Folia Hort. 36(2) (2024): 259–281

DOI: 10.2478/fhort-2024-0016

FOLIA HORTICULTURAE

Published by the Polish Society for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | https://sciendo.com/journal/FHORT

Morphological and physiochemical changes of jojoba under water pollution stress condition

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ABSTRACT

Water security is a major challenge for agricultural sustainability in Egypt. To tackle water scarcity, the reuse of wastewater such as raw agricultural drainage water (RADW) and treated sewage drainage water (TSDW) can be a potential solution. An investigation was conducted over two seasons (2022 and 2023) for 5 months (January–May) on roughly 8-month-old jojoba plants to study their tolerance for irrigation with different concentrations of wastewater (RADW, TSDW) at 25%, 50%, 75% and 100% compared to the control (tap water). The study examined the effect of wastewater irrigation on growth parameters such as plant height, stem diameter, shoot numbers, leaf physical measurements, visual quality (VQ) and leaf chemical components including heavy metals (HMs), antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl [DPPH]), ion leakage (IL%), superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), leaf minerals, pigments, soluble carbohydrate content (SCC), total phenolic content (TPC) and proline performance in jojoba plants. The findings indicated that jojoba plants can tolerate irrigation with RADW up to a concentration of 100% and TSDW up to a concentration of 75%.

Keywords: antioxidant capacity, climate change, heavy metals, ion leakage, jojoba, polluted water, proline performance

Abbreviations: BOD, biochemical oxygen demand; COD, chemical oxygen demand; H_2O_2, hydrogen peroxide; IL%, ion leakage; JHI%, jojoba height increase percentage; K, potassium; MDA, malondialdehyde; N, nitrogen; NSI%, number of shoots increase percentage; $O_2^{\bullet-}$, superoxide anion; P, phosphorus; RADW, raw agricultural drainage water; SCC, soluble carbohydrate content; TE, trace elements; TPC, total phenolic content; TSDW, treated sewage drainage water.

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INTRODUCTION

Due to water limits and the rise in water exhaustion, water security is viewed as one of the major obstacles to sustainable agriculture in the world's arid and semi-arid regions (Zarei et al., 2020). Egypt faces a significant challenge in securing its water supply, particularly with the ongoing construction and filling of the Renaissance Dam in Ethiopia, which could reduce Egypt's annual share of the Nile's water. The amount of water used per person each year has fallen below the critical level of 1000 m^3, indicating water scarcity. The nation's average water consumption per person is 663 m^3, which indicates water poverty (<700 m^3). By 2030, this number is predicted to drop even further to 582 m^3 (Abd Ellah, 2020). As its population continues to grow, Egypt faces a significant challenge in creating and implementing a comprehensive and sustainable water resources plan to meet the fundamental needs of its people (Nasr et al., 2019). Unfortunately, many farmers in areas close to urban or suburban locations have no other choice but to use wastewater to irrigate their crops (Khurana and Singh, 2012; Eissa et al., 2018). The world's largest water usage, agricultural irrigation, is perceived to be solved by the use of substandard water resources (Galavi et al., 2010; Talat, 2020). However, the use of wastewater, such as raw agricultural drainage water (RADW) and sewage drainage water, for irrigation without conducting a risk assessment study and management can seriously endanger water, soil quality and even human health (Mahmoud and Ghoneim, 2016; Abdel-Rahman et al., 2018; El-Hassanin et al., 2020). Wastewater contains heavy metal (HM) pollutants that can pose a significant health risk (Razzak et al., 2022). Effluent containing HMs can cause damage to plants and become toxic to humans and animals when consumed. These metals are poorly soluble in water and can accumulate in plants, especially when affected by stress (Wang et al., 2021; Rashid et al., 2023). HMs are a class of compounds that pose health risks and can be found in many inorganic contaminants, including polluted plants. These contaminants are not biodegradable, resulting in prolonged biological half-lives and undesirable side effects when accumulated in human body organs (Mitra et al., 2022). Organic fertilisers and pesticides are the primary sources of HM contamination of plants, along with contaminated soil, irrigation water and atmospheric pollution from industrial or motor vehicle emissions (EL-Baz et al., 2015; Salama et al., 2015; Alengebawy et al., 2021). Treated or recycled wastewater is used increasingly for irrigation due to decreasing fresh water and its nutrient composition, providing the soil with essential macro and micronutrients such as copper, zinc, iron, manganese, nitrogen and phosphorus, among others (Singh et al., 2012; Ganjegunte et al., 2018). Wastewater contains a significant amount of organic matter which can be used to improve soil quality and promote plant growth (Ofori et al., 2021). jojoba (*Simmondsia chinensis* (Link) Schneider) is a remarkable plant that grows in hot, arid and semi-arid climates, earning its nickname 'green gold'. It is an evergreen perennial woody shrub that naturally grows in the Sonora desert of Mexico and the Southwestern United States. With separate male and female flowers, it is classified as a true xerophyte with roots that can reach depths of up to 9 m, allowing the plant to survive in high temperatures and dry conditions (Benzioni et al., 2006). jojoba seed oil has found favourable commercial uses in cosmetics and as fuel for aircraft and missiles, making it an intriguing alternative crop. The 'oil' content of the seed is substantial, comprising around 50% of its dry weight; it is composed of lipids with uniformly sized straight-chain liquid wax esters (Benzioni et al., 1992). jojoba is regarded as one of the most useful solutions for using poor-quality water resources for irrigation in Egypt and other developing countries (Aboryia et al., 2022). Because of tolerance to high temperatures, drought and salinity, lower probabilities of disease, lower need for fertilisers and plentiful financial income, jojoba cultivation can be done effectively in desert areas (Bafeel et al., 2016).

This study aimed to investigate the effect of irrigation with wastewater (RADW and treated sewage drainage water [TSDW]) in different concentrations on vegetative growth and chemical components of jojoba plants, as well as HM residues in the leaves were evaluated to determine its tolerance to irrigation with polluted water.

MATERIALS AND METHODS

Vegetative experimentation and wastewater applications

Jojoba plants were sourced from the Egyptian Gulf Company Nursery, Egypt. Shoot tip cuttings obtained from female trees were utilised for propagation. These cuttings were placed in plastic cups containing peat moss under the greenhouse; for 6 months. The transplants were placed in plastic bags measuring 40 cm × 20 cm, each containing a mixture of 10 kg of sieved, washed sand, clay and compost at a ratio of 2:1:1. There were three plastic bags for each replication, each containing one plant. After transplantation, plants were irrigated with tap water (tap water analysis results according to Mona et al., 2008 showed Ca, 10.89 mg^{-1}; Mg, 9.98 mg^{-1}; Na, 8.15 mg^{-1}; Zn, 9.08 μg^{-1}; Se, 1.57 μg^{-1}) and fertilised by drenching with 1 g · L^{-1} NPK + TE (20:20:20) + 7 g · L^{-1} humic acid were added once a month for 2 months. RADW was collected from the covered agricultural drainage system in Dakahlia Governorate, Egypt (latitude: 31°54′204″ N and latitude: 31°377′914″ E), and TSDW that treated with pretreatment by aeration and sedimentation was collected from the sewage treatment plant in Mansoura City, Dakahlia Governorate, Egypt (latitude: 31°27′384″ N and latitude: 31°37′140″ E) and they were analysed according to Jones and Case (2018). RADW had pH, 7.52; COD, 48 mg · L^{-1}; BOD, 26 mg ·L^{-1};

NH_4-N, 32.176 ppm; PO_4-p, 19.213 ppm; K^+, 61.99 ppm; Al, 35.300 ppm; Hg, 4.461 ppm; Ag, 0.128 ppm; Ca, 808.160 ppm; Cr, 5.919 ppm; Cu, 1.426 ppm; Fe, 35.838 ppm; Mg, 121.004 ppm; Mn, 1.001 ppm; Ni, 1.832 ppm; Pb, 1.184 ppm; Zn, 19.089 ppm; As, 0.116 ppm; Na, 260.793 ppm; Cd, 0.576 ppm. While TSDW had pH, 7.15; COD, 75.5 mg · L^{-1}; BOD, 39 mg · L^{-1}; NH_4-N, 25.9 ppm; PO_4-p, 10 ppm; K^+, 40.992 ppm; Al, 239.953 ppm; Hg, 1.029 ppm; Ag, 0.291 ppm; Ca, 760.417 ppm; Cr, 5.268 ppm; Cu, 1.942 ppm; Fe, 23.954 ppm; Mg, 74.211 ppm; Mn, 1.010 ppm; Ni, 3.227 ppm; Pb, 3.027 ppm; Zn, 20.076 ppm; Na, 206.040 ppm; Cd, 1.081 ppm. Wastewater treatments at different concentrations (RADW, TSDW) were added once a week for 5 months (November–March) at 25%, 50%, 75% and 100%), wastewater percentages were prepared by mixing wastewater (RADW, TSDW) with tap water at different ratios (v/v).

The experiment had nine treatments, with three replicates for each, as follows: T1 = tap water (Control); T2 = RADW 25%; T3 = RADW 50%; T4 = RADW 75%; T5 = RADW 100%; T6 = TSDW 25%; T7 = TSDW 50%; T8 = TSDW 75%; T9 = TSDW 100%.

Assessment of vegetative development features of jojoba plant

Number of shoots increase percentage (NSI%)

The percentage increase in the number of shoots was recorded at the beginning (BN) and ending (EN) of the trials. The NSI% was calculated using the formula NSI% = [(EN − BN)/BN] × 100.

Jojoba height increase percentage (JHI%)

The length of the jojoba plant's main stem from the ground to the end was measured at the beginning (bH) and end (eH) of the experiment to determine the plant's height in (cm). The formula used to calculate the JHI% could be expressed as

JHI% = [(eH – bH)/bH] × 100.

Jojoba stem diameter increase percentage (JSDI%)

The measuring point was painted with yellow paint on the jojoba stem at the beginning (sd) and end (ed) of the study at the same height beginning from the ground surface in order to ensure accuracy. Using the formula JSDI% = [(ed − sd)/sd] × 100, the diameter of the jojoba stem was estimated.

Number of leaves increase percentage (NLI%), leaf thickness (LT) and leaf area (LA)

For each replication, the number of all mature leaves of $plant^{-1}$ was calculated at the start (SL) and at the end (EL) of the experiment. The following formula was used to calculate the percentage of leaves increasing in number (NLI%): NLI% = [(EL − SL)/SL] × 100. A digital calliper (Digital Calliper Model 500, Fuzhou, China) was used to measure LT. The medial leaves' length and width were measured. Then, LA was computed using the formula provided by Ahmed and Morsy (1999) as follows: LA (cm^2) = 0.70 × (leaf length × leaf width) − 1.06.

