6.49	5.63
Ni (mg/L)	2.61	5.2	2.58	5.31

The experimental design 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-pots; each treatment consisted of thirteen plastic-pots.

2.3. Membrane stability index, Relative water content, and Relative chlorophyll

The membrane stability index (MSI%) was estimated using a 0.2 g sample of fully-expanded leaf tissue. Each sample was placed in a screw-capped vial containing 10 ml of double-distilled water. Vials were then heated at 40 ˚C in a water bath for 30 minutes, and the electrical conductivity of the solution was recorded using a conductivity bridge (C1). The vials were then boiled at 100 ˚C for 10 minutes, and then the electrical conductivity of the solution was recorded using a conductivity bridge (C2). The MSI was calculated using the formula **(Reigosa and Gonzales, 2001)**.

$$MSI\ (\%) = [1- (C1/C2) \times 100.$$

Relative water content (RWC%) was analyzed at full flowering in three leaf samples from the third node from the top of the shoot (three replications per treatment). Leaf discs (n = 20; diameter = 5 mm) were cut from each leaf. After determining the leaf discs (fresh weight), the discs were immersed in distilled water for 8 hours to estimate their turgid weight (TW). The discs were then dried at 70 ˚C for 24 hours to measure their (dry weight). The relative water content was calculated using the following equation **(Reigosa and Gonzales, 2001)**.

$$\mathrm{RWC}\ (\%) = [(\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100]$$

Relative chlorophyll content (SPAD value) was measured in leaf/plant by using a chlorophyll meter (SPAD-502, Minolta, Japan)

2.4. Leaf photosynthetic pigments

Leaf chlorophyll *a* and *b* contents and carotenoid concentrations (mg mm^2 FW) were measured and calculated according to **(Arnon, 1949)**. A 0.1 g of leaf samples were grounded in porcelain mortal using 25 ml of 80 % (v/v) acetone. After filtrating the samples, the absorbance was measured at 663, 645 and 470 nm using a UV-160A UV-visible spectrometer (Shimadzu, Kyoto, Japan).

2.5. Leaf elemental contents

Leaf samples from three randomly selected plants, in each experimental unit, were collected, washed with tap water, rinsed three times with distilled water and dried at 70 C° in a forced-air oven till constant weight. Leaf N (%) was colormetrically determined by using the orange G dye according to the method of **Hafez and Mikkelson (1981)**. Leaf P (mg g^{-1} dry weight) was colorimetrically estimated according to the stannous molybdate chloride method as illustrated in **A.O.A.C. (1995)**. Leaf K^+, Ca and Na^+ (mg g^{-1} DW) were photometrically measured using flam-photometer as mentioned by **Wilde *et al.* (1985)**. Leaf Cu, Ni (ppm) and Mn, Pb, Zn (mg/100g DW) were photometrically measured using atomic absorption spectroscopy.

2.6. Statistical analysis

Appropriate analysis of variance was performed on the 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. Membrane stability index (MSI), relative water content (RWC), and relative chlorophyll content (SPAD value)

A negative relationship was found between membrane stability index (MSI), relative water content (RWC), relative chlorophyll content (SPAD value), and water treatments **(Table 3)**. The MSI, RWC and SPAD values in leaves of kapok seedlings were significantly decreased by irrigation with TSW or ADW at 100%, However irrigating kapok seedlings either by TSW or ADW combination with NIW at a mixing rate of 25%, 50 % did not affect MSI, RWC, and SPAD value. These results align with, **Bañón *et al.* (2011)** on polygala and lantana plants discovered that TSW reduce SPAD compared with well water, as well as mixed water (TSW with NIW 50:50) led to increased leaf SPAD than TSW. In contrast obtained by **Amin (2021)** on *Myrtus comunis*, *Eucalyptus camaldulensis* and *Cupressus sempervirens* indicated that TSW increased chlorophyll *a* in leaves compared with tap water.

Results collectively showed that GB at 50 mM exerted a positive effect on physiological parameters (MSI, RWC and SPAD value) than no spraying with GB **(Table 3)**. In general, previous studies indicated that GB increased the physiological parameters of plants, **Sief Al-Yazal *et al.* (2023)** indicated that GB increased SPAD value and MSI on *Prunus persica* trees under drought stress, **Alamer and Ali (2022)** indicated that GB increased MSI and RWC in leaves of *Tagetes erecta*. Our findings agree with other studies on different crops such **Alasvandyari and Mahdavi (2017)** on safflower (*Carthamus tinctorius*), **Zhang *et al.* (2021)** on *Dalbergia odorifera* seedlings, **Alamer and Ali (2022)** on *Tagetes erecta*, they indicated that GB increased RWC in plants.

