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Response of *Kigelia africana* (Lam.) Benth Transplants to Nano-NPK and Nano-Chitosan under Salinity Stress

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A POT EXPERIMENT was conducted at the Ornamental Department of the Horticulture Research Station in Sakha, Kafr El-Sheikh Governorate, Egypt during two seasons, 2021 and 2022. This study aimed to evaluate the effect of foliar application with nano-NPK and nano-chitosan using different doses under soil salinity stress on the growth and physiochemical attributes of *Kigelia africana* L. transplants. The treatments were mineral NPK using 5 g L^{-1} as a control, nano-NPK at three doses including 1, 2, and 3 ml L^{-1}, as well as nano-NPK at only one dose of 2 ml L^{-1} + nano-chitosan at three doses (i.e., 1, 2 and 3 ml L^{-1}) for each. The obtained results showed that the applied nano-NPK at 2 ml L^{-1} + nano-chitosan at 2 ml L^{-1} significantly increased plant height (cm), stem diameter (cm), fresh and dry weight of vegetative parts per transplant (g), membrane stability index, as well as root length (cm), number of roots and root fresh and dry weights plant with the superiority of nano-NPK at 2 ml L^{-1} + nano-chitosan at 2 ml L^{-1} over control in both seasons. Most treatments recorded significantly higher values in chlorophyll a, b, carotenoids, catalase, and peroxidase activity in the leaves than control, with the superiority of nano-NPK at 2 ml L^{-1} + nano-chitosan at 2 ml L^{-1} transplants compared to the control. The nutrient contents of nitrogen, phosphorus, and potassium in the leaves were significantly increased over the control for all treatments, especially in the treatment of nano-NPK + nano-chitosan at 2 ml L^{-1} under soil salinity stress conditions.

Keywords: Catalase, Carotenoids, Nanofertilizers, Peroxidase, Photosynthetic pigments.

1. Introduction

Kigelia africana (Lam.) Benth, commonly named sausage tree or African sausage tree, is a tropical tree that belongs to the Family Bignoniaceae and is native to Africa (Mann et al. 2003). This tree has many promising attributes because it possesses antioxidant, anti-inflammatory, antidiabetic, antimicrobial, antineoplastic, and anti-urolithic activities, as well as for treating various disorders and drugs (Abbas et al. 2023; Fakudze et al. 2023). The tree produces night-blooming red flowers and bears characteristically long woody fruit that looks like a giant sausage hanging from the crown. This tree is highly valued and culturally important to African communities. Up to 25 m tall, the trunk can reach up to 0.6 m in diameter. It has a round and spreading crown. The bark is grey and thinly flaky. It is, primarily in Singapore, but Singapore can be deciduous during the long dry season. The leaves are compound, odd-pinnate (imparipinnate), and up to 50 cm long. Each leaflet has 7 – 9 lateral veins and sometimes may have rough hairs on both sides (Bello et al. 2016). Each panicle contains 6 – 12, showy flowers with deep red to purple petals. Each flower is about 5 – 10 cm long. They bloom in the evening, lasting only for a night, and are reported to be unpleasantly scented (Oyelami et al. 2012).

Nano-fertilizers are promising candidates for sustainable agriculture and nano-farming approaches, particularly biological nanofertilizers (El-Ramady *et al*. 2023). Foliar nanofertilizers on the vegetative system. Play a great role in plant nutrition (Singh *et al*. 2024). They can increase the activity of photosynthesis processes and raise the ability of plants to withstand various stress conditions (Sári *et al*. 2023; El-Ramady *et al*. 2024; Mahawar *et al*.

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2024). They can also increase plant resistance to phyto-diseases (Sári *et al*. 2024a). The properties of nanoparticles allow them to enter plant cells transfer chemicals and DNA inside the plant, and contribute to the transfer of compounds to the target parts, like leaves, roots, or the rest of the plant parts (Shalaby *et al*. 2022a). Nanofertilizers can increase growth parameters, such as plant height, number of leaves, leaf area, dry matter production, and photosynthesis pigments, which lead to a higher production compared to mineral fertilizers, especially during the seedling growing stage (Shalaby *et al*. 2022b; Sundararajan et al. 2023; Haydar *et al*. 2024; Zhao *et al*. 2024). NPK-nanoparticles (NPK-NPs) can be considered vital for the development of crop production and may play essential roles in food safety. The importance of these nano-NPK fertilizers could be realized from their role in supplying the vital nutrients for plant growth (Mokrani et al. 2018).
Nano-chitosan is a natural polymer derived from the deacetylation of chitin, which may be obtained from crustaceans, insects, fungi, etc. Chitin is a natural polysaccharide, which consists of a copolymer of N-acetyl-D-glucosamine and D-glucosamine residues, linked by b-1,4 glycosidic bonds (Al-Dhabaan *et al*. 2018). It is present in various species in the shells of crustaceans, in cuticles of insects, and the cell walls of fungi and some algae. Chitosan nanoparticles (Ch-NPs) can act as growth enhancers and potent antimicrobial agents against pathogenic fungi and bacteria (Yu *et al*. 2021). Chitosan is a promising bio-stimulant that increases plant productivity and enhance growth rate (Román-Doval et al. 2023). Foliar application of nano-chitosan increases seedling's vegetative growth parameters, photosynthetic pigments, chemical content, and macronutrients in leaves (Elshamy *et al.*2019; Divya *et al*. 2022). Nano-chitosan has antimicrobial properties, so it can be used as an inhibitor of micro-pests such as bacteria and fungi in addition to being used as a plant growth promoter (Maluin et al. 2019). Treated plants with nano-chitosan increased growth compared to control under salinity stress by enhancing vegetative growth, dry matter, and relative water content under stress salinity (Balusamy et al. 2022). A foliar spray of nano-chitosan on *Catharanthus roseus* under salinity enhanced the alkaloid biosynthesis and activity of antioxidant enzymes (Hassan et al. 2021). Many studies confirmed the promising applications of nano-chitosan in different fields such as agriculture (Shrestha et al. 2023), drug delivery (Zaiki et al. 2023), energy storage (Rostami and Khodaei 2023), biomedical attributes (Hassan et al. 2024), bio-sensing and electronic fields (Rana et al. 2024).
Therefore, this investigation aimed to evaluate the effect of foliar application of nano-NPK and nano-chitosan using different doses on the growth and chemical constituents of *Kigelia africana* (Lam.) Benth. During the seedling growing stage. The vegetative parameters, antioxidant activities, photosynthetic pigments, and leaf chemical composition will also be discussed.