Leaf fresh weight (LFW) and leaf dry weight (LDW)

During the research seasons, 20 fully grown leaves were gathered from the fifth and sixth nodes' shoots of each replicate and weighed to calculate the leaf's fresh weight expressed in grams. The average dry weight was determined after drying them at 70°C for 72 hr, or until the weight didn't change in two further weighs.

Visual quality (VQ)

At the end of the investigation, leaf damage severity was recorded in accordance with Sun et al. (2015), using a ranking of 0–5 (visual level), where 0 = corresponds to dead, 1 = corresponds to intense visual injury (injury >90%), 2 = corresponds to medium visual damage (50%–90%), 3 = corresponds to petite visible injury (20%–50%), 4 = corresponds to tiniest visible injury (injury <20%) and 5 = corresponds to no visual injury.

Chemical constituents of jojoba plant

HMs and NPK content analysis

Mature leaves of jojoba were collected at the end of the investigation; cleaned more than once with distilled water to get rid of dust, and then dried at 70°C for 72 h to reach stable dry weight, in the end, it grinds finely and 0.5 g of dry material of each sample was wet digested in digestion flask by concentrated HNO_3 and heated on a hot plate to obtain a delicate refluxing action (Jones and Case, 2018). NPK levels were calculated by wet digesting 0.3 g of dried leaf samples from each replicate with a solution of concentrated H_2SO_4 and $HClO_4$, then calculating N, P and K as previously mentioned (AOAC, 2000). According to Munter et al. (2008), inductively coupled plasma atomic emission spectroscopy (plasma View Duo iCAP7000 Plus Series ICP–OES, Thermo Scientific™, North shore, New Zealand) was next.

Chlorophyll and carotenoids measurements

Five millilitres of dimethylformamide (DMF) was added to 0.5 g of jojoba fresh leaf samples for each replicate. Allowing the DMF to extract the pigments (chlorophyll and carotenoids) from the sample, the suspension was sonicated at 4°C for 15 min. Then it was kept there for 16 hr to eliminate any remaining suspended matter. One millilitre of the supernatant was centrifuged for 5 min at 16000 rpm. Chlorophyll content in the cleared supernatant was then examined using a spectrophotometer at 662 nm (E662) and 650 nm (E650) in accordance with Porra (2002). According to Wellburn (1994), carotenoids were also calculated using a wavelength of 480 nm.

Determination of proline concentration

Five millilitres of 3% sulfosalicylic acid (w/v) was used to homogenise 0.5 g of fresh jojoba leaves. After that,

the mixture was centrifuged at 10000 rpm for 10 min. To determine the proline content, 2.0 mL of the supernatant was collected and combined with the toluene solution and ninhydrin reagent (Bates et al., 1973). This quantity was then quantified using a spectrophotometer at 515 nm. Proline concentration (mg · g^{-1} FW) was determined using the L-proline standard curve.

Soluble carbohydrate content (SCC) and total phenolic content (TPC)

According to Kerepesi et al. (1996), 0.1 g of dried jojoba leaves was cooked for 50 min in 10 mL of distilled water before being strained through a particular filter paper. Using D-(+)-glucose as a standard, an aliquot (0.5 mL) of this filtrate was utilised to calculate the SCC in accordance with Dubois et al. (2002). The acquired results were registered as mg · g^{-1} DW. As for the analysis of TPC, Folin–Ciocalteu reagent was used according to Ainsworth and Gillespie (2007). A standard gallic acid (GAE) solution was used at a concentration of 100–600 ppm. A wavelength of 760 nm was used to measure the absorbance. It was described as mg GAE · g^{-1} FW.

Ion leakage (IL%), malondialdehyde (MDA) accumulation

To estimate IL%, 5 g of fresh leaves were soaked in 20 mL 0.4 M $C_6H_{14}O_6$ for 3 hr at room temperature 24°C, and then firstly detected the electrical conductivity (EC) (L1). Afterwards, all selections were cooked for 30 min at 100°C in a water bath and when the samples reach room temperature, the EC is measured for the second time (L2). IL% was represented as IL (%) = (L1/L2) × 100 according to Hakim et al. (1999). To estimate the level of MDA, about 2.5 g of jojoba leaves were used. A well-crushed leaf sample was mixed with $C_4H_4N_2O_2S$ (TBA), 500 µL of $C_{15}H_{24}O$ (2%, w/v) and 25 mL of metaphosphoric acid (HPO_3) in C_2H_6O (5%, w/v). As an influence of determining the 1,1,3,3-$CH_2(CH(OCH_3)_2)_2$ concentrations of thiobarbituric acid reactive substances varying from 0 mM to 2 mM, which were identical to MDA ranging from 0 mM to 1 mM. Tetraethyoxypropane (1,1,3,3-$CH_2(CH(OCH_3)_2)_2$) is turned into stoichiometrically to MDA through the acid heating stage of the trial (Iturbe-Ormaetxe et al., 1998).

Superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and 2,2-diphenyl-1-picrylhydrazyl (DPPH%)

Three grams of fresh jojoba leaf were mixed with a monobasic potassium phosphate solution (KH_2PO_4, 50 mM · L^{-1}, pH 7.8) after cooling to 4°C. After adding polyvinylpyrrolidone (C_6H_9NO) n (PVP 1% w/v) to the reagent, it was centrifuged at 10000 rpm for 15 min at 4°C. The amount of $O_2^{\bullet-}$ generated was calculated by creating (NO_2^-) from hydroxylamine in the presence of a superoxide anion (Yang et al., 2011).

At a wavelength of 530 nm, the optical density was measured. A standard curve of nitrogen dioxide was used to determine the rate of $O_2^{\bullet-}$ production resulting from the interaction of hydroxylamine with the superoxide anion. The product rate of $O_2^{\bullet-}$ was identified as mM min^{-1} · g^{-1} FW. To determine the H_2O_2, one gram of fresh leaves was mixed with 5 mL of acetone; after that, the mixture was centrifuged for 15 min at 6000 rpm at 4°C. The H_2O_2 content was determined using the method represented by Xu et al. (2012). About 0.2 ml of NH_3 and 0.1 mL of $TiOSO_4$ (5%) were added to 1 ml of the explicit extract, then centrifuged at 6000 rpm for 10 min at 4°C. The final grains (TiO_2^{+2}) were dispersed in 3 ml of 10% H_2SO_4 (v/v) and centrifuged for 10 min at 4°C and 5000 rpm. The following supernatant's optical density was calculated to be 410 nm. Using H_2O_2 as a standard curve, the H_2O_2 content was displayed and then determined as mM · min^{-1} · g^{-1} FW. In terms of antioxidant activity (DPPH), 3 g of jojoba leaves were mixed with 30 ml of methanol (CH_3OH) and then centrifuged at 10000 rpm for 15 min. Three millilitres of 0.1 mM DPPH was added to the resulting extract (1 mL). The combination was kept at room temperature in the dark for 20 min. At 517 nm the absorbance has been selected, and the DPPH radical quashing effect has been registered. The following equation was used [(control(ABs) − sample(ABs))/control(ABs)] × 100, according to Cao et al. (2018).

Statistical analysis

Statistically, the average results for two successive agricultural seasons 2022–2023 were examined. Data were subjected to analysis of variance (ANOVA) in a completely randomised block design (CRBD), by using the statistical program Co-Stat software package, Ver. 6.303 (789 Lighthouse Ave PMB 320, Monterey, CA, 93940, USA), with three replications. A principal component analysis (PCA) was involved to estimate the influence of irrigation jojoba with RADW and TSDW with different concentrations on the characteristics of vegetative growth and chemical properties. Duncan's multiple range test was used to compare the results of all treatments at a significance threshold of $p \leq 0.05$.

RESULTS

Physical parameters of jojoba plant

In Figure 1, the data presented is an average of two successful experimental seasons. The percentage of increases in the number of shoots (NSI%), jojoba height (JHI%) and jojoba stem diameter (JSDI%) all steadily increased with varied levels of RADW at levels 25%, 50%, 75% and 100%. However, when irrigated with TSDW at a level of 100%, a decrease was observed in NSI%, JHI% and JSDI%. The highest rate of increase was achieved when using RADW at a high level of 100%, resulting in 121.73%, 84.17% and 95.79% increases for NSI%, JHI% and JSDI%, respectively, compared to the control. On the other hand, irrigated jojoba plants with a high level of TSDW at 100% resulted in lower increases that reached