The treatments of the combination of water treatments and GB **(Table 3)**, GB at a concentration of 50 mM under irrigation seedlings with TSW and ADW 100% or mixing with NIW enhanced MSI, RWC and SPAD value than no spraying with GB.

Table 3. The effects of water treatments (WT) and foliar application of glycine betaine (GB) on relative chlorophyll content (SPAD value), membrane stability index (MSI), relative water content (RWC), and antioxidant activity of kapok seedlings

Treatment		(SPAD value)		(MSI%)		(RWC%)		Antioxidant activity (%)	
WT	**GB**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**
NIW		45.00 A	45.43 A	64.78 A	65.81 A	79.68 A	78.77 A	53.24 F	53.17 G
ADW 1		41.30 A	42.78 AB	60.48 A	61.26 AB	71.05 AB	73.90 AB	58.99 EF	59.44 FG
ADW 2		43.38 A	42.37 AB	60.43 A	60.00 AB	70.84 AB	66.48 AB	63.62 DE	63.07D-F
ADW 3		35.90 B	38.23 B	49.02 BC	48.07 C	70.05 AB	71.89 AB	68.82 CD	68.87CD
ADW 4		33.87 B	32.17 C	44.96 C	44.93 C	62.93 B	63.04 B	78.09 AB	78.74 AB
TSW 1		40.87 A	42.83 AB	61.46 A	61.35 AB	75.25 AB	70.07 AB	60.56 E	61.00 EF
TSW 2		42.22 A	42.15 AB	61.43 A	59.68 B	69.75 AB	69.41 AB	67.46 D	67.33 C-E
TSW 3		33.65 B	31.93 C	49.81 B	49.52 C	69.30 AB	71.19 AB	74.26 BC	74.39 BC
TSW 4		27.95 C	27.70 C	44.52 C	47.97 C	65.75 B	64.25 B	83.39 A	83.536 A
	0 mM	35.03 B	35.57 B	51.34 B	51.12 B	67.30 B	68.95 B	65.59 B	65.597 B
	50 mM	41.44 A	41.23 A	59.08A	59.68 A	73.95 A	70.82 A	69.62 A	69.860 A
NIW	0mM	40.70 b-d	42.33 a-c	62.03a-c	63.19 a-c	77.10 ab	73.49 ab	51.70 i	55.71 gh
	50mM	49.30 a	48.53 a	67.54 a	70.43 a	91.40 a	78.81 a	54.79 hi	50.63 h
ADW 1	0mM	35.67 d-g	39.07 b-d	53.54 de	55.15 cd	73.65 ab	71.50 ab	57.21 g-i	57.80 f-h
	50mM	46.93 ab	46.50 a	67.43 a	67.37 ab	78.21 ab	78.73 a	60.77 f-h	61.08 d-h
ADW 2	0mM	40.30 b-d	39.33 b-d	56.72 cd	55.30 cd	63.89 b	73.50 ab	62.97 f-h	44.50 e
	50mM	46.47 ab	45.40 ab	64.15 ab	64.69 ab	75.89 ab	76.33 ab	64.27 f-h	62.08 d-h
ADW 3	0mM	34.63 d-g	37.37 cd	44.79 g	45.07 fg	63.00 b	71.46 ab	66.49 e-g	67.80 c-f
	50mM	37.17 c-g	39.10 b-d	53.25 d-f	51.06 d-g	63.62 b	73.63 ab	71.16 b-f	69.94 b-e
ADW 4	0mM	31.50 e-h	29.93 ef	43.17 g	43.55 g	59.11 b	59.46 b	76.37a-e	80.34 ab
	50mM	36.23 d-g	34.40 de	46.75 eg	46.30 e-g	68.04 ab	66.51 ab	79.80 a-c	77.15 a-c
TSW 1	0mM	37.77 c-f	38.83 b-d	54.49 d	54.37 c-e	74.70 ab	67.17 ab	58.13 g-i	58.50 e-h
	50mM	43.97 a-c	46.83 a	68.42 a	68.34 ab	80.66 ab	71.64 ab	63.00 f-h	63.50 d-g
TSW 2	0mM	38.10 c-e	37.77 cd	57.36 b-d	52.70 c-f	71.08 ab	65.00 ab	65.71 fg	65.41 d-g
	50mM	46.33 ab	46.53 a	65.50 a	66.66 ab	78.70 ab	70.28 ab	69.20 d-f	69.26 b-f
TSW 3	0mM	30.60 f-h	29.17 ef	46.18 g	45.97 e-g	61.56 b	65.55 ab	70.75 c-f	70.82 a-c
	50mM	36.70 c-g	34.70 de	53.44 de	53.07 c-f	64.30 b	70.89 ab	77.78 a-d	46.92 a-d
TSW 4	0mM	26.03 h	26.30 f	43.82 g	46.73 d-g	62.41 b	60.09 b	80.96 ab	80.19 ab
	50mM	29.87 gh	29.10 ef	45.22 g	49.20 d-g	63.90 b	59.94 b	85.82 a	86.88 a

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%.

