Moringa oleifera Lam. seed extract enhances tolerance to water stress by regulating photosynthesis and antioxidant defence mechanism in Lessertia frutescens L.

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Research Article

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Abstract

Drought is a predominant environmental stress that limits plant growth and yield. Biostimulants including moringa (*Moringa oleifera* Lam.) seed extract (MSE) can alleviate adverse plant responses triggered by drought stress. Nonetheless, there is limited information regarding the functions of MSE in promoting drought tolerance in plants. Consequently, the current study investigated the effect of MSE on the enhancement of drought tolerance in cancer bush (*Lessertia frutescens* L.) plants under deficit irrigation (DI). The 6% MSE foliar spray was applied to cancer bush plants subject to standard (80% of soil water holding capacity; SWHC) and deficit irrigation (DI) (60% of SWHC) in a terracotta pots experiment which was conducted twice sequentially in a tunnel. Plants that were not treated with MSE were used as control. The application of MSE effectively alleviated the adverse effect of water stress on cancer bush by improving plant growth and yield characteristics, photosynthesis attributes, soluble protein and proline contents. The MSE mitigated lipid oxidation (malondialdehyde; MDA) of drought affected plants and enhanced the antioxidant enzyme activities. These results demonstrated that MSE application effectively alleviated drought stress in cancer bush plants. Therefore, MSE is an economical and eco-friendly biostimulant for enhancing plant performances under water stress.

Introduction

Abiotic stresses including drought, salinity and extreme low or high temperatures significantly limit agricultural productivity by reducing crop quality and yield by more than 50% (Campobenedetto et al. 2021; Francesca et al. 2021; Tinte et al. 2022). Recently, droughts have led in an economic loss of approximately 30 billion US dollars globally (Gupta et al. 2020; Repke et al. 2022). Water stress can affect crop production by triggering changes in the plant's physiological, biochemical and molecular processes (Tinte et al. 2022; Sujata et al. 2023). Furthermore, drought adversely affects photosynthesis by damaging chloroplasts, restricting the electron transfer and generating cellular oxidative stress (Petropoulos et al. 2020). In addition, photosynthesis is also inhibited due to the stomatal closure (Banerjee et al. 2019; Antonucci et al. 2021), chloroplast degradation (Hamedeh et al. 2022) and decreased transpiration (Ahmad et al. 2021; Seleiman et al. 2022) initiated by drought conditions which ultimately leads to crop yield loss as well as plant death (Repke et al. 2022; El Boukhari et al. 2023).

Drought promotes overproduction of reactive oxygen species (ROS) primarily superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) in cell organelles (Pinciroli et al. 2018; Tinte et al. 2022), leading to the initiation of oxidative stress through the peroxidation of cellular membranes (Desoky et al. 2021a) and the deterioration of cell structures including proteins, nucleic acids, membrane lipids, photosynthetic pigments and activate programmed cell death (Desoky et al. 2021b). Plants adapt to drought through enzymatic and non-enzymatic antioxidant mechanisms that involve morphological, physiological and biochemical changes that eliminate ROS-induced oxidative stress (Babaei et al. 2021; Sujata et al. 2023).

During drought conditions, plants can accumulate osmoprotective molecules including proline and other amino acids, soluble sugars and essential minerals which function to decrease cellular osmotic potential, thus, contributing to preservation of water absorption and cell turgor (Annunziata et al. 2017; Antonucci et al. 2021). Another mechanism of plants to cope with drought comprise reduction of water loss through regulation of stomatal closure and production of small leaves, enhancing water uptake by promoting the root system growth and accumulating osmoprotective substances (Francesca et al. 2021; Amiri Forotagheet al. 2022). Furthermore, recovering from stress has also been regarded as a drought resistance response strategy for the plant, allowing vital metabolic functions such as transpiration and photosynthesis to resume (Desoky et al. 2021a, b; Sujata et al. 2023).