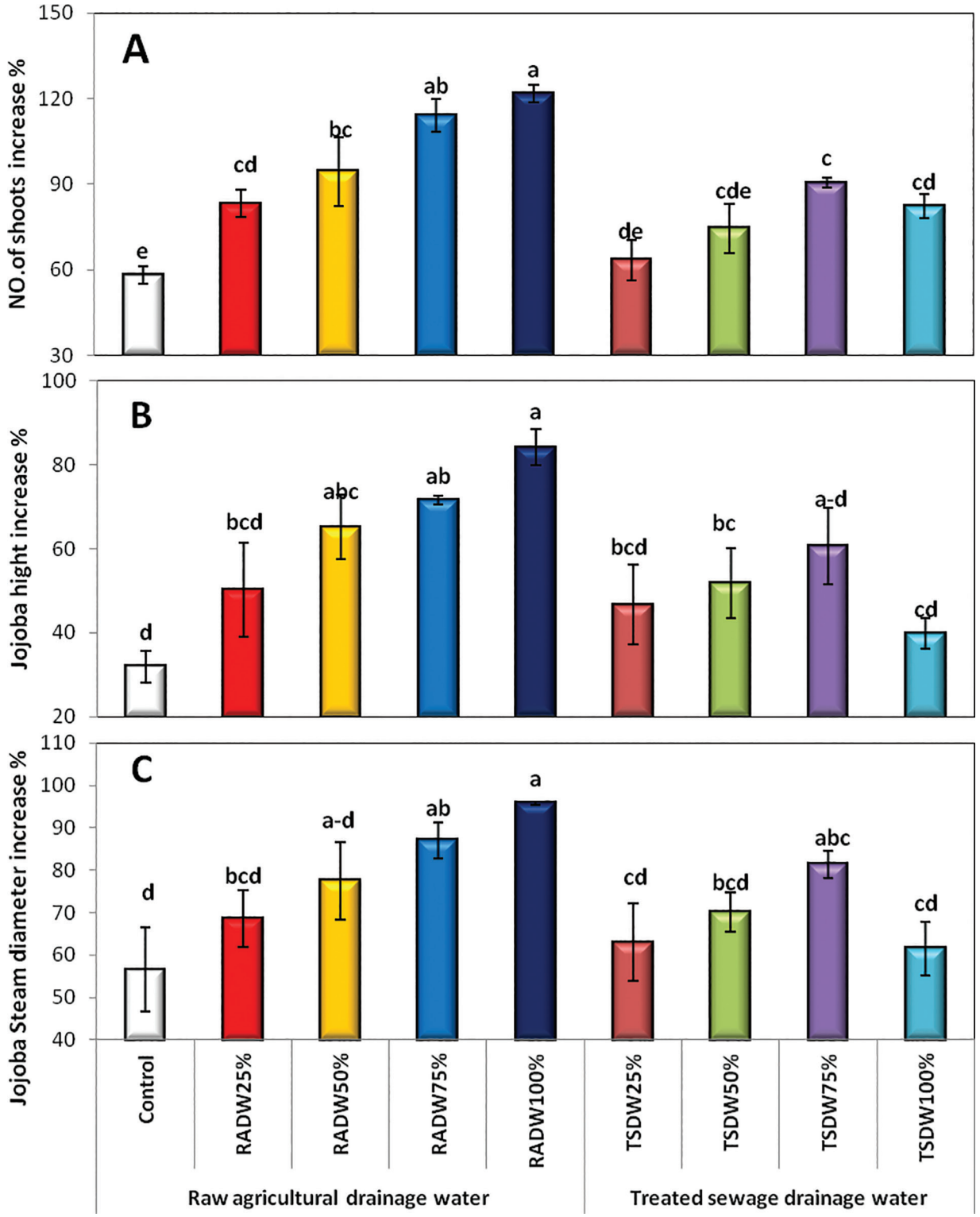


Figure 1. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100%, on the NSI% (A), JHI% (B) and JSDI% (C). The results are the average of two subsequent seasons (2022 and 2023), with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. JHI%, jojoba height increase percentage; JSDI, jojoba stem diameter increase percentage; NSI%, number of shoots increase percentage; RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

82.24%, 39.83% and 61.53%, respectively, compared to RADW at 100%. However, it was higher than the control. It was noticed in Figure 2 that the percentage of jojoba plants' leaf number increase (LNI%) significantly increased (84.22%) when irrigated with 100% RADW. The LT (0.82 mm) and LA (16.65 cm²) also increased significantly compared to irrigation with TSDW at 100%, which recorded 39.73%, 0.61 mm and

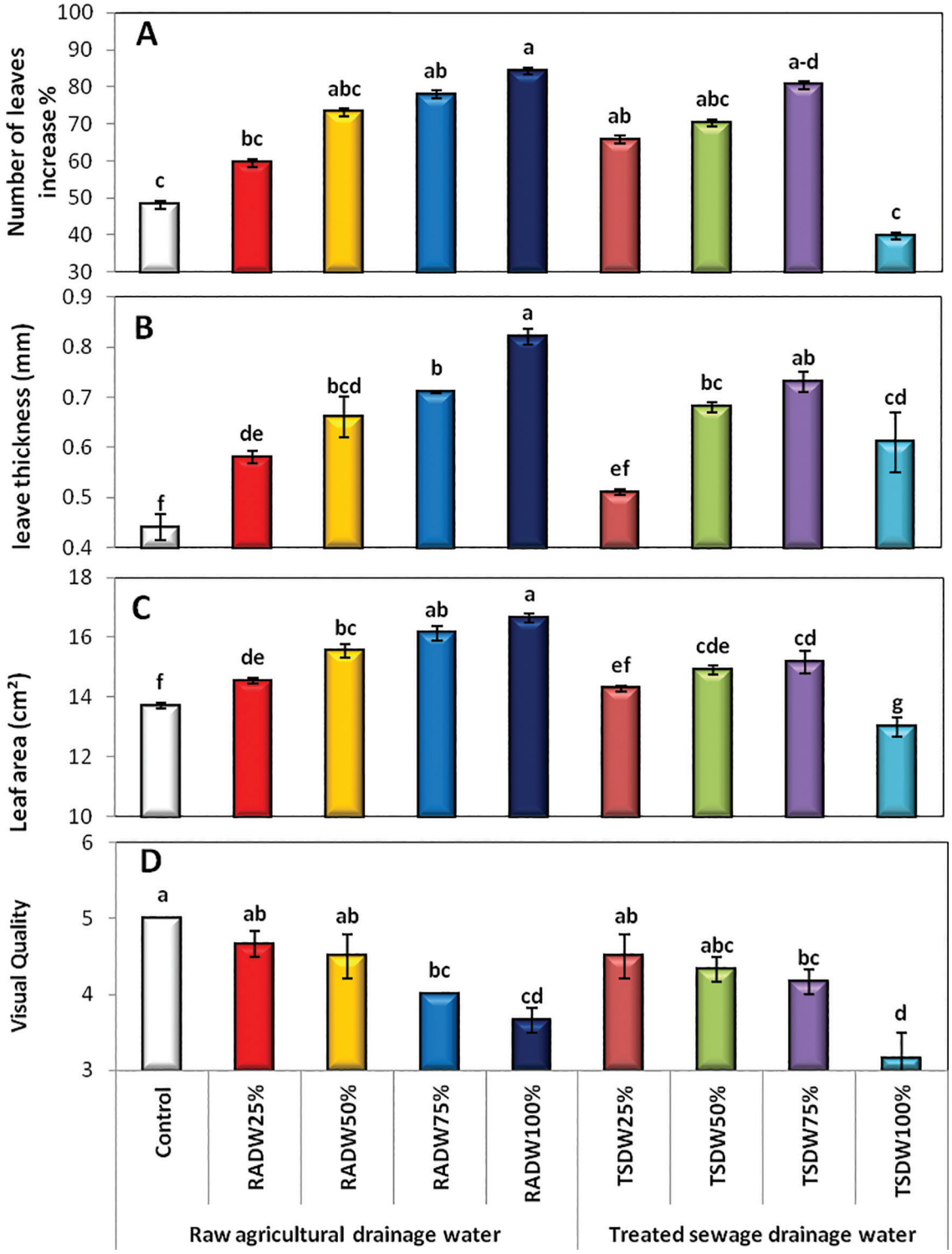


Figure 2. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100%, on the NLI% (A), LT (mm) (B), LA (cm²) (C) and VQ (D). The results are the average of two subsequent seasons (2022 and 2023), with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. LA, leaf area; LT, leaf thickness; NLI%, Number of leaves increase percentage; RADW, raw agricultural drainage water; TSDW, treated sewage drainage water; VQ, Visual quality.

13 cm², respectively. The control recorded increases of 48.14%, 0.44 mm and 13.7 cm² for LNI%, LT and LA, respectively. However, the VQ decreased with the increase in the concentration of RADW and TSDW, where the VQ value was 3.66 and 3.16 at the level of 100% in each of RADW and TSDW, respectively compared to the control. The results showed that irrigating jojoba plants with different concentrations of RADW up to 100% led to a significant increase in the NSI%, JHI%, JSDI%, LT and LA. On the other hand, irrigating jojoba plants with TSDW led to a significant increase in all the characteristics of vegetative growth at concentrations of 25%, 50% and 75%, but not at 100% concentration, which led to a decrease in all the characteristics of vegetative growth compared to the use of RADW.

Leaf biomass and SCC

According to the data presented in Figure 3, it is noticeable that there is a gradual increase in the leaf biomass, leaf fresh weight (LFW) and LDW of jojoba leaves when the concentration of RADW is increased up to 100%. At this concentration, the fresh weight and the dry weight of the leaves were 9.55 g and 3.50 g, respectively. On the other hand, when the jojoba plant was irrigated with TSDW, the fresh and dry weight of the leaves decreased significantly at 100% concentration, where the fresh weight and dry weight of leaves were 7 g and 2.99 g, respectively.

These results show that the fresh and dry weight of leaves increased with the use of different concentrations of RADW (25%, 50%, 75% and 100%) compared to the control (tap water) and other treatment (TSDW). While the SCC increased significantly by increasing the level of RADW from 25% to 100% compared to the control (tap water) and treatment of jojoba plant with TSDW, the SCC decreased gradually. Compared to the control, an increase in TSDW from 25% to 100% was observed.

Chemical parameters of jojoba plant

HM accumulation

Figure 4 shows that the accumulation of HMs, such as nickel, aluminium, chromium and cadmium, increased in jojoba plant leaf tissues when RADW was used for irrigation at various levels up to 100%. The concentrations of HMs were 0.678 ppm for nickel, 5.16 ppm for aluminium, 0.347 ppm for chromium and 0.08 ppm for cadmium. On the other hand, the accumulation of HMs in jojoba leaf tissues significantly increased when TSDW was used for irrigation at different levels of 25%, 50%, 75% and 100%. The concentrations of HMs were 1.923 ppm for nickel, 7.84 ppm for aluminium, 0.692 ppm for chromium and 0.216 ppm for cadmium. Significantly, the most concentrated HM in jojoba plant leaf tissues is aluminium whether RADW or TSDW is used to irrigate the plant, compared to control.

Nitrogen, phosphors and potassium concentration

Based on the data shown in Figure 5, it is evident that the use of RADW and TSDW resulted in higher concentrations of nitrogen, phosphorus and potassium in jojoba leaves compared to the control group. The jojoba plants that were irrigated with both RADW and TSDW at 100% had the highest concentration of nitrogen, phosphorus and potassium, with values of 49 mg · 100 g^{-1} DW, 45 mg · 100 g^{-1} DW, 52 mg · 100 g^{-1} DW, and 53 mg · 100 g^{-1} DW, 36 mg · 100 g^{-1} DW, 45 mg · 100 g^{-1} DW, respectively. In contrast, the control group that used tap water had lower concentrations of these nutrients.

Chlorophyll and carotene contents

Results demonstrate how variable levels of RADW and TSDW influence the concentration of jojoba leaf pigments as chlorophyll a and b, total chlorophyll and carotene are shown in Figure 6. The data indicated that the concentration of chlorophyll a, chlorophyll b and total chlorophyll in jojoba leaves increased with the increase in the level of RADW and TSDW used in irrigation at 25%, 50% and 75% levels, but decreased at a level of 100%. On the contrary, the level of carotenoids was increased at 100% of the wastewater RADW and TSDW used. The highest concentration of chlorophyll a and b and total chlorophyll was found at the level of 75% of RADW and TSDW which recorded 92 μg · cm^{-2}, 31.5 μg · cm^{-2} and 125.5 μg · cm^{-2}. However, the largest concentration of carotenoids was noticed at the 100% RADW and TSDW, which were 26.5 μg · cm^{-2} and 31.5 μg · cm^{-2}, respectively.