2. Materials and Methods

A series of pot experiments were conducted at the Ornamental Department of the Horticulture Research Station in Sakha, Kafr El-Sheikh Governorate (the site is located at 31.07º N latitude and 30.57º E longitude for two summer seasons of 2021 and 2022. Data on climatic conditions of this study were obtained from the Agriculture Research Center during the two experimental seasons and presented in **Table (1).** The soil used in each season was analyzed according to **Sparks *et al.* (2020)** before cultivation (**Table 2**). The used soil was collected from the Station in Sakha, Kafr El-Sheikh Governorate.

2.1 Plant materials

At six months produced, transplants were obtained from a private nursery on March 25th for two seasons at a uniform length of 18 ±1 cm. Then, on April 5th, the plants were planted in pots of 25 cm diameter using clayey loam soil (8 kg). The pots were manually watered every 3 days by using fresh water. Randomized completely block design was performed seven treatments were assessed. Each treatment consisted of three replicates as every replicate contained 10 plants. One months later, seedlings were foliar sprayed with mineral NPK (5g L^{-1}), as a control, nano-NPK at 1, 2, and 3 ml L^{-1} and nano-NPK at 2 ml L^{-1} + nano-chitosan at 1, 2, 3 ml L^{-1} for each (**Fig. 1**). During the two experimental seasons at intervals of 15 days, the foliar spraying was done in the early morning until runoff. Nano-chitosan was foliar sprayed on the second day from adding NPK at 2 ml L^{-1}.

2.2 Source of applied treatments

The mineral NPK fertilizer (EGY FLEX, as commercial fertilizer 20:20:20) is composed of total nitrogen 20 % N, phosphorus (20% P_2O_5), and potassium (20 % K_2O) produced by Egyptian Chem International for Agrochemicals". Nano-NPK (NPK-NPs; 19:6:20) was and created by Biota EG Company with a concentration of nitrogen (3.8% N), phosphorus (1.2 % P_2O_5), and potassium (4% K_2O). Nano-chitosan (Ch-NPs), solution at 2%, was produced by Biota EG Company. Transmission Electron Microscopy (TEM) of both nano-NPK and nano-sulfur can be observed in **Figure 2**. These nanoparticles were measured directly by TEM (Model Talos L120CG2 – TEM – Thermo- Fisher, Europe). The mean diameters of applied

nanomaterials were **308 and 302.6 nm** for nan-NPK and nano-chitosan, respectively.

Table 1. Monthly air temperature (max., min., and mean °C), relative humidity (RH %) at the experimental during the two growing seasons of 2021 and 2022.

Months	Air temperature (°C)			RH, %	Air temperature (°C)			
	Max	Min	Mean		Max	Min	Mean	RH, %
	First season (2021)				Second season (2022)			
March	22.3	17.6	19.9	62.6	21.7	16.8	19.2	63.5
April	26.9	20.4	23.6	59.6	27.9	19.7	23.8	61.0
May	32.4	24.7	28.5	58.3	31.0	21.8	26.4	59.5
June	30.9	25.5	28.2	65.1	33.0	25.7	29.3	64.7
July	33.9	27.9	30.9	69.6	33.4	26.6	30.0	71.7
August	35.6	28.3	31.9	66.2	34.5	25.9	30.2	72.7
September	32.5	25.1	28.8	66.8	32.9	25.9	29.4	69.3
October	28.5	22.3	25.4	69.6	29.6	20.4	25.0	74.9
November	26.6	19.9	23.2	72.1	25.7	18.5	22.1	74.3

Table 2. Physical and chemical analysis of the used soil in the 1st and 2nd two experimental seasons, 2021 and 2022.

Season	Soil physical properties				Soil chemical properties					
	Sand	Silt	Clay	Texture class	pH*	EC* (dS m^{-1})	OM (%)	Available nutrients (ppm)		
								N	P	K
First season	24.58	37.79	37.63	Clay loam	8.46	4.52	1.02	17.06	7.03	212.4
Second season	24.30	35.28	40.42	Clayey	8.40	4.55	1.15	22.16	8.34	209.8

* In both seasons, pH and soil electrical conductivity (EC) were determined in soil suspension (1:5) and saturated soil paste extract, respectively.