3.2. Leaf photosynthetic pigments

Data shown in **Table 4** indicate that irrigating kapok seedlings with 100% TSW or ADW significantly lowers the contents of chlorophyll *a*, *b*, and carotenoids. However, kapok seedlings irrigated with TSW or ADW mixed with NIW at 25, 50% compared with NIW, no significant changes were recorded. These findings were confirmed by **Belhaj *et al.* (2016)** who worked on three vegetable crops (tomato, radish, and lettuce) and reported that TSW decreased chlorophyll *a*, *b*, and total carotenoids compared with fresh water, which may be with an increase in heavy metal content in the irrigation water. The study attributed such a negative effect to the toxic levels of heavy metals in the wastewater. Additionally, **Tangahu *et al.* (2011)** and **Jiwan and Ajah (2011)** indicated that elevated levels of cadmium in water soils may inhibit soil productivity, while a very low cadmium concentration may decrease the biological processes in plants including the photosynthesis process. **Hashish *et al.* (2017)** indicated that TSW decreased total carotenoids in leaves of *Terminalia angustifolia*, *Rademachera ignea*, and *Ficus mango* compared with tap water.

Foliar application of GB significantly increased leaf chlorophyll *a*, *b*, and carotenoids, with higher increments recorded at a concentration of 50 mM. Similar results were obtained by **Hu and Hu (2012)** on *Lolium perenne*, **Shala and Mahmoud (2018)** on *Hibiscus sabdariffa*, **Huang *et al.* (2022)** on *Camellia sinensis* seedlings, **Safwat and Abdel Salam (2022)** on *Ocimum basilicum* and **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings. They indicated GB increased chlorophyll *a* and *b* content in leaf plants. Furthermore, **Cisse *et al.* (2021)** noticed that plant under stress reduces water loss by closing their stomata. GB plays a role in improving the reopening of stomata, thereby increasing the net photosynthesis rate. Additionally, GB can directly scavenge ROS, thereby alleviating oxidative damage and stabilizing osmotic differences between the surroundings of the cell and the cytosol. Furthermore, stress affects negatively the concentration of proteins in plant cells, so GB increases the concentration of proteins under stress conditions. In contrast, **Cisse *et al.* (2022)** found that GB decreased chlorophyll a and b content in *Dalbergia odorifera* plants. Our findings align with **Cisse *et al.* (2021)** and **Alamer and Ali (2022)**, who found that GB increased, total carotenoids in leaves of *Dalbergia odorifera*, and *Tagetes erecta*, respectively.

The interaction between water treatment and GB on leaf photosynthetic pigment content was significant. Kapok seedlings irrigated with TSW or ADW at 25, 50% plus GB at 50 mM recorded the highest values of Chl a, Chl b, and carotenoids.

Table 4. The effects of water treatments (WT) and foliar application of glycine betaine (GB) on leaf chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations of kapok seedlings