Proline, IL% and MDA

The data displayed in Figure 7 proves a significant increase in proline content, IL% and MDA in jojoba plants under the effect of RADW and TSDW at different levels of 25%, 50%, 75% and 100% compared to the control. Inclusion, irrigation with a higher level (100%) of RADW and TSDW caused a significant increase in proline content, IL% and MDA, which achieved 4.68 mg · g^{-1} DW and 5.65 mg · g^{-1} DW, 13.83% and 17.5%, 26.33 μM · g^{-1} FW and 30.66 μM · g^{-1} FW, respectively.

Superoxide anion (O_2•−), hydrogen peroxide (H_2O_2) and DPPH%

Data in Figure 8 revealed that jojoba plants irrigated with RADW and TSDW affected the concentration of superoxide anion ($O_2^{\bullet-}$), H_2O_2 and DPPH% antioxidant capacity. After the studied period of wastewater stress, $O_2^{\bullet-}$ and H_2O_2 concentration was noticeably elevated in all various wastewater treatments in comparison to the control, which reached 0.33 mM · min^{-1} · g^{-1} FW and 0.33 mM · min^{-1} · g^{-1} FW at level 100% RADW, 0.14 mM · min^{-1} · g^{-1} FW and 0.23 mM · min^{-1} · g^{-1}

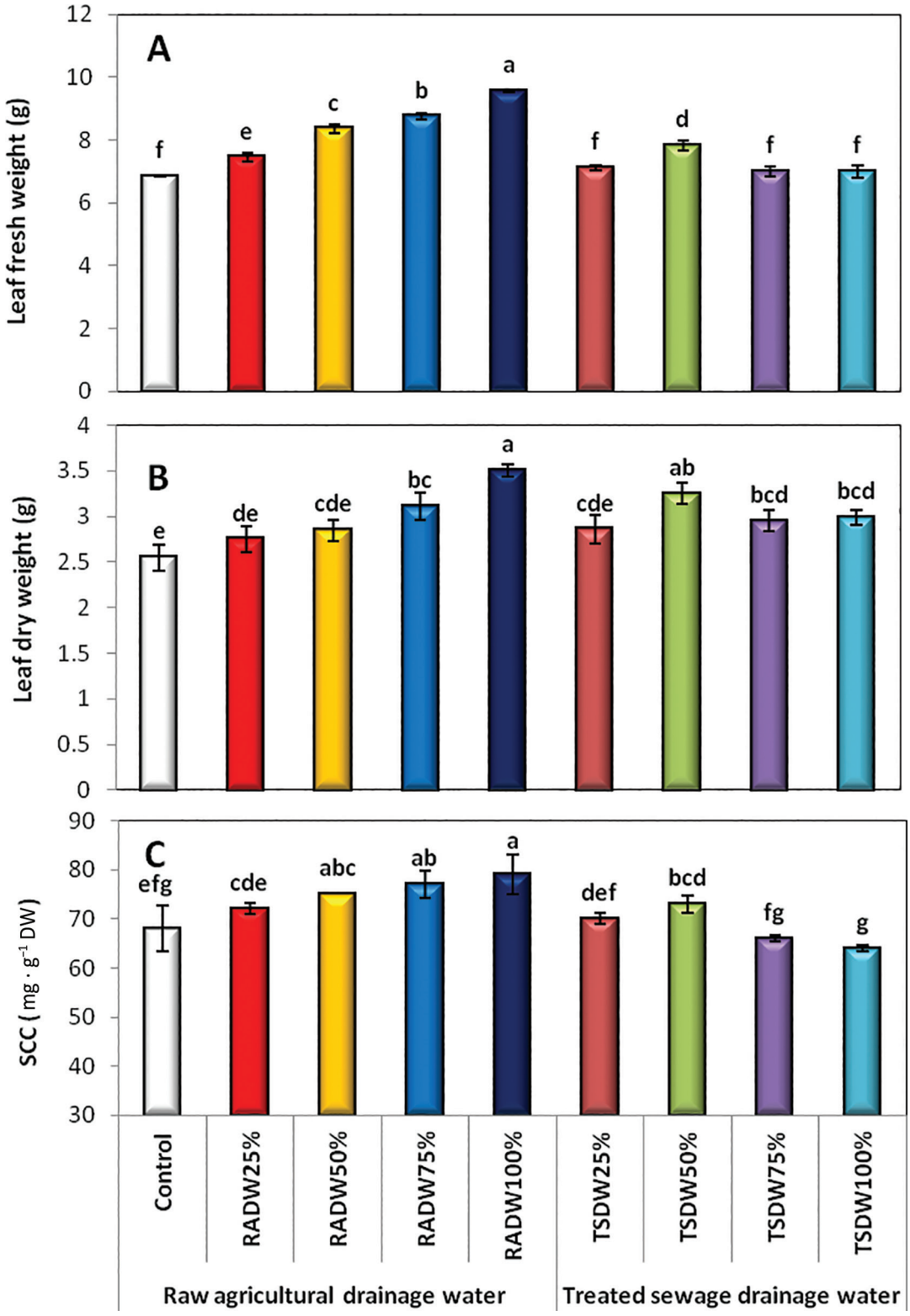


Figure 3. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100%, on LFW (g) (A), LDW (g) (B) and SCC (mg · g^{-1} DW) (C). The results are the average of two subsequent seasons (2022 and 2023), with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. LDW, leaf dry weight; LFW, leaf fresh weight; RADW, raw agricultural drainage water; SCC, soluble carbohydrate content; TSDW, treated sewage drainage water.

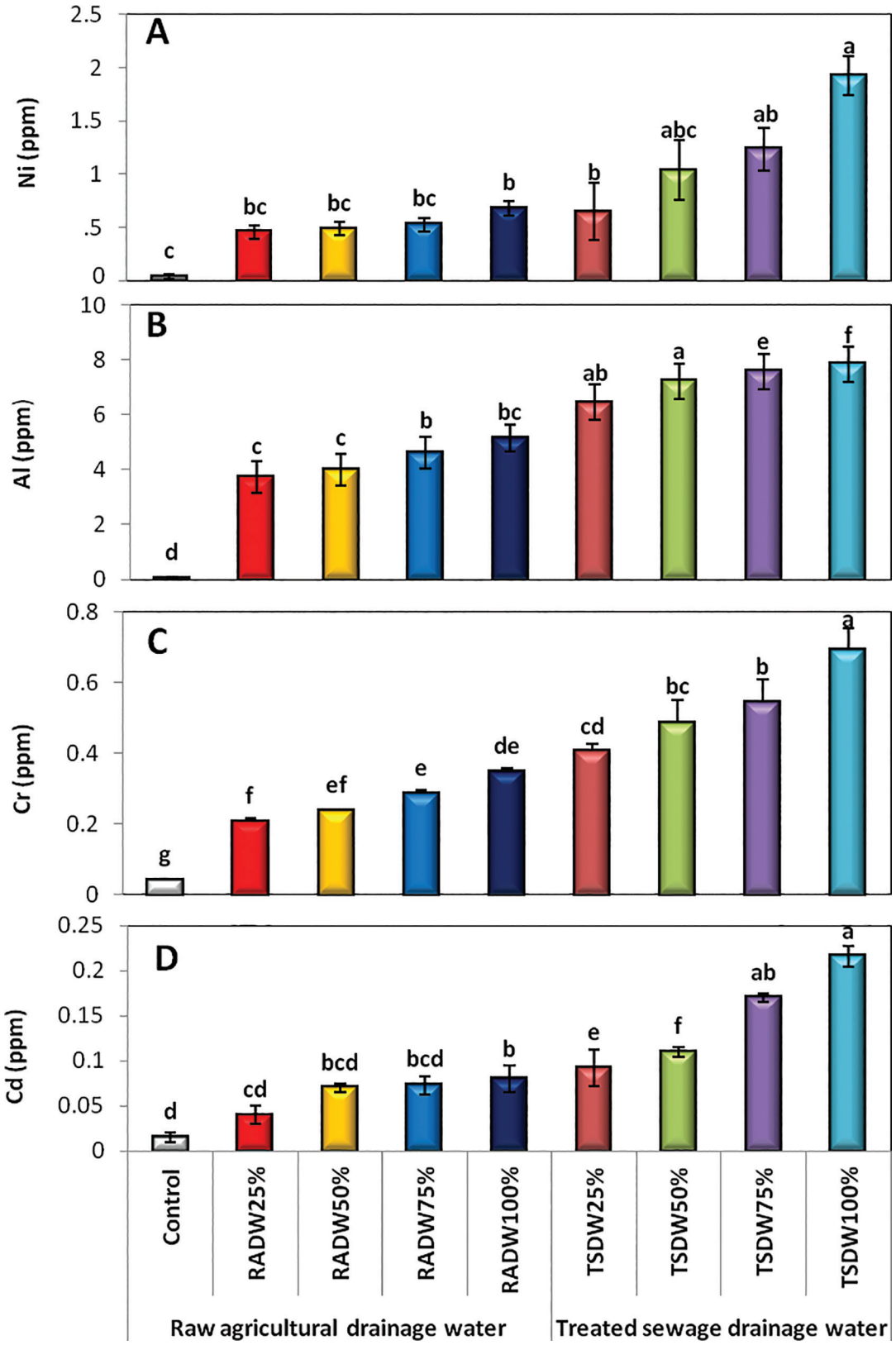


Figure 4. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100%, on HM concentrations as Ni ppm (A), Al ppm (B), Cr ppm (C) and Cd ppm (D). The results are the average of two subsequent seasons (2022 and 2023), with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. HM, Heavy metal; RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