In addition to adaptation to water deficit conditions, activation of the antioxidant capacity is a vital mechanism to scavenge excessive ROS and reduce ROS-induced damages which involves antioxidant enzymes and non-enzymatic antioxidants including phenolic compounds, carotenoids, ascorbic acid and glutathione (Amir et al. 2021; Amiri Forotagheet al. 2022). Synthetic fertilizers which are expensive, toxic and cause environmental pollution have been effectively used worldwide to improve plant productivity and help plants cope with the effect of climate change including high temperatures, drought and salinity (Bibi et al. 2023; Piyasena et al. 2023). Although chemical fertilizers are an effective input to get higher crop productivity under water deficiency conditions, excessive application of synthetic fertilisers is associated with soil compaction and acidification, heavy metals pollution and changes in soil microbiome (Duddigan et al. 2023; Piyasena et al. 2023). The application of synthetic fertilisers over long turn alters the bacterial composition of soil, decreases soil pH and microbial metabolic activity, leading to the decrease of beneficial bacteria (Fincheira et al. 2021; Piyasena et al. 2023). In addition, the overuse of inorganic fertilisers causes emissions and water contamination and reduces crop yields over time (Seleiman et al. 2020; Duddigan et al. 2023). Therefore, the modern agricultural sector needs to explore more environmentally friendly techniques that promotes agricultural sustainability and plant yield.

The environmentally friendly alternative to enhance crop yield during drought stress is the application of plant growth regulators and stress alleviators such as biostimulants (Goñi et al. 2018; Tinte et al. 2022). Biostimulants are involved in various processes including membrane stability, stomatal conductance, osmoprotection, ROS scavenging, nutrient and water use efficiency and phytohormonal signalling (Ali et al. 2021; García-Sánchez et al. 2022) and activate stress responsive genes by the signal transduction and transcriptional regulations (Monteiro et al. 2022; Arif et al. 2023). Biostimulant application can alleviate the adverse effect of drought stress in plants by stabilizing the levels of the photosynthetic pigments, regulating the accumulation of osmoprotectants and conserving the cell wall lipid bilayer from oxidation by adjusting the transcriptional response of genes involved in source to sink carbohydrate metabolism and translocation, stomatal closure and cell homeostasis (Arif et al. 2023; Gupta et al. 2023).

Amongst natural biostimulants, moringa (*Moringa oleifera* Lam) leaf extracts (MLE) is an important biostimulator due to the presence of essential mineral nutrients, vitamins, antioxidants and phytohormones that function to facilitate plant growth and development (Zulfiqar et al. 2020; Arif et al. 2023). The presence of nutrients such as calcium and potassium in MLE may improve plant growth and development through enzyme activation, osmoregulation and photosynthesis (Rana et al. 2019; Zulfiqar et al. 2020). Antioxidants and phytohormones in MLE effectively decrease environmental stresses in plants including salinity, drought and heat stress by promoting the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) which aid in scavenging ROS and enhancing plant growth and yield attributes (Latif et al. 2016; Zulfiqar et al. 2020; Arif et al. 2023). Abd El-Mageed et al. (2017) and Maswada et al. (2018) stated that 3% MLE stimulated defence mechanism and enhanced tolerance in Squash (*Cucurbita pepo* L.) and maize (*Zea mays* L.) plants against abiotic stresses including drought and salinity and improved plant growth, yield and various physio-biochemical and anatomical traits. Hala et al. (2017) stated that 2, 4 and 6% MLE foliar spray enhanced pepper (*Capsicum annuum* L.) fruit size, total vitamin C, carbohydrate, potassium and calcium content. Similar, Ismail et al. (2021) showed that 2, 4 and 6% MLE foliar application improved fruit weight, firmness, total soluble solids, maturity index and anthocyanin content of strawberry (*Fragaria × ananassa* Duch.). These findings demonstrate that plant derived biostimulants such as MLE may be an effective technique to enhance plant drought tolerance. Although all plant parts of moringa such as roots, leaves, flowers, fruit, pods, and seeds have high nutrient values due to the presence of essential phytochemicals and phytohormones (Gopalakrishnan et al. 2016; Ismail et al. 2021), limited previous studies mainly investigated the biostimulant potential of moringa leaves, thus, little is known about the effect of moringa seeds on abiotic stress tolerance and growth of plants. Therefore, there is a need for future studies to investigate biostimulant potential of other plant parts of moringa on plant growth under abiotic stress conditions.