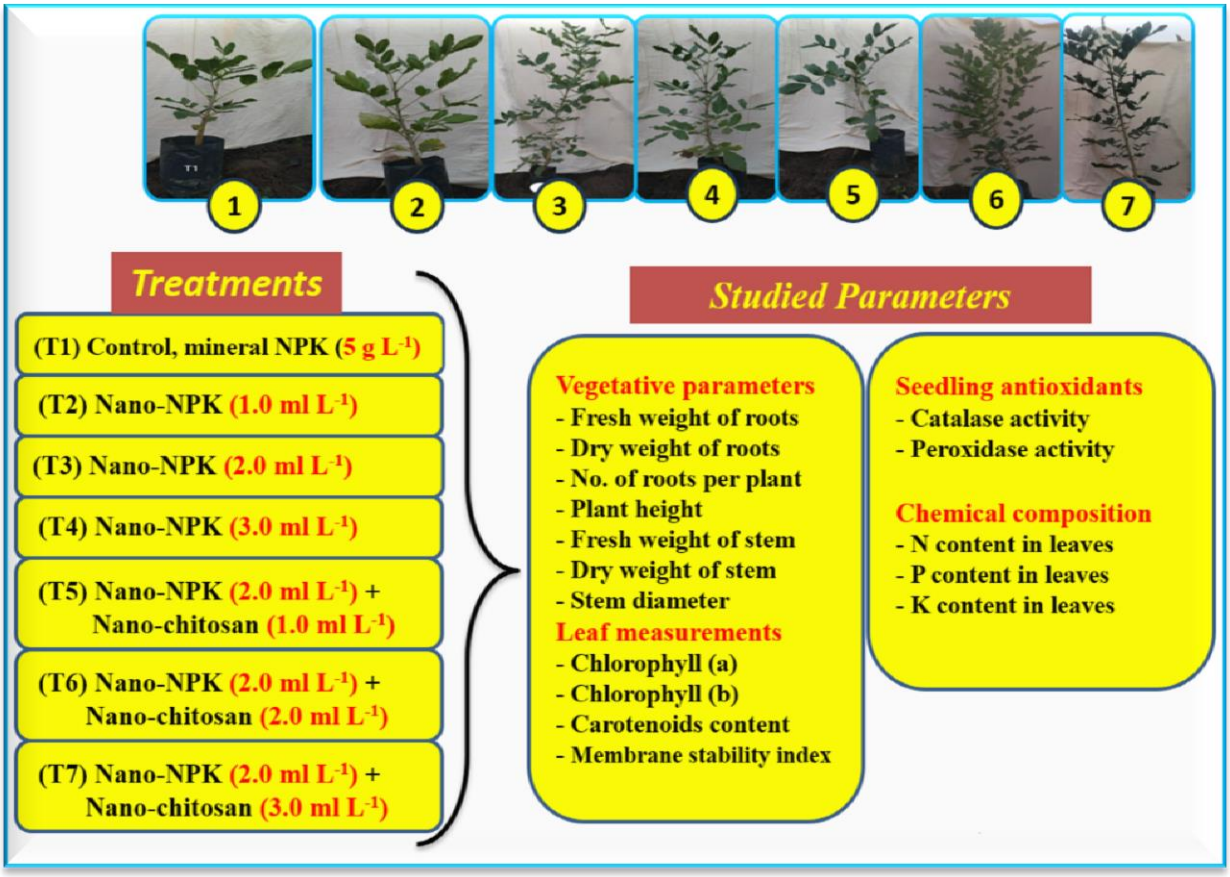

Fig. 1. An overview of the main treatments and measurements during the study.

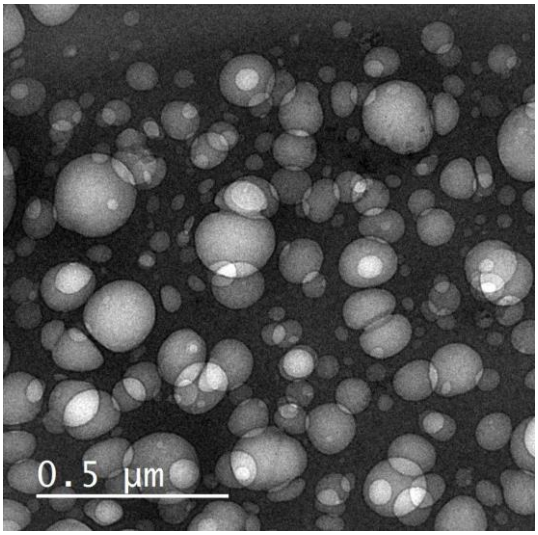

Fig. 2. TEM imaging of nano-chitosan .

2.3 Vegetative growth parameters

At the end of experimental seasons on November 10^{th} to $15^{th,}$ 2021 and 2022, respectively, the following data were recorded, including plant height (cm), stem diameter (cm), fresh and dry weights of the stem (g), as well as root length (cm), number of roots and root fresh and dry weights (g) per plant.

2.4 Estimation of chlorophyll content

Chlorophyll content was gauged by adding 20 ml of acetone 80% to 1.0 g leaf tissue and 0.5 g of ($MgCO_3$) powder were was append and then gently grinded. The admixture was incubated at 4ºC for 3 hrs. The mixture was centrifuged at 2500 rpm for 5 min. After that, supernatant fluid was taken to a 100 ml volumetric flask. The volume was increased to 100 ml by adding acetone 80%, and the mix was used to evaluate chlorophyll a and b by using the following equations according **to** Rajalakshmi and Banu (2015):

Chlorophyll a (mg g^{-1} FW) =

$$[12.7(A_{663}) – 2.69 (A_{645})] V/1000W$$

Chlorophyll b (mg g^{-1} FW) =

$$[22.9(A_{645}) – 4.68 (A_{663})] V/1000W$$

Where A = Absorbance of specific wavelength,

V = Final volume of chlorophyll extract in 80% acetone and

W = Fresh weight of tissue extract**.**

2.5 Evaluation of total carotenoids

The content of carotenoids was evaluated using the same chlorophyll extract and measured at 470 nm in a spectrophotometer (Jenway 6405, the UK) to estimate the total content of carotenoids (including both xanthophylls + carotene) as follows equation as studied by Sumanta *et al*. (2014).

Total carotenoids (mg g^{-1} FW) = (1000A470 - 1.82 Chl. a – 85.02Chl. b)/198

Where A = Absorbance at respective wave length, Chl. a= chlorophyll a and Chl. b= chlorophyll-b.