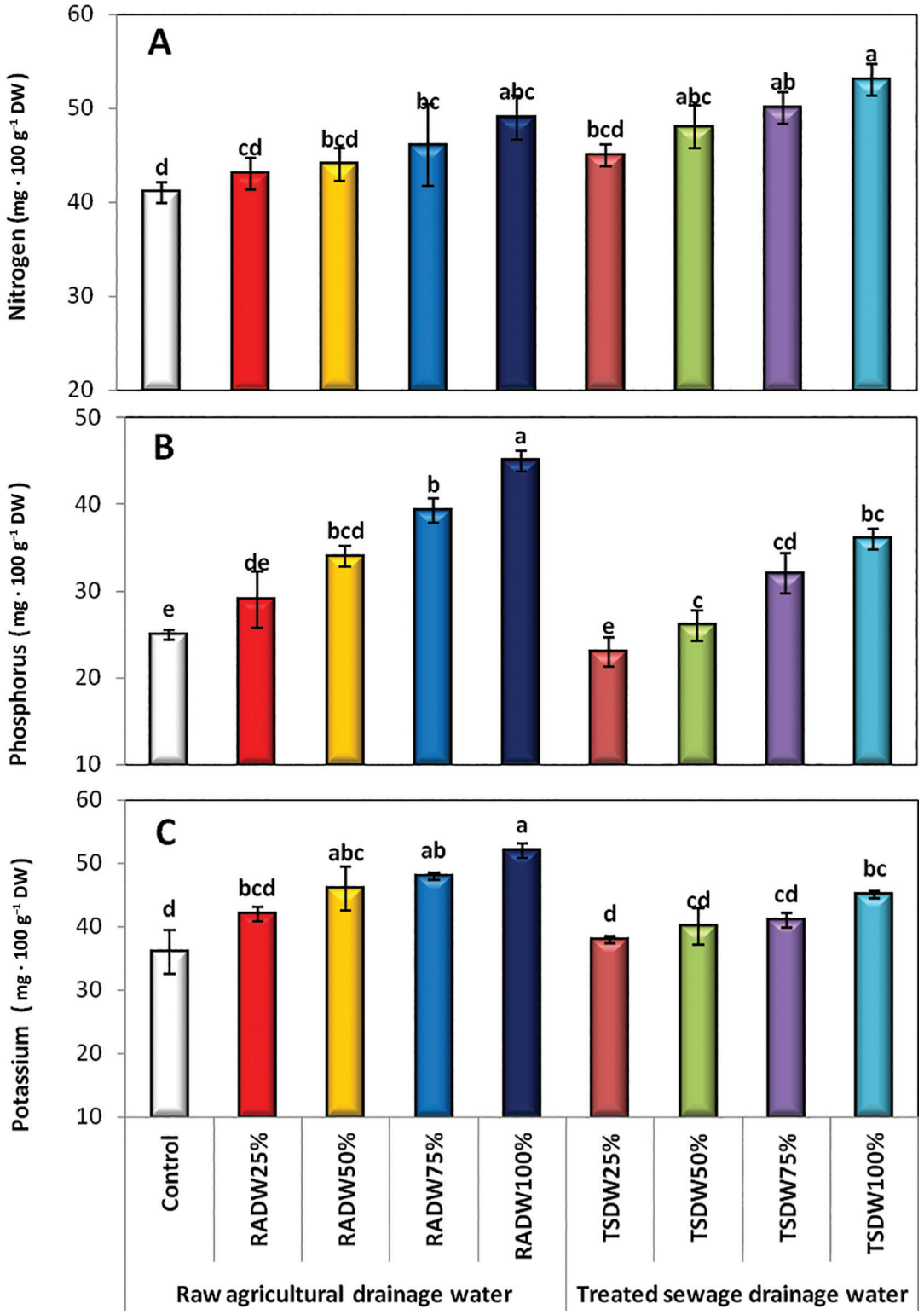


Figure 5. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100%, on nitrogen content (mg · 100 g^{-1} DW) (A), phosphorus content (mg · 100 g^{-1} DW) (B) and potassium content (mg · 100 g^{-1} DW) (C). The results are the average of two subsequent seasons (2022 and 2023), with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

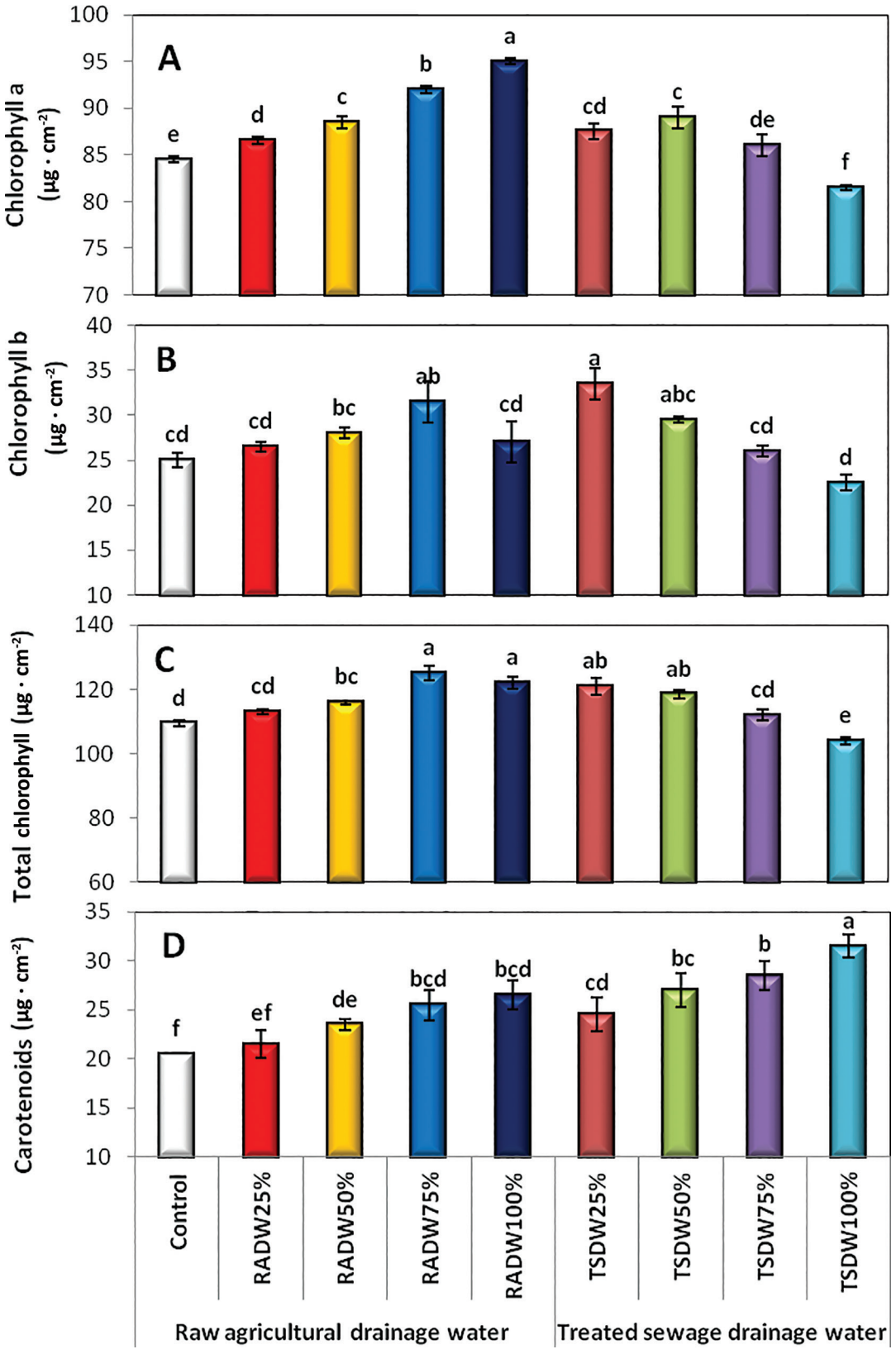


Figure 6. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100% on: chlorophyll a (µg · cm^{-2}) (A), chlorophyll b (µg · cm^{-2}) (B), total chlorophyll (µg · cm^{-2}) (C) and carotenoids (µg · cm^{-2}) (D). The results are the average of two subsequent seasons (2022 and 2023) with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

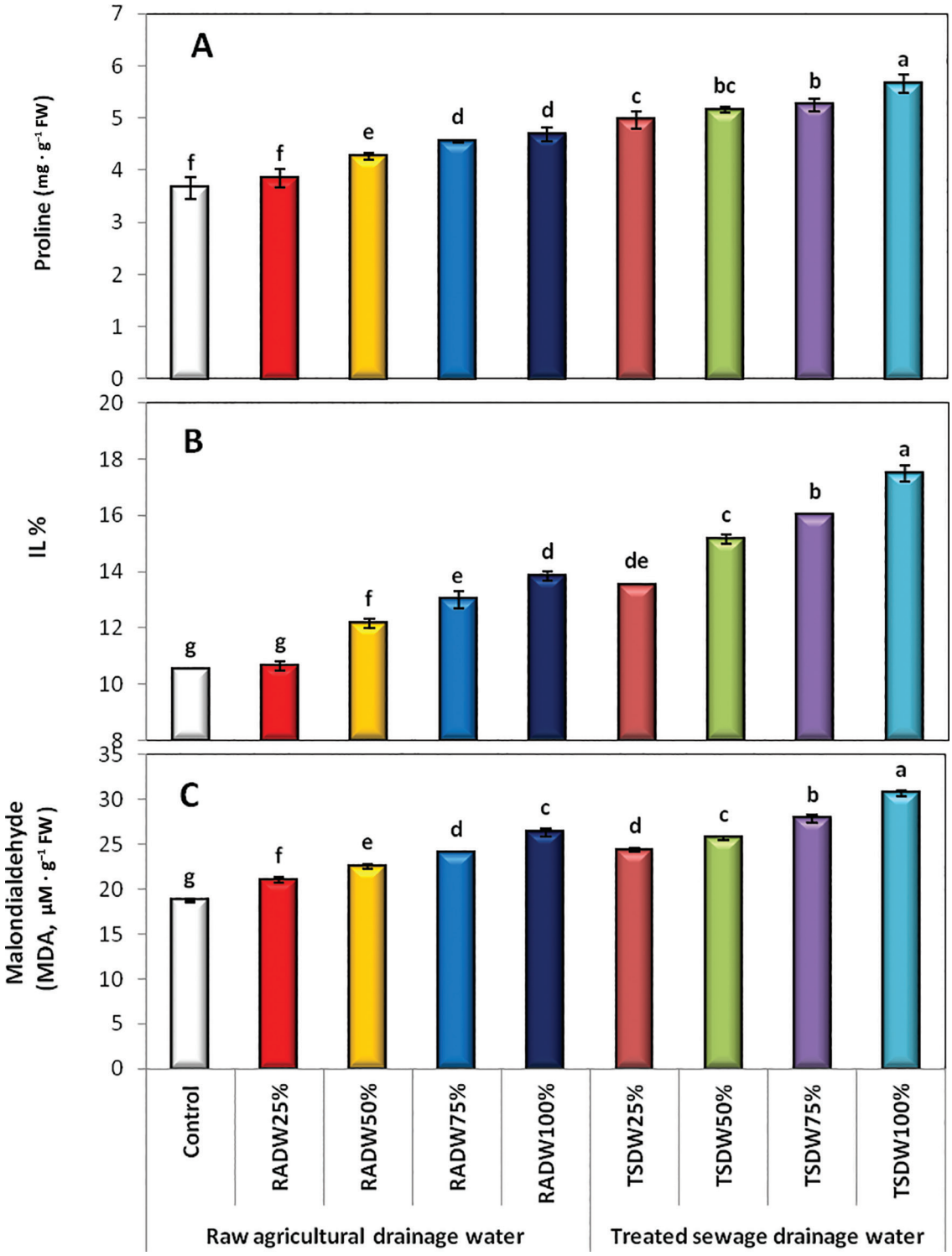


Figure 7. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100% on proline (mg · g^{-1} FW) (A), IL (%) (B) and MDA (µM · g^{-1} FW) (C). The results are the average of two subsequent seasons (2022 and 2023) with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. IL%, ion leakage; MDA, malondialdehyde; RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