Cancer bush [*Lessertia frutescens* L. (syn. *Sutherlandia frutescens* L.)] is an important medicinal plant native to South Africa (Govender et al. 2023). The leaves of cancer bush comprise a diversity of bioactive compounds including pinitol, triterpenoid saponins, flavonoids and amino acids (Avula et al. 2010; Govender et al. 2023) to which several pharmacological activities such as analgesic, anti-inflammatory, hypoglycemic and anticonvulsant activities have been ascribed (Avula et al. 2010; Nsibanyoni et al. 2023). For over 200 years, cancer bush has been widely used in traditional African medicine to treat various diseases such as human immunodeficiency virus/acquired immunodeficiency syndrome, diabetes, cancer, tuberculosis, osteochondrosis, influenza, viral hepatitis, asthma and chronic bronchitis, rheumatoid arthritis, hot flashes and irritability in menopause (Hlongwane et al. 2023; Nosov et al. 2023). Commercial plantations of cancer bush were established in the late 1990s in South Africa, particularly at Sanniesh of in the North-West Province (Van Wyk 2011; Hlongwane et al. 2023). The first branded products were tablets made from powdered leaves of a selected chemotype that became known as the SU1 type (Hlongwane et al. 2023; Govender et al. 2023), thus, cancer bush is a valuable medicinal plant both in the modern and traditional medicine. However, the growth of cancer bush is mostly limited by drought stress which extensively inhibits plant growth and yield, resulting to economic loses. Although the role of MLE biostimulant in enhancing the growth and yield of plants have been documented, little is known regarding the effect of moringa seed extracts (MSE) on water stress tolerance and productivity of medicinal plants. Therefore, this study investigated the effect of MSE on drought stress tolerance and productivity of cancer bush plants.

Materials and methods

Plant growth conditions

This experiment was conducted twice sequentially under an experimental setup as described by Buthelezi et al. (2022) at Sefako Makgatho Health Sciences University (SMU), South Africa (25°37'8" S, 28°1'22" E) during winter–summer seasons of 2021. Plants were grown in the tunnel that was 4 m high, 8 m long and 4 m wide covered with a green coloured net with 40% shading (ChromatiNet™, Carports and Pergola Builders (Pty) Ltd., Pretoria, South Africa). During the experiment, the average temperature and relative humidity in the tunnel were 21.08 C and 43.01%, correspondingly.

The seedlings of cancer bush were purchased from the Plantland Akasia Garden Centre Nursery, Pretoria, South Africa (25°39'41.2" S, 28°04'34.5" E). The seedlings were then transported and kept in the tunnel at SMU where they were watered three times a day with an average of 200 mL tap water per plant for a week. Afterwards, healthy seedlings were transplanted into plastic terracotta pots (40 cm in diameter and 50 cm depth), containing 5 kg of culterra potting soil per pot.

The culterra potting soil used in the current study was obtained from Builders Express, Pretoria, South Africa (25° 40' 28.49" S, 28° 6' 31.22" E). This product was composed from raw organic material such as general 2:3:2 (22), lawn 8:1:5 (25), LAN (28%), ammonium sulfate (21%), vita flora 5:1:5 (33) SRN and vital flora 3:1:5 (26) SRN per 30 kg of soil (Buthelezi et al. 2023).