Treatment		Chlorophyll *a* (mg/mm^2)		Chlorophyll *b* (mg/mm^2)		Carotenoids (mg/mm^2)	
WT	GB	2020/21	2021/22	2020/21	2021/22	2020/21	2021/22
NIW		0.309 A	0.304 A	0.137 A	0.187 A	0.110 A	0.112 A
ADW 1		0.287 B	0.280 AB	0.132 A	0.133 A-C	0.106 A	0.105 B
ADW 2		0.276 B	0.276 AB	0.123 A	0.163 AB	0.102 A	0.102 B
ADW 3		0.233 C	0.239 C	0.108 A	0.107 BC	0.088 B	0.083 D
ADW 4		0.217 C	0.215 C	0.224 A	0.107 BC	0.079 C	0.081 D
TSW 1		0.292 AB	0.285 AB	0.138 A	0.142 ABC	0.107 A	0.105 B
TSW 2		0.273 B	0.273 B	0.134 A	0.134 ABC	0.104 A	0.101 B
TSW 3		0.235 C	0.238 C	0.105 A	0.115 BC	0.089 B	0.092 C
TSW 4		0.217 C	0.216 C	0.093 A	0.096 C	0.085 BC	0.083 D
	0 mM	0.243 B	0.243 B	0.110 A	0.125 B	0.090 B	0.089 B
	50 mM	0.277 A	0.275 A	0.155 A	0.138 A	0.104 A	0.103 A
NIW	0 mM	0.300 ab	0.293 a-c	0.132 b	0.146 a-c	0.102 bc	0.103 cd
	50 mM	0.317 a	0.315 a	0.142 b	0.227 a	0.119 a	0.121 a
ADW 1	0 mM	0.268 c-e	0.266 b-d	0.120 b	0.125 bc	0.097 cd	0.095 de
	50 mM	0.306 ab	0.295 a-c	0.140 b	0.142 bc	0.116 a	0.116 ab
ADW 2	0 mM	0.252 d-f	0.257c-e	0.115 b	0.124 bc	0.095 c-e	0.090 e-g
	50 mM	0.299 ab	0.296 a-c	0.132 b	0.202 ab	0.110 ab	0.113 ab
ADW 3	0 mM	0.221 f-i	0.226 d-f	0.098 b	0.119 bc	0.082 f-h	0.081 gh
	50 mM	0.246 e-g	0.252 de	0.118 b	0.120 bc	0.094 c-f	0.086 e-h
ADW 4	0 mM	0.204 hi	0.201 f	0.081 b	0.096 c	0.076 h	0.078 h
	50 mM	0.229 f-h	0.230 d-f	0.166 a	0.094 c	0.083 e-h	0.084 f-h
TSW 1	0 mM	0.278 b-d	0.264 cd	0.135 b	0.134 bc	0.101 bc	0.095 de
	50 mM	0.307 ab	0.306 ab	0.141 b	0.150 a-c	0.114 a	0.114 ab
TSW 2	0 mM	0.248 d-g	0.253 de	0.131 b	0.127 bc	0.095 c-e	0.091 ef
	50 mM	0.297 a-c	0.294 a-c	0.138 b	0.141 bc	0.114 a	0.111 bc
TSW 3	0 mM	0.219 g-i	0.221 ef	0.098 b	0.108 c	0.085 d-h	0.090 e-g
	50 mM	0.251 d-g	0.255 c-e	0.113 b	0.121 bc	0.094 c-f	0.095 de
TSW 4	0 mM	0.194 i	0.200 f	0.079 b	0.089 c	0.080 gh	0.077 h
	50 mM	0.240 e-g	0.232 d-f	0.108 b	0.103 c	0.090c-g	0.089 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%.