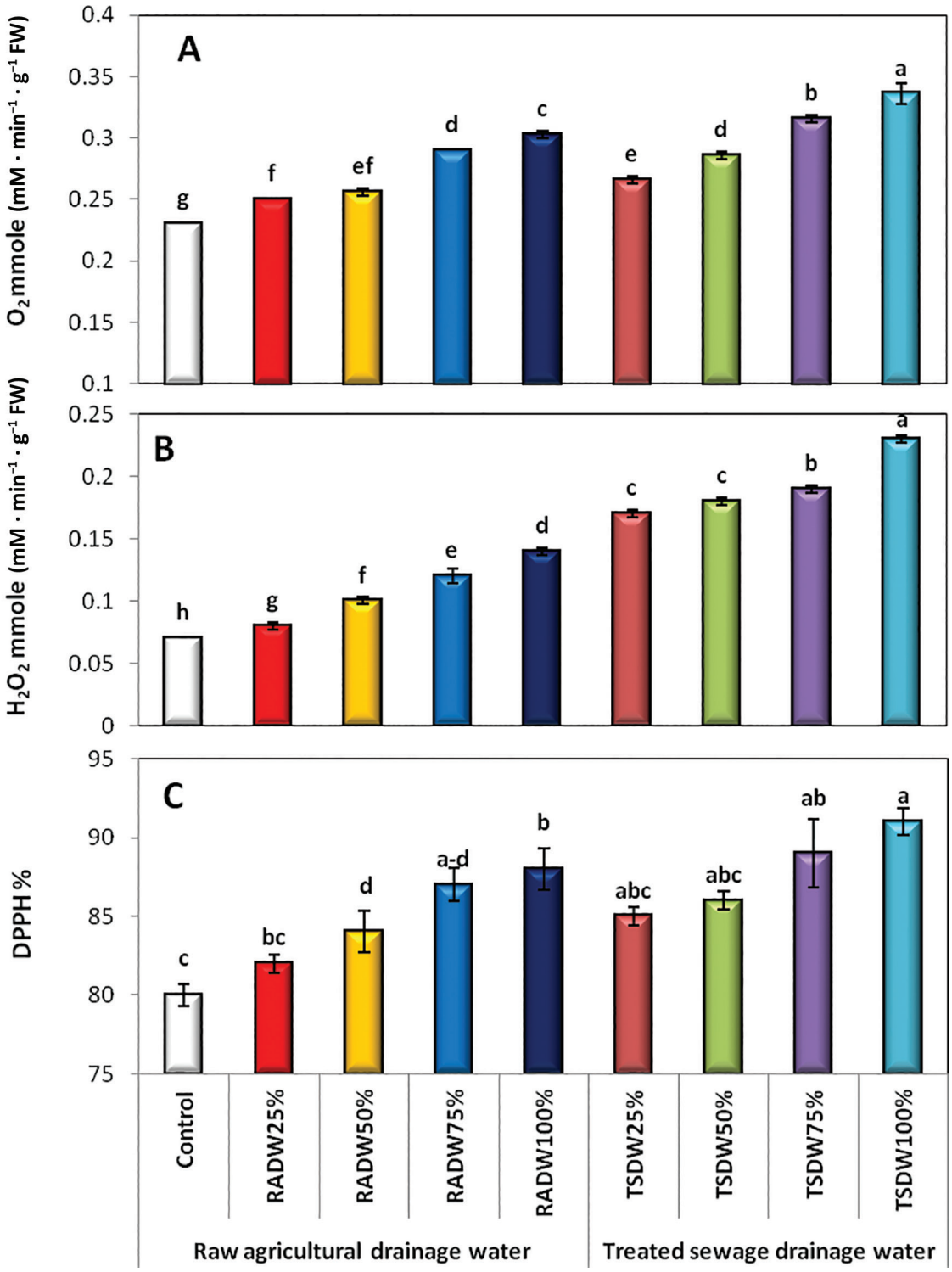


Figure 8. The effect of different concentrations of RADW and TSDW at four levels, 25%, 50%, 75% and 100% on $O_2^{\bullet-}$ (mM · min^{-1} · g^{-1} FW) (A), H_2O_2 (mM · min^{-1} · g^{-1} FW) (B) and DPPH% (C). The results are the average of two subsequent seasons (2022 and 2023) with $n = 3$ for each season. According to Duncan's multiple range test, each parameter's mean values and standard error (±SE) are significantly different when each is followed by a different alphabetical letter at $p \leq 0.05$. DPPH, 2,2-diphenyl-1-picrylhydrazyl; RADW, raw agricultural drainage water; TSDW, treated sewage drainage water.

FW at level 100% TSDW, respectively. Meanwhile, the antioxidant capacity (DPPH) decreased slightly under the influence of different concentrations of RADW and TSDW, reaching 83% when using a level of RADW at 100%, while it reached 80% when using a level of TSDW at 100% compared to the control and other levels.

Multivariate analysis of jojoba plant resistant parameters

During the experiment, the effect of RADW and TSDW on the resistant parameters of jojoba was evaluated using Pearson's correlation matrix (Figure 9) and PCA (Figure 10). The multivariate space of the two principal components (PCs) showed that the jojoba-resistant parameters were differently influenced by treatments during the experiment over the period of 5 months. DPPH, O_2, H_2O_2, MDA, Cd, Ni, Cr, Al, N, LDW and carotenoids were on the left side of the PC1 axis, while JHI, JSDI, NSI, LA, NLI, LT, SCC, cha, chb, total ch and VQ were on the right side of the same axis. Shifts in the PC average values from negative DPPH, O_2, H_2O_2, MDA, Cd, Ni, Cr, Al, N, LDW and carotenoids to positive JHI, JSDI, NSI, LA, NLI, LT, SCC, Cha, chb, total ch and VQ were observed with advances in treatments and control. The results indicate that there is stability of DPPH in all irrigation treatments which helps to scavenge free radicals (O_2 and H_2O_2), which has been confirmed by the increase of all vegetative characteristics under the influence of all treatments compared to the control. Pearson's correlation coefficient among the studied

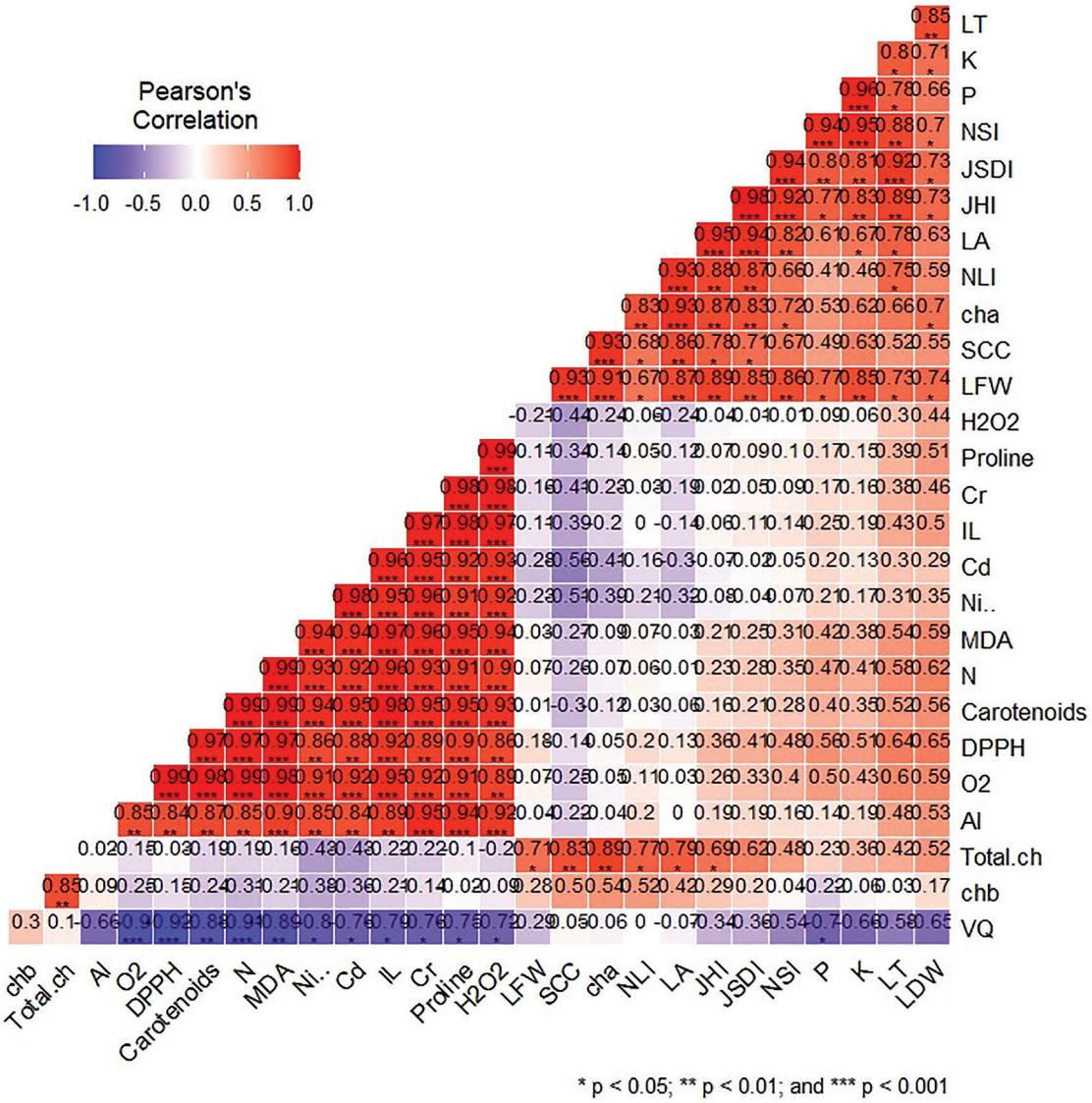


Figure 9. Person's correlation matrix among the resistant-related parameters on jojoba. Values express the average values of the wastewater treatments on jojoba plant. The correlations are calculated by the Row-wise method. DPPH, 2,2-diphenyl-1-picrylhydrazyl; IL%, ion leakage; JHI%, jojoba height increase percentage; JSDI, jojoba stem diameter increase percentage; LA, leaf area; LDW, leaf dry weight; LT, leaf thickness; MDA, malondialdehyde; NLI, Number of leaves increase percentage; NSI%, number of shoots increase percentage; SCC, soluble carbohydrate content; VQ, Visual quality.