Irrigation water applied

Soil water-holding capacity (SWHC) was evaluated according to Taha et al. (2020), with slight modifications using Eq. 1 as follows:

$$SWHC\,(\%) = totalporosity\,(\%) - airspace\,(\%)\,(1)$$

Two irrigation treatments; 80% which was calculated as ideal irrigation water for optimum growth of cancer bush plants grown in plastic terracotta pots containing 5 kg of culterra potting soil) and 60% of SWHC was used during the experiment after transplanting. All pots were well irrigated with 200 mL of tap water before and after transplanting. The well irrigated treatment (80% of SWHC) was used as a control whereas 60% of SWHC) was regarded as a deficit irrigation (DI) treatment. In our preliminary study (data not shown), water deficiency in 40% of SWHC significantly reduced growth and yield of cancer bush even when treated with MSE. The DI levels were applied to cancer bush plants as drought stress three times a day. Culterra potting soil moisture content per terracotta pots was observed daily (HH2 moisture meter Version 4.0, Delta-T Devices Ltd., UK.) and maintained through water application where applicable.

Moringa seed extract (MSE) preparation and treatments

Moringa seeds used in this experiment was the remainder from our previous study (Buthelezi et al. 2023) which were harvested in bulk from commercial orchards of Afrinest Moringa Farm in Tzaneen, Limpopo, South Africa (23°49'15.3" S, 30°10'08.7" E). The harvested seeds were then ground into fine powder using a blender (Mellerware Fusion 1L 300W, Makro, Pretoria, South Africa) and extracted according to El-Sappah et al. (2023), with minor modifications. A 100 g of moringa seed powder was mixed with 1 L 80% aqueous ethanol and vortexed (vortex mixer, Oxford LP Benchmate Digital Vortex Mixer VM-D, Selectech Lab Equipment Supplier, Pretoria, South Africa) for 1 min. Afterwards, the mixture was kept at ambient conditions overnight. Then, the solution was centrifuged (laboratory centrifuge-TD4C, Labtex Co., Ltd., Bangladesh, China) at 8000 × *g* for 20 min and filtered twice using Whatman® no. 1 filter paper and then with non-absorbent cotton. Consequently, the supernatant was mixed with distilled water (v/v) to attain the desired extract at 6% MSE to use as a foliar spray. In our preliminary study (data not shown), 6 and 8% MSE successfully enhanced plant growth and development of cancer bush with no and little significant differences compared to 2 and 4% MSE. Freshly prepared extract was then used within 24 h or kept in the refrigerator at -20 ˚C for further use. Untreated plants which were considered as control were sprayed with tap water at the same time of MSE treatment.

Experimental design and plant management

The two experiments were each conducted on 50 individual plants which were irrigated with 200 mL tap water three times a day for two weeks after transplanting. Thereafter, using a randomised complete block design, plant pots were set up with an intra-row pot of 40 cm and inter-row spacing of 50 cm (Buthelezi et al. 2023). The fifty pots of each one of the two irrigation groups (DI-stressed plants; 60% of SWHC and unstressed plants; 80% of SWHC) were divided into two subgroups for MSE foliar application. The treatments of this experiment were irrigation with 80% of SWHC + foliar spray with distilled water (control), irrigation with 80% of SWHC + foliar spray with MSE application, irrigation with 60% of SWHC + foliar spray with distilled water (control) and irrigation with 60% of SWHC + foliar spray with MSE. Overall, each treatment had 25 pot plants, making a total of 100 pots plants of the entire experiment. The MSE foliar pray was applied to cancer bush plants at 7 days intervals during the 10 weeks of the experiment.

Measurements of plant growth, ornamental and root morphology characteristics

At Harvest (10 weeks after transplanting), the growing medium (culterra potting soil) was carefully washed from the roots and the plants were divided into shoots, leaves, flowers and roots. Five plants per treatment were used to determine shoots and roots fresh weight (FW) using a precision balance (ME3002T, Labotec (PTY) Ltd., Johannesburg, South Africa). Afterwards, these were oven dried (DON-H, Bioevopeak, Co., Ltd., Shandong, China) at 70 ˚C until a constant weight was attained (dry weight; DW) (Buthelezi et al. 2022). The number of inflorescences was counted. Fruit yield attributes such as fruit number, individual fruit mass and overall fruit yield per plant were also recorded. Root scanning was performed following a method of Ertani et al. (2018), with minor adjustments. Fresh root systems were gently washed with tap water after harvest, placed on a transparent tray and scanned before the sampling process with a root scanner (Epson Expression 10000XL 1.0 system, Regent Instruments Inc., Québec, Canada) to measure total root length, average diameter, volume, tips, forks, crossings, projected and surface area. Roots of three plants were scanned per replicate and treatment.