2.6 Membrane stability index

Membrane stability index (MSI) or electrolyte leakage (EL) was determined as described by Sairam *et al*. (1997) using 200 mg leaf disks in two test tubes including 10 ml of distilled water. The first set was put at 40 ºC in a water bath for 30 min, and electrical conductivity (C1) was measured. Another set was kept at 100ºC for 15 min and measured electrical conductivity (C2). MSI was stated as the following formula:

$$\textbf{MSI (\%) = (1- C1/C2) ×100}$$

2.7 Leaf chemical composition

Estimating N, P, and K content in the dried leaves was performed by using 0.2 g as the digested solution was used to determine N, P, and K by Kjeldahl device, spectrophotometer (Jenway 6405), and flame photometer (Jenway PFP7, Staffordshire, UK) of the previous elements, respectively**.**

2.8 Determination of enzyme activities

Catalase (CAT) and peroxidase (POX) activities were estimated using 0.3 g of leaves in liquid nitrogen. Then they added 2.0 mL of homogenized medium (0.1 M potassium phosphate buffer, pH 6.8, 0.1 Mm EDTA, 1 mM phenyl methyl sulfonyl fluoride, and 1.0 % polyvinyl-pyrrolidone (w/v) and centrifugated at 12,000×g for 15 min at 4 °C according to Peixoto et al. (1999). Catalase activity (EC 1.11.1.6) was assessed by mixing 0.1 mL of enzymatic extract with 2.9 mL of reaction solution that was composed of 50 mM sodium phosphate

buffer (pH 7.0) and 12.5 mM H_2O_2 (Havir and McHale 1987). The decreasing absorbance was measured after a minute reaction at 240 nm at 25 °C. Catalase activity was calculated using a molar extinction coefficient of 36 mol L^{-1} cm-1 and expressed by µmol of H_2O_2 min^{-1} mg^{-1} FW proposed by Anderson *et al*. (1995). Peroxidase was determined by spectrophotometer (Jenway 6405, Japan). POX was determined through the production rate of purpurogallin at 420 nm according to Nakano and Asada (1981). The enzymatic activity was expressed in µmol purpurogallin min^{-1} g^{-1} FW. CAT and POX were determined using a spectrophotometer (Jenway 6405, Japan).

2.9 Statistical analyses

Data were statically analyzed by analysis of variance (ANOVA) using Costat (Version 6.303, Co Hort, USA, 1998–2004)] program for the data set of the two independent experimental and combined analysis was carried out after checking the homogeneity of the variance Bartlett's test. Duncan's multiple range test (Duncan, 1955) was used to compare the mean at the $P \leq 0.05\%$ probability level, according to Gomez and Gomez (1984). Resulted were presented as the average mean of the two independent seasons ±SE.

3. Results

3.1 Vegetative characteristics

Data of *Kigelia* growth parameters included plant height, stem diameter, fresh and dry weight of aerial parts, and leaf number, are listed in **Table (3).** The treated plants with N, P, and K-nano fertilizer at different levels and plus nano-chitosan were significantly superior in all traits compared to the control (mineral fertilizer). Treatment of nano-NPK at 2 ml L^{-1} plus nano-chitosan at 2 ml L^{-1} gave the longest plant height, recording an increase rate of 58.8% compared to the control. The previous treatment was followed by treated plants with nano-NPK at 2 ml L^{-1} plus nano-chitosan at 3 ml L^{-1} with an increasing rate of 32.6%, compared the control treatment. NPK-nano fertilizer and nano-chitosan at different levels significantly increased in all studied vegetative parameters compared to the control. Nano-NPK (2 ml L^{-1}) plus nano-chitosan at 2 ml L^{-1} recorded the highest value of stem diameter with an increase of 30.2 %, followed by nano-NPK at 2 ml L^{-1} plus nano-chitosan at 3 ml L^{-1} with an increased rate of 17.7 % compared to the control treatment. Concerning the fresh and dry weights of plants, the same previous trend was observed, which showed significant superiority in treated plants with NPK-NPs and Ch-NPs at an applied dose of 2 ml L^{-1} compared to the control (**Table 3)**. The highest increase values in fresh and dry weights (160 and 124 %, respectively) were recorded at the applied dose of 2 ml L^{-1} of both studied nanomaterials, followed by the applied dose of NPK-NPs at 2 ml L^{-1} plus Ch-NPs at 3 ml L^{-1}, which recorded increase rate for fresh and dry weights of 100 and 85.6 %, respectively comparing with the control treatment.

Table 3. Effect of nano-NPK and nano-chitosan foliar application on stem growth of *Kigelia africana* (Lam.) plants (average of two seasons, 2021 and 2022).

Treatments	Plant height (cm)	Stem diameter (cm)	Fresh weight of stem (g)	Dry weight of stem (g)
(T1) Control (mineral NPK 5 g L^{-1})	79.17g	20.60g	96.65g	29.17g
(T2) NPK-NPs 1 ml L^{-1}	80.42f	21.15f	104.42f	32.27f
(T3) NPK-NPs 2 ml L^{-1}	112.82b	24.73b	220.65b	60.45b
(T4) NPK-NPs 3 ml L^{-1}	89.85e	23.15e	165.55e	44.83e
(T5) NPK-NPs 2 ml L^{-1} + Ch-NPs 1 ml L^{-1}	99.95d	23.78d	179.61d	51.17d
(T6) NPK-NPs 2 ml L^{-1} + Ch-NPs 2 ml L^{-1}	125.77a	26.83a	250.76a	65.42a
(T7) NPK-NPs 2 ml L^{-1} + Ch-NPs 3 ml L^{-1}	104.98c	24.25c	192.53c	54.15c
F-test	**	**	**	**

The mean followed by the same letter in the same column does not differ significantly from Duncan's multiple range test at the 5% level.