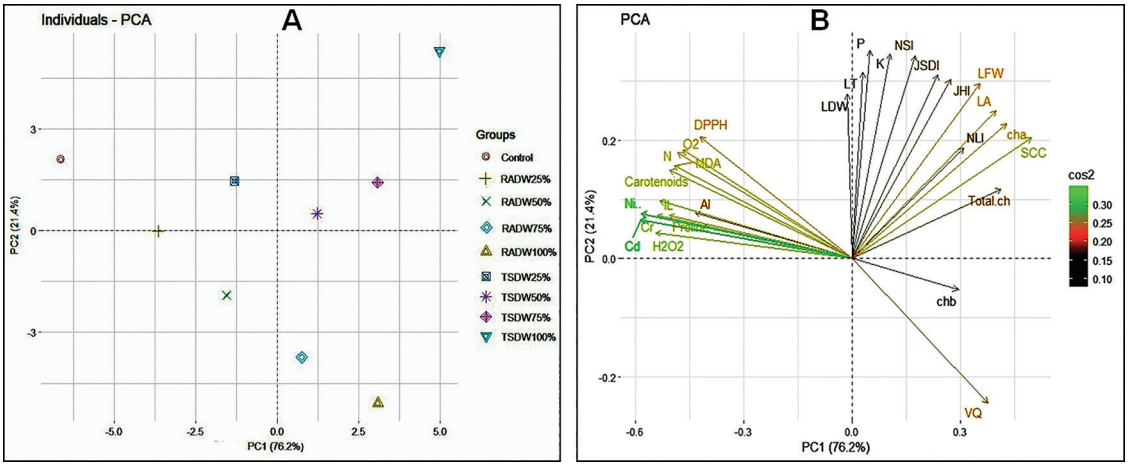


Figure 10. First and second PCA scores plot of different waste water treatments (A). First. and second PCA scores plot of the correlation in analysed parameters (B). DPPH, 2,2-diphenyl-1-picrylhydrazyl; IL%, ion leakage; JHI%, jojoba height increase percentage; JSDI, jojoba stem diameter increase percentage; LA, leaf area; LDW, leaf dry weight; LT, leaf thickness; MDA, malondialdehyde; NLI, Number of leaves increase percentage; NSI%, number of shoots increase percentage; PCA, principal component analysis; RADW, raw agricultural drainage water; SCC, soluble carbohydrate content;TSDW, treated sewage drainage water; VQ, Visual quality.

parameters shows the correlation and indicates that these results according to PC1 and PC2 show the relationship between treating different wastewater concentrations and the physical and chemical properties.

DISCUSSION

Freshwater scarcity is a major issue in Egypt due to the construction and filling of the Renaissance Dam in Ethiopia. It is predicted that by 2025 there will be a shortage of about 13.5 million cubic meters of freshwater annually. To tackle this problem, it is important to focus on reusing wastewater for plant irrigation. However, this can cause plant stress. Water stress occurs when there is an imbalance between the water supply and the plant water demand (Pomar and Moussa, 2016; Abd Ellah, 2020). Salt stress significantly limits crop growth and development, posing a threat to global food production. It mainly induces osmotic stress, ionic and nutritional imbalance and reactive oxygen species (ROS), resulting in a significant loss in plant growth (Dustgeer et al., 2021; Seleiman et al., 2022; Sultan et al., 2022). Salt stress leads to a reduction in crop productivity by decreasing photosynthetic efficiency (Alghamdi et al., 2021). Drought is a significant abiotic stress that greatly limits crop growth and global food production (Hassan et al., 2020; Memon et al., 2022). The reduced water availability causes severe changes in plant physiological processes, leading to significant yield losses (Hassan et al., 2021). Water scarcity results in a significant increase in abscisic acid accumulation in plants. The increased presence of abscisic acid in plants can cause oxidative stress, leading to lipid peroxidation, electrolyte leakage and chlorophyll degradation (Jiang et al., 2020). Salt and drought stress can induce the production of ROS in plants, which are regulated by different plant growth regulators. These ROS serve as the plant's internal defence system, initiating the scavenging of ROS and reducing oxidative stress by enhancing the activities of antioxidant enzymes (Liang et al., 2019; Khan et al., 2022; Wongla et al., 2023). Plants have developed various strategies to cope with limited water availability. These strategies include minimising water loss, supplying water to essential members and maintaining the water content of cells during dry spells (Kern et al., 2020). Water shortage tolerance is the capacity of plants to detect and adapt to water stress. Plant water deficit tolerance is a multifaceted characteristic that can take the form of three main approaches: increasing water use efficiency to avoid drought stress and tissue damage, shortening their lifecycle to escape stress and managing water deficiency by preserving low water content and little activity to live with the least amount of loss (Bolger et al., 2014). The jojoba plant's ability to withstand irrigation with wastewater as RADW and TSDW at various levels (25%, 50%, 75% and 100%) was investigated. The data showed that the jojoba plants did not experience severe effects or death when exposed to water contaminated with HMs and organic residues as agreed with Rasheed et al. (2019). These findings are also consistent with previous research which found the jojoba plant can grow in polluted soil with HMs (Nasr et al., 2019). These abiotic stresses disrupt plant physiological and metabolic functioning development processes (Jeandroz and Lamotte, 2017) and induce the production of ROS, lipid peroxidation accumulation of various osmolytes and significant yield losses (Batool et al., 2022). The accumulation of abscisic acid and jasmonic acid (phytohormones) in the leaves of plants under water stress activates the regulation of stomatal closure (Iqbal et al., 2014). Stomatal closure inhibits transpiration, preserves water content and suppresses

carbon dioxide diffusion to the chloroplast (Ma and Bai, 2021). This reduction in carbon dioxide assimilation can harm photosynthesis activity and lead to the generation of ROS such as $O_2^{\bullet-}$ and H_2O_2 (Hasanuzzaman et al., 2021). The excessive production of ROS causes oxidative stress, damaging DNA, proteins and lipids, reducing plant yield and growth and ultimately leading to plant apoptosis (Afzal et al., 2023). At the physiological, cellular and molecular levels, plants have evolved a variety of dehydration resistance mechanisms to offset these detrimental consequences. Enzymatic (superoxide dismutase [SOD], catalase [CAT], peroxidase, glutathione reductase) and non-enzymatic (tocopherol, ascorbic acid, carotenoids, glutathione, phenolics, flavonoids) antioxidant components can reduce the detrimental effects of accumulated ROS, creating an essential defence system (Zandi and Schnug, 2022). Plants that produce more antioxidant molecules are better able to reduce the amount of ROS they produce (Laxa et al., 2019).

Furthermore, plants accumulate osmolytes such as proline, glycine betaine, sugars and sugar alcohols in their cells as a result of the fast formation of ROS, which acts as a stress signal (Mukherjee and Choudhuri, 1983). Osmolytes are essential for preserving the osmotic balance of plants and for balancing the composition and activity of several enzymes and macromolecules in the face of elevated ROS levels (Huang et al., 2009). Osmolytes are tiny, soluble substances that insulate cells from damage by maintaining the water content of cells, and protecting the integrity of the cell membrane (Sarma et al., 2023). Auxins, a type of phytohormone that the plant produces in response to its constant need for water, are important in changing the roots (Ilyas et al., 2021). The primary sites of auxin biosynthesis are developing leaves and leaf primordia. Auxins, however, go to the root tips under conditions of water stress via the phloem, cells or auxin-transport proteins such as PIN-FORMED (PIN) (Merelo et al., 2017). Plant roots become more active as a result of this movement when there is water stress. As a result of abiotic and biotic stresses, there is an increase in membrane leakage due to elevated lipid peroxidation. Recall that, other significant macromolecules such lipids, proteins, nucleic acids and pigments involved in photosynthetic processes are also impacted by ROS. Antioxidants can lower the likelihood of experiencing oxidative stress situations by mediating the scavenging of ROS. They comprise both enzymatic (SOD, CAT, ascorbate peroxidase [APX], etc.) and non-enzymatic components (Wu et al., 2019). Reusing wastewater for plant irrigation can reduce the need for freshwater resources and costly chemical fertilisers, but it has been found that it may also have negative effects on soil and plant physiochemical properties (Pomar and Moussa, 2016; Alnaimy et al., 2021). Nonetheless, it contributes to soil fertility and plant growth because it contains high quantities of nutrients (Tarchouna et al., 2010; Saliba et al., 2018). It's also important to remember that using wastewater for irrigation can lead to soil salinisation, which poses potential risks. Higher levels of sodium compared to other cations, along with contaminated soil and plants containing HMs, can have long-term health implications. The extent of risk depends on the origin of the wastewater and the methods used for treatment (Abd-Elwahed, 2018; Ganjegunte et al., 2018; El-Hassanin et al., 2020). Low molecular weight non-enzymatic antioxidants, such as proline, ascorbic acid and glutathione, can neutralise ROS. On the other hand, enzymatic antioxidants, such as SOD, POD, GST and CAT, can convert ROS into H_2O_2, which is then further converted into water and oxygen. These two categories of antioxidants protect the cell structure from oxidative stress and can also deactivate harmful oxygen by-products. In the data reported herein, the amount of proline increased as the levels of waste water increased. When plants are under HM stress, proline helps to remove harmful ROS in *Brassic juncea* and *Cajanus cajan*. It also helps to regulate water potential, reduce metal uptake and maintain osmotic balance for cellular health (Kalaivanan and Ganeshamurthy, 2016). Several studies have shown that proline acts as an osmolyte under stressful conditions and can enhance antioxidant enzymes to mitigate the harmful effects of oxidative stress. Proline also helps to regulate plant growth and maintain osmotic balance, protecting cells from ROS when exposed to high levels of cadmium (Shak and Dubey, 1997; Islam et al., 2009). The EC in the leaves, which indicates IL, increased with high levels of RADW and TSDW. Salt-induced water stress leads to a reduction in chloroplast stroma volume, resulting in the generation of ROS and inhibition of the photosynthetic rate (Rasool et al., 2022). While carotenoids and Chlb considerably decreased at high salt concentrations, pigment levels in the leaves of jojoba plants significantly decreased at intermediate salt concentrations in Chla. This implies that at low and moderate levels of salt stress in jojoba leaves, stroma volume and photosynthetic rate were unaffected, suggesting that jojoba can withstand moderate levels of salt stress (Inoti, et al., 2016). It has been observed that using TSDW at a concentration of 100% led to a decrease in all vegetative characteristics compared to other concentrations (25%, 50% and 75% RADW and TSDW). This decrease was also noted in biomass production, LFW, LDW and SCC (Redha et al., 2021). The reason for this is that agricultural wastewater has a higher concentration of nutrients such as nitrogen, phosphorus, potassium, magnesium and calcium, with a lower concentration of HMs than TSDW, especially at a concentration of 100%. HMs pose a severe threat to global food production. Their concentration in agricultural soil is rapidly increasing due to human activities (Rehman et al., 2022). Plant contamination with HMs can occur due to various sources, such as irrigation with polluted water (Alia et al., 2015), pesticides (Shaheen et al., 2016), chemical fertilisers, motor vehicle emissions and industrial discharges