Photosynthetic parameters quantification

Leaf gas exchanges based on the CO_2 net assimilation rate (A), stomatal conductance (Gs), transpiratory rate (E) and intercellular CO_2 concentration (Ci) were performed on fully expanded leaves using an infrared gas analyzer (IRGA; portable model LI-6400xt, Thermo Fisher Scientific Inc., Johannesburg, South Africa). Measurements were taken between 09:00 and 11:30 in the morning with constant photosynthetically active radiation (PAR) (1000 μmol photons m^{-2} s^{-1}), atmospheric CO_2 concentration, room temperature and humidity (Kałużewicz et al. 2017; Agliassaet al. 2021). Water use efficiency (WUE) and carboxylation efficiency (CE) were calculated based on the A/E and A/Ci ratio, respectively (Agliassaet al. 2021).

Determination of soluble protein

The total soluble protein concentration was determined according to Contartese et al. (2015) using the Bradford assay (1976), with bovine serum albumin as a standard. Plant extract (1 mL supernatant) was added to 0.1 mL water and 2 mL Bradford reagent in a test tube. The absorbance was read at 595 nm using a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan).

Determination of proline

A 0.05 g ground leaf tissue was homogenized in 0.5 mL of ethanol (v/v) and incubated overnight at 4 ˚C in the dark. The homogenates were centrifuged (FC5706 230V, United Scientific (Pty) Ltd., Johannesburg, South Africa) at 20,000 × *g* for 15 min at 4 ˚C. Samples of 100 µL of the extract were added to 200 µL of acidic ninhydrin solution and the mixture was incubated at 100 ˚C for 1 h. After cooling at ambient temperature, the absorbance was measured at 520 nm using a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan). After preparing a calibration curve standard curve with L-proline (Sigma-Aldrich), proline content was expressed as mg g^{-1} DW (Contartese et al. 2015).

Determination of lipid peroxidation

Lipid peroxidation was measured by the quantification of malondialdehyde (MDA) in leave tissues. The MDA concentration was analyzed by the reaction with thiobarbituric acid (Jacomassi et al. 2022). Briefly, the MDA was extracted with 10% trichloroacetic acid and then the extract was mixed with 0.6% thiobarbituric acid and centrifuged at 15,000 × *g* at 4 ˚C for 25 min. Afterwards, 0.5 mL aliquots of the supernatants were added to 1.5 mL of 0.5% thiobarbituric acid (TBA) solution in 20% TCA and incubated at 90 ˚C in a shacking water bath (Monochrome LCD, Thermo Fisher Scientific, Johannesburg, South Africa) for 30 min and the reaction was stopped in an ice bath (17763, Sigma-Aldrich® Solutions, Johannesburg, South Africa) for 15 min. Thereafter, the samples were centrifuged at 10,000 × *g* for 5 min and the absorbance was read at 532 nm and 600 nm. The MDA concentration was measured by subtracting the non-specific absorption 600 nm from the absorption at 532 nm. The value for non-specific absorption at 600 nm was subtracted using an absorbance and absorptivity coefficient of 155 mM^{-1} cm^{-1}. The results were expressed in MDA nmol g^{-1} FW.

Determination of antioxidant enzyme activities

To measure the activities of antioxidant enzymes, the total proteins were extracted using a method of Rahimi et al. (2022), with some alterations. Briefly, 0.3 g samples of leaf tissue were macerated in liquid nitrogen, homogenized with 0.1 M potassium phosphate buffer (pH 7.8), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM, phenylmethylsulfonic fluoride (PMSF) and 1% (w/v) polyvinylpyrrolidone (PVPP) for extraction of the enzymes superoxide dismutase (SOD – EC 1.15.1.1), catalase (CAT - EC 1.11.1.6), peroxidase (POX - EC 1.11.1) and ascorbate peroxidase (APX − 1.11.1.11). The mixture was centrifuged for 20 min at 12,000 × g at 4 ˚C and the supernatant used as crude enzyme extract.