3.2 Root growth parameters

There is a significant increase in all studied parameters with an increase in the applied dose of nano-NPK and chitosan nanoparticles, including the root length, root number, root fresh, and dry weight compared with the control treatment (**Table 4**). The highest root length was observed after applying foliar of NPK-NPs at 2 ml L^{-1} plus Ch-NPs at the same level (2 ml L^{-1}), with an increment rate of 47.9%, which was followed by 30 % in the case of nano-NPK at 2 ml L^{-1} and nano-chitosan at 3 ml L^{-1} comparing with the control treatment. The same direction was recorded for all parameters of roots, as shown in **Table (4),** compared to the control treatment (mineral NPK foliar spray).

3.3 Photosynthetic pigments

Data illustrated in **Fig. 3 (A, B**, and **C**) that all NPK-nano and NPK-nano plus nano-chitosan at different rates increased the values of studied photosynthetic pigments (i.e., chlorophyll a, b, and carotenoids) comparing with the control. The same trend for previous parameters could be noticed, which recorded the highest values in studied photosynthetic pigments resulting from the foliar

spraying with nano-NPK (2 ml L^{-1}) plus nano-chitosan (2 ml L^{-1}), except the values of carotenoids, which the highest values were recorded after applying NPK-NPs at 2 ml L^{-1} plus 3 ml L^{-1} nano-chitosan (T7).

Table 4. Effect of nano-NPK and nano-chitosan foliar application on root growth parameter of *Kigelia Africana* L. plants (average of two seasons, 2021 and 2022).

Treatments	Root length (cm)	No. of roots	Root fresh weight (g)	Root dry weight (g)
(T1) Control (mineral NPK 5 g L^{-1})	49.97g	25.17e	48.30g	15.11g
(T2) NPK-NPs 1 ml L^{-1}	52.77f	27.00e	59.99f	16.68f
(T3) NPK-NPs 2 ml L^{-1}	71.68b	44.67b	111.62b	50.16b
(T4) NPK-NPs 3 ml L^{-1}	60.68e	33.00d	76.93e	29.07e
(T5) NPK-NPs 2 ml L^{-1} + Ch-NPs 1 ml L^{-1}	63.27d	38.00c	90.20d	36.97d
(T6) NPK-NPs 2 ml L^{-1}+ Ch-NPs 2 ml L^{-1}	73.95a	48.67a	149.23a	60.45a
(T7) NPK-NPs 2 ml L^{-1}+ Ch-NPs 3 ml L^{-1}	64.98c	39.33c	98.50c	40.47c
F-test	**	**	**	**

The mean followed by the same letter in the same column does not differ significantly from Duncan's multiple range test at the 5% level.

3.4 Membrane stability index (MSI)

Data presented in **Fig. 3**D showed that the height values were recorded as the previous parameters from nano–NPK (2 ml L^{-1}) plus nano-chitosan (2 ml L^{-1}) with an increasing rate of 10.6% followed by nano-NPK alone or plus 3 ml L^{-1} nano-chitosan as listed 8.6 and 6.9%. On the other hand, the lowest values resulted from nano-NPK at 1 ml L^{-1} and control treatment with non-significant differences among them.