(Abou-Arab et al., 2015). Additionally, certain products like fungicides, inorganic fertilisers and phosphate fertilisers may contain varying levels of cadmium, chromium, nickel, lead and zinc, depending on their sources (Tőzsér et al., 2017). There have been numerous investigations examining the plant species that can resist HMs, either by accumulating or translocating them, so they can be used in phytoremediation (Redha et al., 2021). Based on the results obtained, it was found that when using TSDW at a concentration of 100% compared to other concentrations of RADW and control, the concentration of HMs in the leaves of jojoba plants was high. This may be due to the jojoba plant's ability to concentrate HMs in its root system. Similar trends have been documented in previous studies where HM concentrations, such as lead, were found to increase in roots instead of leaves (Qasim et al., 2016). Plants have developed various mechanisms to cope with the harmful effects of HMs. One such mechanism is the limited movement of HMs from roots to leaves, which helps plants tolerate the toxic effects of HMs that mostly damage the pigments of the photosynthesis system. Different plant species have different ways of absorbing, translocating and accumulating HMs. The concentration of HMs in the soil also affects these mechanisms (Radojčić Redovniković et al., 2022). HM toxicity in plants leads to the formation of ROS, which in turn causes the peroxidation of essential cellular components (Awad et al., 2021). To combat these harmful effects, plants have evolved a strong defence system consisting of enzymatic and non-enzymatic antioxidants. Exposure to HMs has been reported to increase the percentage of IL% due to the generation of ROS (Tamás et al., 2006; Ofori et al., 2021). Polyunsaturated fatty acids, which are the main components of membrane lipids, are particularly sensitive to oxidation. The lipid peroxidation reaction is initiated by –OH radicals and results in the formation of highly reactive lipid peroxyl radicals that can react with another fatty acid (Prisacaru, 2016; Radwan et al., 2010). The chain reaction can be terminated when two radicals combine to form a non-radical compound, and MDA is usually the end product of the process (Martemucci et al., 2022). Therefore, an increase in MDA concentration is generally considered to be the main biomarker for the severity of oxidative stress (Sharma and Dietz, 2009). In biological systems, oxygen molecules can produce oxygen free radicals when they take electrons from other molecules. Additionally, several intracellular processes can convert oxygen to $O_2^{\bullet-}$ or H_2O_2. Hydroxyl radicals (–OH) are responsible for most oxidative damage in biological systems, even though these molecules are not particularly reactive (Sharma et al., 2012). Therefore, the balance between the production of free radicals and antioxidants (DPPH) activity is crucial for plants to adapt to unfavourable environmental conditions. An increase in free radicals can inhibit the formation of photosynthetic pigments like chlorophyll and carotenoids, as the chloroplast membrane becomes oxidised (Malar et al., 2014). HMs can affect physiochemical processes and generate ROS instantly in humans, animals and plants, leading to the stopping of some proteins and glutathione (Abdulaal et al., 2017). To evaluate antioxidant capacity, different techniques have been used to estimate the antioxidants' ability to quench ROS. The stable DPPH radical quenching is a widely used technique to assess antioxidant capacity (Shahidi and Zhong, 2015).

Under water stress, ion buildup and functional abnormalities observed during stoma opening and closure may also reduce the total chlorophyll content (Molazem et al., 2010; Nawaz et al., 2010). When salt stress occurs, genotypes that are susceptible to salt show a more pronounced reduction in chlorophyll concentration than cultivars that are more tolerant, like jojoba (Ali et al., 2013; Sharkey, 2020). When exposed to a salt stress of 100 mM NaCl, the chlorophyll-a level somewhat decreased, but at 200 mM NaCl, it increased. Conversely, the outcomes for carotenoids and chlorophyll-b showed significant drops at 200 mM NaCl salt stress but rises at 100 mM NaCl. These findings suggest that the genes linked to salt stress response to produce chlorophyll a took longer (day 30) to respond than the genes responsible for producing carotenoids or chlorophyll b (day 25) (Inoti et al., 2016). Water-stress-related genes were found in a recent study, which may help explain the mechanisms underlying jojoba's remarkable salt tolerance. The genes linked to water stress found in jojoba may aid in defining the plant's resistance to salt and offer a useful supply of genes that can help other species develop a tolerance to water stress (Al Dossary et al., 2024).

Increases in carotenoids and chlorophyll-(a, b) were evident in our results. This is because, in response to salt stress, the jojoba plant produces more transketolase as a defence mechanism to keep the amount of carotenoids and chlorophyll-(a, b) in the leaves of the plant constant. Because (S)-2-hydroxy-acid oxidase is activated during the conversion of glycolate to glyoxylate and is increased in jojoba under salt stress, it is expected that H_2O_2 will be generated extensively throughout this process. During the synthesis of CAT, the H_2O_2 level in jojoba is dramatically lowered as it is transformed to $O_2^{\bullet-}$. As a result, the latter greatly increased under salt stress in jojoba in favour of H_2O_2 reduction. Both in the physiological and molecular analyses, APX increased dramatically. The crosstalk between the 'ascorbate and alternate metabolism' and 'glyoxylate and dicarboxylate metabolism' pathways is complemented by the high expression of APX in jojoba, where tartronate semialdehyde functions as a transitional step. The most significant stable non-radical ROS is H_2O_2. (Ślesak et al., 2007; Sofo et al., 2015). Along with other ROS, the concentration of H_2O_2 in cells is a reliable indicator of the degree of oxidative stress. Therefore, the primary enzymatic H_2O_2 scavenging mechanism in plants, the balance of APX and CAT activity is essential for the suppression of hazardous H_2O_2 levels in a cell (Sofo et

al., 2015). Plants that collect ROS will have reduced light-harvesting efficiency and slowed down signal transduction and protein synthesis as a result of osmotic pressure fluctuations. ROS can be scavenged by APX and other peroxidases including CAT and SOD (Sofo et al., 2015). Molecular investigation of the gene encoding the enzyme that demonstrated an increase under salt stress supports the enhanced activity of the APX enzyme in jojoba under salt stress. Tartronate semialdehyde in the 'ascorbate and alternate metabolism' pathway produces APX. Research conducted by Hasanuzzaman et al. (2019) has shown that an increase in the formation of ROS leads to an increase in antioxidant capacity, which aids in the detoxification of H_2O_2. Previous studies (Bratovcic, 2020) have also found that the creation of antioxidants protects against the generation of free radicals, which act as superoxide generated in many cells under HM stress. The experiment demonstrated that as the amount of TSDW and RADW treatments with high concentrations of HMs increased, the production of antioxidant capacity (DPPH) gradually increased as well (Figure 8). The jojoba plants triggered their antioxidant capacity along with the accumulation of superoxide anion $O_2^{\bullet-}$ and H_2O_2, which was demonstrated through the parallel production of antioxidant capacity (DPPH) and H_2O_2 and $O_2^{\bullet-}$. However, the level of HMs plays a crucial role in either energising or inhibiting antioxidant activity (Emamverdian et al., 2023). Generally, HM toxicity is registered to raise the activity of antioxidant defence systems in plants grown in polluted soil (Emamverdian et al., 2023).

CONCLUSIONS

Climate change has a significant impact on the environment and living organisms, and it can cause water scarcity. One way to adapt to these changes is by reusing wastewater to irrigate plants that are capable of withstanding it. The jojoba plant, *S. chinensis* (Link) Schneider, was studied to determine its ability to tolerate wastewater rich in HMs and organic residues. The results of the study showed that the physical and chemical properties of the jojoba plant were only slightly affected by using different concentrations of RADW and TSDW (25%, 50%, 75% and 100%). The jojoba plant has a robust antioxidant defence system that can mitigate the harmful effects of HM-contaminated water. It can also reduce the transfer of HMs from the roots to the leaves, which preserves the photosynthetic pigments and the photosynthetic system from damage, maintains the cell wall and reduces the production of ROS such as H_2O_2, superoxide anion ($O_2^{\bullet-}$), MDA and IL%. Based on these findings, we recommend irrigating the jojoba plant with RADW and TSDW at a concentration of 100% and 75%, respectively. For future studies, it would be helpful to use some phytohormones external treatments to study their effect on water stress genes in the jojoba plant. The obtained information can also aid in the development of new economically important crop plants with improved salt tolerance through metabolic engineering.

ACKNOWLEDGEMENTS

The authors extend their appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-53). This research was supported by the Lab of the Horticulture Department, Damietta University, Egypt. And We would like to thank Eng, Ismail Mohammed manager of the Egyptian Gulf Company, Desert Land Reclamation for its contribution to providing jojoba plants.

FUNDING

This research was funded by Taif University, Saudi Arabia, Project No. (TU-DSPP-2024-53).

AUTHOR CONTRIBUTIONS

M.S.A – methodology, formal analysis, investigation, software, data curation, writing – original draft, writing – review & editing, validation, visualisation; M.S.M.A. – resources, data curation, editing, visualisation; A.F.El.S. – supervision, software, validation, formal analysis, visualisation; B.M.A – drafted the design chart, editing; A.M.F. – software, editing, validation; I.E.E. – chemical analysis; A.A.H and F.R.I. – software, editing, validation; EA.S. – software, editing, validation; H.G.A. – software, editing; S.F.M. – validation, editing; and E.-S.A.E.-B. – investigation, software, data curation, methodology, validation. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest that could have appeared to influence the work.

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Received: February 29, 2024; accepted: June 19, 2024