3.3. Elemental status of kapok seedling

Kapok seedlings irrigated with TSW and ADW at 100% decreased significantly percentage of N, P, K, and Ca but also irrigation with 50% and 25% combination with NIW gave no significant percentage for these elements. As well irrigation at 50, 75, and 100 % from TSW and ADW increased a significant percentage of Na, Mn, Pb, Ni, Cu, and Zn than seedlings irrigated with NIW at 100% (**Tables 5-7**). This finding is in agreement with **Amin (2021)** on (*Myrtus comunis*, *Eucalyptus camaldulensis,* and *Cupressus sempervirens*), **Abd El-Naim *et al.* (2002)** on bermuda grass, **El-Arby and Elbordiny (2006)** on *Taxodium distichum*, *Cupressus sempervirens*, *Khaya senegalensis*, *Simmondsiaco hinensis*, they found total Pb, Mn, Cu, Zn, Ni content increased with irrigated plants with secondary TSW compared with well water. Also **Ali *et al***. **(2011)** on *Swietenia mahagoni* seedlings and **El-Sayed (2005)** on *Acacia saligna*, *Acacia stenophylla, and Ceratonia siliqua*, they found secondary TSW increased Pb, Mn, Cu, Zn, Ni content in leaves plant compared with tap water, might be attributed to an increase in the occupancy root zone by applying TSW that reflected on their uptake by roots. Data showed that TSW and ADW affected element contents in leaf plants. These findings were confirmed by **Hashish *et al,* (2017)** on *Terminalia angustifolia*, *Rademachera ignea*, and *Ficus mango* they indicated TSW decreased total N, P content in leaves compared with well water, **Ahmed *et al.* (2000)** indicated stress might be implicated indirectly in decreasing N concentration of plant due to the role played by chloride ions. In contrast, results obtained by **El-Sayed (2005)** on *Acacia saligna*, *Acacia stenophylla*, and *Ceratonia siliqua* indicated that total N, P, K, Ca, and Na content increased with secondary TSW than well water. **Ashraf and O, leary (1996)** indicated that total K^+ content decreased with secondary TSW than with tap water. The reduction of K^+ concentration in plants may be due to the Na^+ ions that can substitute K^+ ions partially in plant tissue, hence the more concentrated salinity treatment, the more Na^+ substitution to consequently K^+ ions decrease in plant tissue. GB application improved N, P, K, Ca, phenolic, and proline content in Kapok seedlings **(Tables 5 and 6)**, likewise decreased Na^+ content. In a study reported by **Amin and Al-Atrash (2020)** on *Populus nigra* seedlings, they indicated GB increased N, P, and K content in leaf plants. **Abdel-Mawgoud (2017)** reported that GB-treated plants could absorb more nutrients compared to untreated ones and this was reflected in overall plant growth. As well as **Sobahan *et al.* (2009)** found GB may play a role in maintaining cytosolic K^+ homeostasis by suppressing Na^+-enhanced apoplastic flow to reduce Na^+ uptake. **Amin and Al-Atrash (2020)** on *Populus nigra* seedlings, and **Hu and Hu (2012)** on perennial ryegrass, indicated GB decreased Na^+ content in leaf plants. **Sobahan *et al.* (2009)** found GB may play a role in suppressing Na^+-enhanced apoplastic flow to reduce Na^+ uptake. **Shehzad *et al.* (2019)** on oat *Avena sativa*, **Alamer and Ali (2022)** on *Tagetes erecta* plants, and **Safwat and Abdel Salam (2022)** on *Ocimum basilicum*, found that GB increased total phenolic in plants. In contrast to the results of **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings, and **Cisse *et al.* (2022)** on *Dalbergia odorifera* plants, found that GB decreased total phenolic. **Amin and Al-Atrash (2020)** on *Populus nigra* seedlings, **Alasvandyari and Mahdavi (2017)** on safflower (*Carthamus tinctorius*), **Cisse *et al.* (2021)** on *Dalbergia odorifera* seedlings and **Shehzad *et al.* (2019)** on oat (*Avena sativa*

L.), found that 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*, found that GB had no significant effect on proline content in plants.

Foliar application of GB at 50 mM on kapok seedlings gives positive effects on all elements by increasing the percentage of N, P, K, and Ca than seedlings sprayed with water in **Tables 5-7**. Likewise decreased concentration of heavy metals in kapok leaves (Cu, Ni, Pb, Zn, Mn, and Na)

The interaction between water treatment and foliar application of GB was significant **(Tables 5-7)**. It can be inferred that spraying kapok seedlings with GB at 50 mM and irrigation with TSW or ADW at 25% or 50%, respectively increased the percentage of N, P, K, and Ca, whereas it decreased the concentration of (Cu, Ni, Pb, Zn, Mn, and Na)

Table 5. The effects of water treatments (WT) and foliar application of glycine betaine (GB) on leaf elemental contents (N, P, K, and Ca) of kapok seedlings:

Treatment		**N (%)**		**P (mg g^{-1})**		**K (mg g^{-1})**		**Ca (mg g^{-1})**	
WT	**GB**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**
NIW		2.783 AB	2.749 A	0.224 A	0.224 A	2.63 A	2.66 A	8.113 A	8.22 A
ADW 1		2.742 AB	2.671 A	0.217 AB	0.215 A	2.49 C	2.52 B	7.838 A	7.95 AB
ADW 2		2.670 C	2.639 A	0.210 B	0.212 A	2.51 BC	2.50 B	7.762 A	7.83 AB
ADW 3		2.282 D	2.292 B	0.182 C	0.167 B	2.38 D	2.39 C	6.460 B	6.48 C
ADW 4		2.234 D	2.224 B	0.161 D	0.152 C	2.30 D	2.28 DE	5.530 C	5.73 B
TSW 1		2.835 A	2.744 A	0.218 AB	0.216 A	2.58 AB	2.55 AB	7.990 A	8.17 AB
TSW 2		2.783 AB	2.694 A	0.212 B	0.214 A	2.52 BC	2.52 B	8.053 A	8.13 AB
TSW 3		2.255 D	2.240 B	0.179 C	0.172 B	2.34 D	2.34 CD	6.607 B	6.60 D
TSW 4		1.966 E	2.069 C	0.164 D	0.164 BC	2.31 D	2.26 E	5.612 C	5.61 D
	0mM	2.401 B	2.402 B	0.192 B	0.188 B	2.38 B	2.38 B	6.690 B	6.73 B
	50mM	2.582 A	2.558 A	0.200 A	0.198 A	2.52 A	2.52 A	7.519 A	7.59 A
NIW	0mM	2.655 bc	2.647 a-d	0.217 b	0.220 a	2.56 b	2.64 ab	7.723 bc	7.94 bc
	50mM	2.911 a	2.852 a	0.231 a	0.228 a	2.69 a	2.69 a	8.503 a	8.49 ab
ADW 1	0mM	2.551 c	2.586 cd	0.217 b	0.214 a	2.44 c-e	2.46 cd	7.517 bc	7.51 cd
	50mM	2.833 a	2.757 a-d	0.224 a	0.217 a	2.54 bc	2.58 b	8.160 ab	8.38 ab
ADW 2	0mM	2.561 c	2.625 b-d	0.206 b	0.208 a	2.42 de	2.42 cd	7.117 c-e	7.13 de
	50mM	2.779 ab	2.653 a-d	0.214 b	0.217 a	2.59 ab	2.61 ab	8.407 a	8.32 ab
ADW 3	0mM	2.233 de	2.207 ef	0.181 c	0.155 c	2.35 ef	2.35 de	6.280 fg	6.14 g
	50mM	2.331 d	2.377 e	0.183 c	0.180 b	2.40 e	2.43 cd	6.640 e-g	6.82 ef
ADW 4	0mM	2.217 de	2.217 ef	0.155 e	0.154 c	2.24 g	2.21 gh	5.063 h	5.11 h
	50mM	2.251 de	2.232 ef	0.167 de	0.150 c	2.37 e	2.35 de	5.997 g	6.04 g
TSW 1	0mM	2.791 ab	2.674 a-d	0.217 de	0.213 a	2.53 b-d	2.48 c	7.527 bc	7.62 cd
	50mM	2.879 a	2.813 ab	0.223 a	0.220 a	2.64 ab	2.63 ab	8.453 a	8.71 a
TSW 2	0mM	2.611 c	2.581 d	0.208 b	0.209 a	2.44 c-e	2.45 cd	7.470 b-d	7.55 cd
	50mM	2.802 ab	2.807 a-c	0.217 b	0.218 a	2.60 ab	2.59 ab	8.637 a	8.71 a
TSW 3	0mM	2.195 de	2.148 f	0.170 d	0.158 c	2.26 fg	2.26 eg	6.393 fg	6.35 fg
	50mM	2.314 d	2.331 ef	0.189 c	0.187 b	2.41 e	2.43 cd	6.820 d-f	6.84 ef
TSW 4	0mM	1.797 f	1.936 g	0.161 de	0.159 c	2.20 g	2.13 h	5.170 h	5.20 h
	50mM	2.136 e	2.202 ef	0.167 de	0.168 bc	2.42 de	2.39 cd	6.053 g	6.02 g

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Table 6. The effects of water treatments (WT) and foliar application of glycine betaine (GB) on leaf elemental contents (Na, Pb, Cu, and Mn) of kapok seedlings