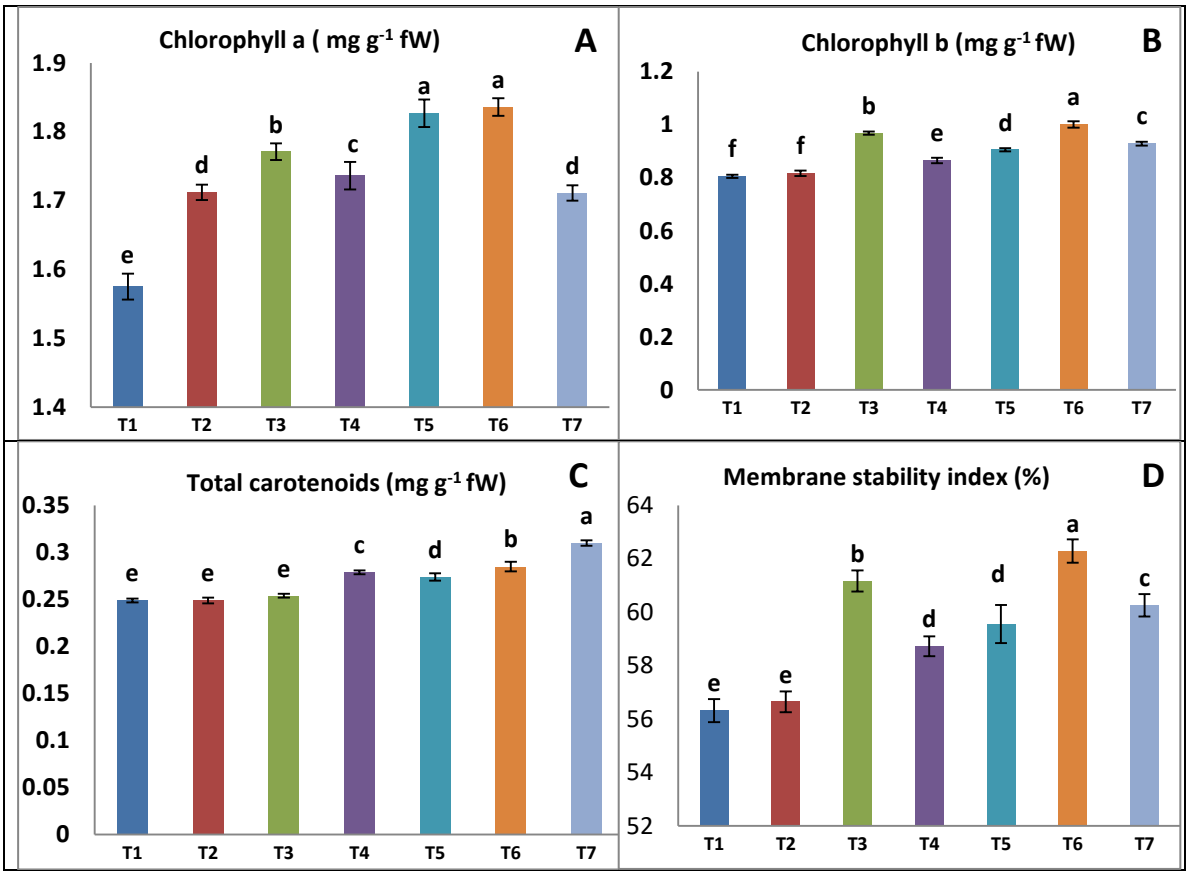

Fig. 3. Effect of nano-NPK and nano-chitosan foliar application on photosynthetic pigments and membrane stability index (MSI) of *Kigelia africana* L. as (A) chlorophyll a (mg g-^{1} FW), (B) chlorophyll b (mg g^{-1} FW), (C), carotenoids (mg g^{-1} FW) and (D) membrane stability index (%). Data are mean value ± SE. Bars at the same letter are insignificant at the P≤0.05 level. (T1) Control (mineral NPK 5 g L^{-1}), (T2) NPK-NPs 1 ml L^{-1}, (T3) NPK-NPs 2 ml L^{-1}, (T4) NPK-NPs 3 ml L^{-1}, (T5) NPK-NPs 2 ml L^{-1} + Ch-NPs 1 ml L^{-1}, (T6) NPK-NPs 2 ml L^{-1} + Ch -NPs 2 ml L^{-1}, (T7) NPK-NPs 2 ml L^{-1} + Ch -NPs 3 ml L^{-1} (average of two seasons, 2021 and 2022).

3.5 Chemical composition of leaves

It is indicated from results in **Fig. 4 (A, B, and C)** that all treatments, including nano-NPK alone or plus Ch–NPs, augmented the content of nitrogen, phosphorus, and potassium in the leaves with increasing applied doses. The significantly highest values of N, P, and K content in plant leaves were achieved from the treatment of nano-NPK with nano-chitosan at 2 ml L^{-1} as recorded at 4.15, 0.5 and 3.63%, respectively, followed by treated plants with nano-NPK (2 ml L^{-1}) alone or plus nano-chitosan (3 ml L^{-1}) against the control treatment, which gave the lowest values of N, P and K content (3.11, 0.48 and 2.73%, respectively).

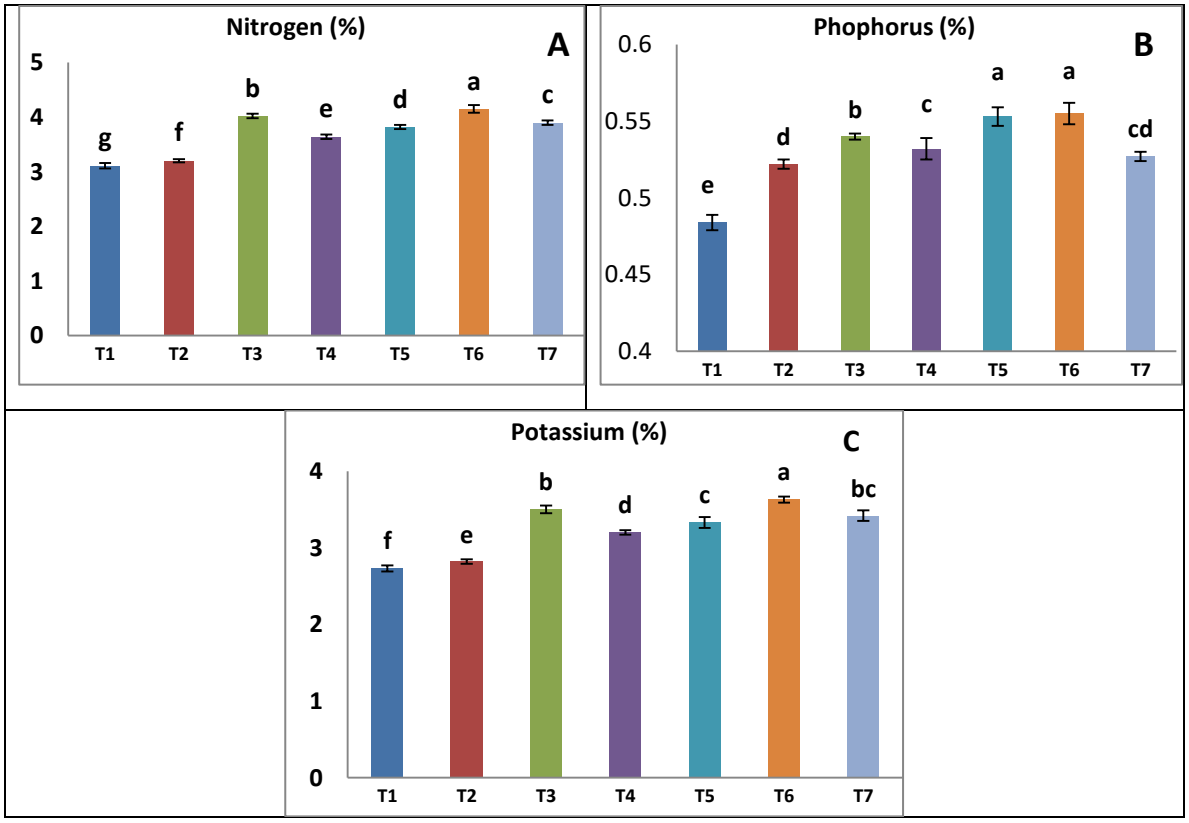

Fig. 4. Effect of nano-NPK and nano-chitosan foliar application on chemical composition nitrogen, phosphorus, and potassium of *Kigelia africana* L in leaves (%) for N, P, K (A), (B), and (C), respectively. Data are mean value ± SE. Bars at the same letter are insignificant at the P≤0.05 level. (T1) Control (mineral NPK 5 g L^{-1}), (T2) NPK-NPs 1 ml L^{-1}, (T3) NPK-NPs 2 ml L^{-1}, (T4) NPK-NPs 3 ml L^{-1}, (T5) NPK-NPs 2 ml L^{-1} + Ch-NPs 1 ml L^{-1}, (T6) NPK-NPs 2 ml L^{-1}+ Ch -NPs 2 ml L^{-1}, (T7) NPK-NPs 2 ml L^{-1}+ Ch -NPs 3 ml L^{-1} (average of two seasons, 2021 and 2022).