Superoxide dismutase (SOD – EC 1.15.1.1) activity

Superoxide dismutase (SOD) activity was evaluated using the method outlined by Repke et al. (2022) and Rahimi et al. (2022) with slight modifications. Briefly, SOD activity was determined in a reaction medium consisting of 50 mM sodium phosphate buffer (pH 7.8) containing 13 mM methionine, 75 µM p-nitro tetrazolium (NBT) blue, 0.1 mM EDTA and 2 µM riboflavin together with the enzyme extract. The absorbance was detected at 560 nm (Shimadzu, UV-2700, Kyoto, Japan) after 15 min of light exposure (4000 lux). One unit of total SOD activity was calculated as the amount of protein per milligram causing 50% inhibition of NBT reduction. Enzymatic activity results were expressed as U mg^{-1} proteins.

Catalase (CAT- EC 1.11.1.6) activity

Catalase (CAT) activity was determined according to the decomposition of hydrogen peroxide (H_2O_2) using a method of Rahimi et al. (2022), with some alterations. Briefly, an aliquot of 50 µL of crude extract was added to 950 µL of reaction medium containing 50 mM sodium phosphate buffer (pH 7.0) and 12.5 mM H_2O_2. The absorbance was obtained at 240 nm using a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan) after 1 min. CAT activity was calculated as µmol of decomposed H_2O_2 per minute. Enzymatic activity results were expressed as nmol H_2O_2 min^{-1} mg^{-1} proteins.

Peroxidase (POX - EC 1.11.1) activity

Peroxidase (POX) activity was measured in aliquots of crude extract added to the reaction medium consisting of 25 mM potassium phosphate buffer (pH 6.8), 20 mM pyrogallol and 20 mM H_2O_2. The production of purpurogallin was measured at 420 nm using a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan). Enzyme activity was calculated using the absorbance and molar extinction coefficient of 2.47 mM^{-1} cm^{-1} (Panda et al. 2003; Akensous et al. 2022) and expressed as µmol of purpurogaline min^{-1} mg^{-1} protein.

Ascorbate Peroxidase (APX – 1.11.1.11) activity

Ascorbate peroxidase (APX) activity was determined according to Akensous et al. (2022), with slight modifications. An aliquot of 100 µL of crude extract was added to 900 µL of reaction medium, consisting of 0.05 M sodium phosphate buffer (pH 7.0), 0.8 mM ascorbic acid and 1.0 mM H_2O_2. Enzymatic activity was measured at 290 nm using a spectrophotometer (Shimadzu, UV-2700, Kyoto, Japan), considering the molar extinction coefficient of 2.8 Mm^{-1} cm^{-1}. Results were expressed as µmol of ascorbic acid min^{-1} mg^{-1} protein.

Statistical analysis

The collected data were subjected to analysis of variance (ANOVA) using GenStat statistical software GenStat®, 18.1 edition, VSN International, UK. Means were compared using Duncan's Multiple Range Test ($p < 0.001$) and standard error ($p < 0.001$).

Results

Plant growth and yield characteristics

The foliar application of MSE under ideal irrigation (80% of SWHC) significantly ($p < 0.001$) improved all measured growth and yield parameters of cancer bush plants compared to corresponding control (Table 1). Deficit irrigation (DI; 60% of SWHC) significantly ($p < 0.001$) decreased all evaluated plant growth and yield characteristics compared to normal irrigation (80% of SWHC). However, the application of MSE effectively ($p < 0.001$) increased all assessed growth and yield characteristics of DI-stressed plants compared to corresponding controls. As shown in Table 1, MSE significantly ($p < 0.001$) increased shoot dry and fresh weight, root fresh and dry weight, number of inflorescences per plant, number of fruit per plant, individual fruit mass per plant and overall yield per plant of water stressed plants by 50.04, 60.75, 30.29, 74.22, 36.22, 23.44, 36.98 and 19.70%, respectively in comparison to corresponding controls. In addition, Fig. 1 shows that roots parameters such as total length, diameter, volume, tips, crossings and forks of DI-stressed plants treated with MSE were increased by 49.45, 37.27, 35.81, 43.19, 41.47 and 72.76%, respectively compared to corresponding controls. The positive effect of MSE treatment was more evident under DI than its application under normal irrigation and yielded minor to no significant differences between all evaluated traits of ideal-irrigated plants and those of DI-stressed plants treated with MSE.