Treatment		Na (mg g^{-1})		Pb (mg/100 g)		Cu (ppm)		Mn (mg/100 g)	
WT	GB	2020/21	2021/22	2020/21	2021/22	2020/21	2021/22	2020/21	2021/22
NIW		2.39 E	2.37 D	41.98 F	41.82 F	7.91 F	8.05 G	0.1155 E	0.115 D
ADW 1		2.48 D	2.52 C	43.96 E	44.00 E	11.10 E	10.93 F	0.144 D	0.143 C
ADW 2		2.52 D	2.51 C	45.35 D	45.01 DE	16.48 D	16.60 D	0.207 C	0.210 B
ADW 3		2.74 C	2.76 B	45.86 CD	45.79 CD	16.58 D	16.64 D	0.224 C	0.225 B
ADW 4		2.84 AB	2.83 AB	46.79 BC	46.80 BC	19.10 C	18.94 C	0.256 B	0.257 A
TSW 1		2.47 D	2.50 C	45.76 CD	45.8 B-D	12.53 E	12.56 E	0.132 DE	0.133 CD
TSW 2		2.53 D	2.53 C	46.73 BC	46.87 BC	16.54 D	16.39 D	0.210 C	0.208 B
TSW 3		2.77 BC	2.76 B	47.22 B	47.24 AB	24.46 B	24.64 B	0.255 B	0.264 A
TSW 4		2.90 A	2.93 A	48.32 A	48.38 A	33.34 A	32.42 A	0.288 A	0.280 A
	0 mM	2.71 A	2.71 A	46.25 A	45.21 A	18.83 A	18.42 A	0.2152 A	0.217 A
	50 mM	2.55 B	2.56 B	44.29 B	43.30 B	16.30 B	16.51 B	0.1914 B	0.191 B
NIW	0 mM	2.46 fg	2.44 gh	42.31 f	42.04 f	7.89 h	7.93 h	01160 g	0.115 i
	50 mM	2.31 h	2.31 h	41.65 f	41.59 f	7.93 h	8.17 h	0.1150 g	0.115 i
ADW 1	0 mM	2.55 d-f	2.57 d-g	45.31 c-e	45.58 c-e	11.32 fg	10.99 g	0.1430 fg	0.142 hi
	50 mM	2.41 gh	2.42 gh	42.62 f	42.43 f	10.88 g	10.88 g	0.1430 fg	0.145 hi
ADW 2	0 mM	2.59 de	2.59 d-f	45.10 de	44.50 e	16.99 d	17.03 de	0.2273 cd	0.234 de
	50 mM	2.49 gh	2.42 gh	45.60 c-e	45.52 c-e	15.97 de	15.98 e	0.1867 e	0.186 fg
ADW 3	0 mM	2.84 bc	2.89 ab	46.58 b-d	46.39 b-e	17.03 d	17.03 de	0.2093 de	0.212 ef
	50 mM	2.62 de	2.62 de	45.13 de	45.18 de	16.13 de	16.26 de	0.2393 b-d	0.239 c-e
ADW 4	0 mM	2.87 a-c	2.89 ab	47.32 ab	47.32 a-c	19.90 c	19.90 c	0.2753 b	0.279 ab
	50 mM	2.91 ab	2.78 bc	46.26 b-e	46.27 b-e	18.30 cd	17.97 d	0.2360 cd	0.236 de
TSW 1	0 mM	2.50 e-g	2.51 d-g	46.66 b-d	46.94 a-d	13.72 ef	13.79 f	0.1483 fg	0.150 g-i
	50 mM	2.43 fg	2.49 e-g	44.87 e	44.83 de	11.34 fg	11.33 g	0.1153 g	0.115 i
TSW 2	0 mM	2.60 de	2.59 d-f	46.61 b-d	46.84 a-d	16.73 d	16.50 de	0.2420 b-d	0.239 c-e
	50 mM	2.46 fg	2.46 fg	46.84 bc	46.90 a-d	16.34 de	16.28 de	0.1770 ef	0.177 f-h
TSW 3	0 mM	2.90 ab	2.87 ab	47.56 ab	47.55 a-c	29.02 b	29.01 b	0.2590 bc	0.277 a-c
	50 mM	2.64 d	2.66 cd	46.88 bc	46.92 a-d	19.90 c	20.24 c	0.2517 bc	0.251 b-d
TSW 4	0 mM	2.98 a	3.00 a	48.83 a	48.76 a	36.90 a	37.00 a	0.3163 a	0.304 a
	50 mM	2.82 bc	2.86 b	47.81 ab	48.01 ab	29.95 b	29.89 b	0.2587 bc	0.255 b-d

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Table 7. The effects of water treatments (WT) and foliar application of glycine betaine (GB) on leaf elemental contents (Zn and Ni), proline, and phenolic content of kapok seedlings.