3.6 Antioxidant activity

The same trend can be observed in the case of antioxidants, including catalase and peroxidase activities, after applying different doses of nano-NPK and nano-chitosan (**Fig. 5 A and B).** Concerning the catalase activity, the highest value (32.05 µmol of H_2O_2 min^{-1} mg^{-1} FW) of CAT was recorded after applying nano-NPK (2 ml L^{-1}) and nano-chitosan (2 ml L^{-1}) compared to the control. For peroxidase activity, a similar trend like CAT was observed, where the highest value (0.97 µmol of H_2O_2 min^{-1} mg^{-1} fw) of peroxidase was recorded after applying the dose of 2 ml L^{-1} of NPK-NPs and nano-chitosan compared with the control.

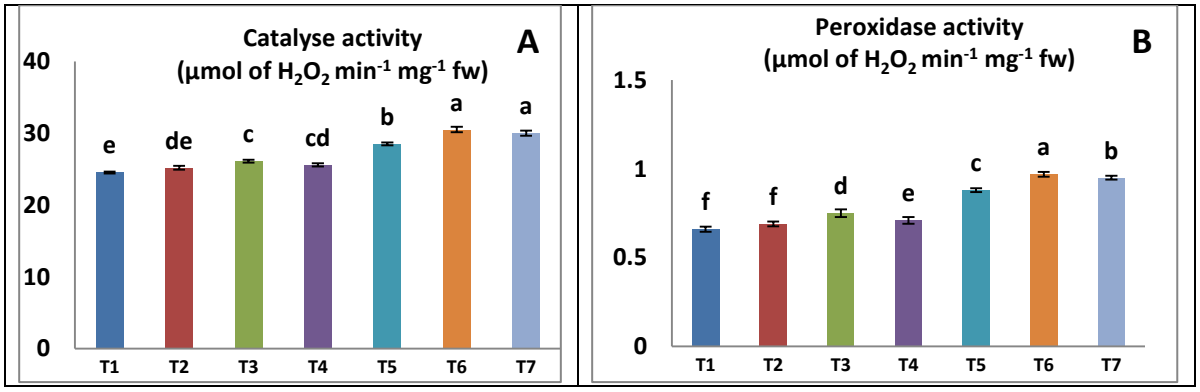

Fig. 5. Effect of nano-NPK and nano-sulfur foliar application on antioxidant activity of *Kigelia africana* L. as (A) catalase activity (µmol of H_2O_2 min^{-1} mg^{-1} FW) and (B) peroxidase activity (µmol purpurogallin min^{-1} g^{-1} FW). Data are mean value ± SE. Bars at the same letter are insignificant at the P≤0.05 level. (T1) Control (mineral NPK 5 g L^{-1}), (T2) NPK-NPs 1 ml L^{-1}, (T3) NPK-NPs 2 ml L^{-1}, (T4) NPK-NPs 3 ml L^{-1}, (T5) NPK-NPs 2 ml L^{-1} + Ch-NPs 1 ml L^{-1}, (T6) NPK-NPs 2 ml L^{-1}+ Ch -NPs 2 ml L^{-1}, (T7) NPK-NPs 2 ml L^{-1}+ Ch -NPs 3 ml L^{-1} (average of two seasons, 2021 and 2022).

4. Discussion

Under arid and semi-arid regions, soil salinity increases yearly, causing a serious global problem for food security. Few studies were done using nanofertilizers on the growth of woody transplants. Therefore, this study aimed to evaluate the effects of foliar-applied nano-NPK and nano-chitosan at different doses on the growth and chemical compounds of *Kigelia africana* (Lam.) Benth transplants. This section will answer the following questions:

- What is the role of applied nanomaterials in ameliorating the salinity stress?
- What is the suggested mechanism of nano-chitosan under salinity stress?
- To what extent can seedlings of *Kigelia africana* (Lam.) Benth to be tolerant to soil salinity stress?
- What is the recommended applied dose of nanomaterials under such studied stress?
- What is further research needed in the future on such a topic?

The applied nanomaterials have many benefits under different stresses, such as soil salinity stress. The role of studied materials, including nano-fertilizers and nano-amendments like nano-chitosan in ameliorating the salinity stress was confirmed on many crops (Balusamy et al. 2022; Singh et al. 2023; Rehman et al. 2024). The suggested mechanism under salinity may go back to optimizing phytohormone and phenolic levels, protecting the photosynthetic apparatus, enhancing photosynthetic efficiency, boosting the uptake of nutrients and the activity of antioxidants, as well as regulating gene expression, thereby strengthening the plant's resilience to such stress (Rehman et al. 2024). Another suggested mechanism involved the role of nano-chitosan in reducing the deleterious impacts of salinity on plants by enhancing plant growth through regulating cellular osmotic pressure, increasing the bioavailability and uptake of essential nutrients and water (Aazami et al. 2023).

In the current study, applied Ch-NPs increased many studied vegetative and biochemical parameters by enhancing the metabolic processes, increasing photosynthetic efficiency, and antioxidative enzymatic resistance. These results are in harmony with the report of many studies on different crops such as *Catharanthus roseus* (L.) G. Don. (Hassan et al. 2021), wheat (Hajihashemi and Kazemi 2022), and grapes (Aazami et al. 2023). This may be attributed to the fact that the nano fertilizers have a smaller diameter than the pores of the plant cell, and nanoparticles easily permeate through the plant cell wall and reach the plasma membrane (Mohamed *et al*. 2022).

Recently, more studies on the combined application of nano-NPK and nano-chitosan on different crops were published with a focus on the productivity of French bean (Hasaneen *et al*. 2016), wheat (Abdel-aziz *et al*. (2016), potato (Elshamy et al. 2019), coffee (Ha et al. 2019), field crops (Ashraf et al. 2022), and intercropped maize-soybean (Abou El-Enin et al. 2023). The previous studies are in harmony with the results that this combination is a promising nano-based fertilizer which can be applied under normal and stressful conditions.

As far as we know, this is the first report on the combined foliar application of nano-NPK and nano-chitosan on *Kigelia africana*. Based on the obtained results from the current study, it is obvious that the seedlings of *Kigelia africana* (Lam.) Benth is considered to be tolerant to soil salinity stress until the studied salinity level (4.5 dS m^{-1}). More studies are needed to investigate the higher levels of soil salinity in different growing stages, including the seedlings and others.

The recommended applied dose of nano-NPK and nano-chitosan was 2 ml L^{-1} from each nano-form under such studied conditions. It could be summarized the suggested mechanisms of applied nano-chitosan in Fig. 6. It is reported that nano-chitosan can upregulate the metabolism of both C and N in soybean plants under elevated levels of atmospheric carbon dioxide (Abuelsoud et al. 2023). So, it is expected the integrated impact of both nano-NPK, and nano-chitosan in promoting the growing plants under salinity stress in the recent study.

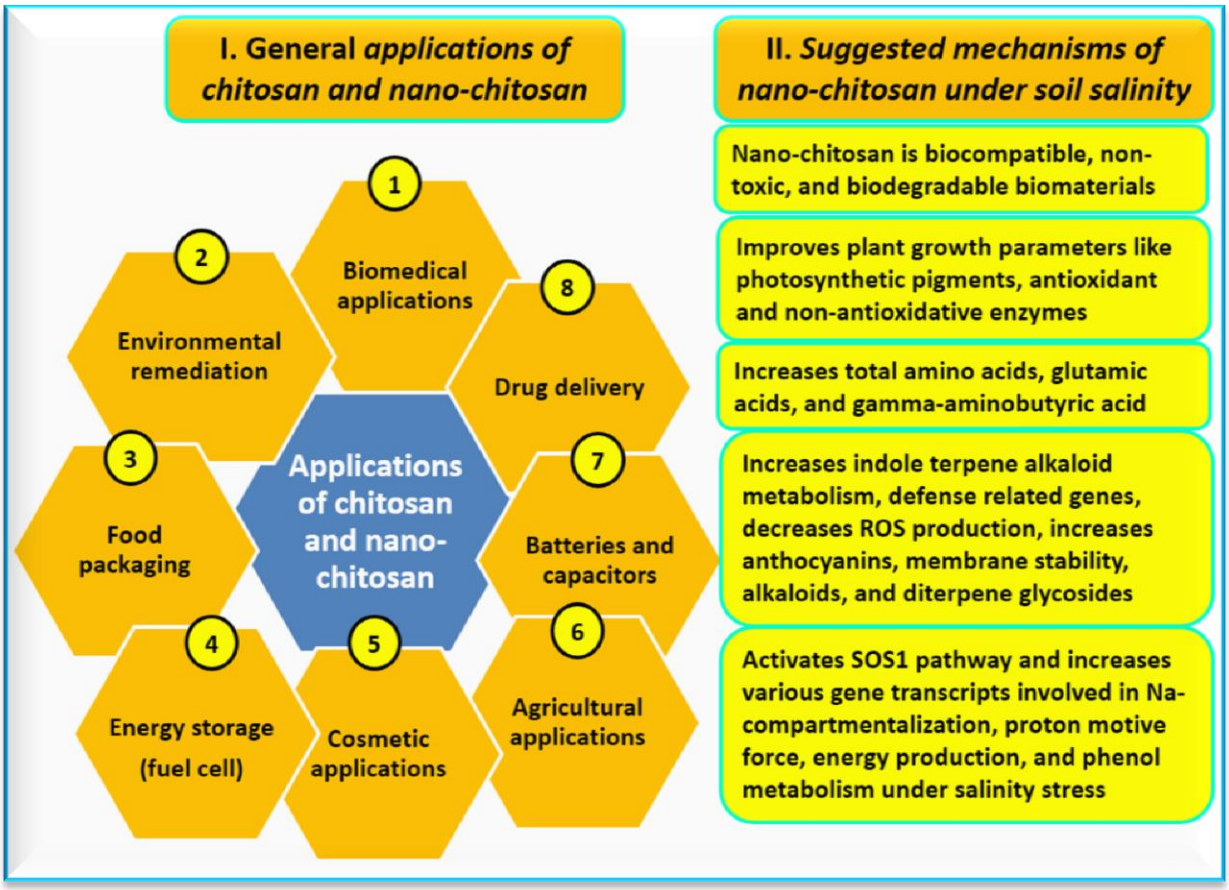

Fig. 6. The main applications of nano-chitosan (Part I) and the suggested mechanisms resulted from applying nano-chitosan (Part II).

Conclusions

Soil salinity is one of the most important global issues that threatens global food production, especially under climate change. Study the growing *Kigelia Africana* under salinity stress in the presence of different doses using foliar application of nano-NPK and nano-chitosan was investigated in the current study. With a focus on these seedlings' morphological, physiological, and biochemical characteristics, foliar spraying of a combined dose of 2 ml L^{-1} from both nano-chitosan and nano-NPK recorded the highest studied parameters under salinity stress. This previously applied dose is the recommended combined dose under such salinity stress conditions. Further investigations are needed on this crop, focusing on the molecular attributes. The economic and ecological aspects are very important and should be considered during an investigation such as nano-based fertilizers.

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