Treatment		Zn (mg/100 g)		Ni (ppm)		Proline (mg g^{-1})		Phenolic (mg g^{-1})	
WT	**GB**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**	**2020/21**	**2021/22**
NIW		0.210 D	0.211 D	56.15 G	55.85 E	0.553 C	0.721 BC	0.463 E	0.497 D
ADW 1		0.219 DE	0.238 BC	67.45 F	67.53 D	0.611 BC	0.618 D	0.519 DE	0.541 CD
ADW 2		0.232 C	0.237 BC	72.31 DE	71.77 D	0.752 A	0.742 BC	0.549 D	0.577 C
ADW 3		0.241 C	0.242 B	68.72 EF	68.74 D	0.792 A	0.798 AB	0.654 C	0.686 B
ADW 4		0.250 B	0.249 B	76.79 CD	76.80 C	0.811 A	0.819 A	0.759 A	0.787 A
TSW 1		0.219 DE	0.229 CD	76.39 CD	75.86 C	0.575 C	0.579 DE	0.505 DE	0.511 D
TSW 2		0.238 C	0.244 B	79.50 C	79.30 C	0.665 B	0.708 C	0.570 D	0.581 C
TSW 3		0.251 B	0.252 AB	87.53 B	87.81 B	0.765 A	0.76 ABC	0.680 BC	0.720 B
TSW 4		0.269 A	0.269 A	94.69 A	94.68 A	0.811 A	0.821 A	0.724 AB	0.732 AB
	0 mM	0.240 A	0.245 A	75.54 A	77.34 A	0.702 B	0.704 B	0.510 B	0.531 B
	50 mM	0.233 B	0.236 B	73.47 B	72.41 B	0.728 A	0.732A	0.695 A	0.720 A
NIW	0 mM	0.212 h	0.215 de	57.61 h	57.00 f	0.534 g	0.517 g	0.372 h	0.399 g
	50 mM	0.209 h	0.207 e	54.68 h	54.70 f	0.572 fg	0.566 fg	0.555 c-e	0.594 c-e
ADW 1	0 mM	0.220 f-h	0.256 ab	69.33 fg	69.53 de	0.619 e-g	0.616 ef	0.436 f-h	0.467 fg
	50 mM	0.219 gh	0.219 de	65.56 g	65.56 e	0.602 fg	0.619 ef	0.602 cd	0.615 cd
ADW 2	0 mM	0.232 ef	0.234 b-e	76.23 de	75.63 cd	0.760 a-d	0.750 a-d	0.479 e-g	0.499 ef
	50 mM	0.232 ef	0.240 b-d	68.39 fg	67.90 e	0.743 a-d	0.735 b-d	0.619 c	0.655 c
ADW 3	0 mM	0.240 e	0.243 b-d	69.64 e-g	69.64 de	0.795 ab	0.807 a-c	0.553 c-e	0.598 cd
	50 mM	0.241 de	0.240 b-d	67.80 fg	67.84 e	0.789 a-c	0.788 a-c	0.756 b	0.774 b
ADW 4	0 mM	0.254 b-d	0.255 ab	79.11 d	79.11 c	0.811 ab	0.822 ab	0.630 c	0.655 c
	50 mM	0.245 b-e	0.243 b-d	74.47 d-f	74.49 cd	0.8102 ab	0.816 a-c	0.888 a	0.918 a
TSW 1	0 mM	0.226 fg	0.222 c-e	78.48 d	77.45 c	0.572 fg	0.583 e-g	0.420 gh	0.433 fg
	50 mM	0.211 h	0.218 de	74.31 d-f	74.27 cd	0.578 fg	0.575 e-g	0.589 cd	0.588 c-e
TSW 2	0 mM	0.243 c-e	0.243 b-d	80.78 cd	80.76 c	0.653 d-f	0.750 a-d	0.515 d-f	0.520 d-f
	50 mM	0.233 ef	0.244 b-d	78.23 d	77.85 c	0.677 c-f	0.666 de	0.625 c	0.642 c
TSW 3	0 mM	0.255 bc	0.253 a-c	88.33 b	88.57 b	0.729 b-e	0.7220 cd	0.577 cd	0.600 cd
	50 mM	0.246 b-e	0.252 a-c	86.73 bc	87.06 b	0.802 ab	0.808 a-c	0.783 b	0.839 ab
TSW 4	0 mM	0.279 a	0.279 a	98.33 a	98.34 a	0.848 a	0.843 a	0.608 cd	0.606 cd
	50 mM	0.258 b	0.259 ab	91.06 b	91.02 b	0.801 ab	0.799 a-c	0.839 ab	0.859 ab

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4. CONCLUSIONS

Results reported in this investigation indicated that the physiological parameters, chemical constituents, and nutrient content of kapok seedling leaves were effectively negative to irrigation by TSW and ADW 100%. This could be due to the accumulation of heavy metals and soluble salts. As GB at 50 mM concentrations proved to have remarkably positive effects on physiological parameters and chemical constituents of kapok seedlings under water stress, it could be attributed to its protective effect on peroxidation-linked membrane deterioration and scavenging free radicals and duo to osmotic protection. But also, mixing TSW or ADW with NIW percentages 25:75 and 50:50 gives better results on physiological parameters, chemical constituents, and nutrient content of kapok seedling leaves similar to those irrigated with NIW. The interaction between the two studied factors, the irrigation with TSW, ADW at 25, 50 % with spraying GB at 50 mM significantly improved the physiological parameters and chemical constituents of kapok seedlings than other treatments. Considering all of the above, irrigation of kapok seedlings with TSW or ADW preferably was mixing with NIW percentage 50:50. Because mixing any type of water with NIW gives better results on physiological parameters, chemical constituents and nutrient content of kapok leaves seedlings similar to those irrigated with NIW, and spraying of GB principally at the concentration of 50 mM can enhance and improve the quantity and quality characters of kapok.

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