Table 1
The effect of foliar moringa seed extracts (MSE) application on morphological growth and yield attributes of cancer bush plants under drought stress

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Number of inflorescences per plant	Number of fruit per plant	Individual fruit mass per plant (g)	overall yield per plant (g)
80% of SWHC	20.06 ± 1.17 [b]	13.04 ± 0.56 [b]	14.67 ± 0.88 [b]	38.33 ± 0.88 [b]	24.01 ± 1.15 [a]	721.01 ± 45.32 [ab]
80% of SWHC + MSE	30.09 ± 1.08 [c]	17.16 ± 0.42 [c]	18.33 ± 0.33 [c]	45.33 ± 2.60 [b]	35.01 ± 0.52 [b]	850.98 ± 48.82 [b]
60% of SWHC	12.71 ± 1.02 [a]	8.05 ± 0.55 [a]	11.33 ± 0.67 [a]	29.67 ± 2.19 [a]	20.01 ± 5.29 [a]	600.35 ± 60.20 [a]
60% of SWHC + MSE	19.07 ± 0.58 [b]	12.94 ± 0.87 [b]	15.33 ± 1.20 [b]	37.00 ± 2.08 [ab]	27.41 ± 0.71 [ab]	715.21 ± 56.05 [ab]

SWHC, soil water holding capacity; MSE, moringa seed extract. Data presented as mean ± SE. Different letters among treatments for each attribute are significantly different ($p < 0.001$) according to Duncan’s multiple range test.

Photosynthetic parameters

Well-watered (80% of SWHC) cancer bush plants treated with MSE had effectively ($p < 0.001$) improved A, Gs, E, Ci, CE and WUE in comparison to control (Fig. 2). Nevertheless, DI (60% of SWHC) treatment significantly decreased all the abovementioned parameters compared to well-irrigated (80% of SWHC). However, MSE treatment significantly ($p < 0.001$) enhanced all aforementioned parameters of DI-stressed plants compared to the corresponding controls. The A, Gs, E, Ci, CE and WUE were increased by 56.81, 92.31, 31.96, 30.02, 99.59 and 84.62%, respectively compared with corresponding controls. Overall, the application of MSE was effective in improving all the assessed photosynthetic parameters of cancer bush plants which was more demonstrated under DI than under normal irrigation. In addition, there were no significant differences between all assessed parameters of well-irrigated plants and those of DI-stressed plants treated with MSE.

Soluble protein and proline concentration

The MSE treatment significantly ($p < 0.001$) induced the accumulation of soluble protein and proline contents which was increased by 7.45 and 49.38% in well-watered (80% SWHC) cancer bush plants, respectively compared to corresponding controls (Fig. 3). Subjecting plant to DI (60% of SWHC) significantly reduced the above-mentioned parameters in comparison to well-irrigated (80% of SWHC) plants. However, MSE effectively ($p < 0.001$) increased soluble protein (31.88%) and proline (66.67%) of DI-stressed in comparison to corresponding controls. The effectiveness of MSE was more apparent under

DI than under ideal irrigation. This is further supported by no significant differences between the contents of soluble protein and proline of well-irrigated plants and that of DI-stressed plants treated with MSE.

Lipid peroxidation

As shown in Fig. 4, the application of MSE under ideal irrigation (80% of SWHC) significantly ($p < 0.001$) reduced MDA concentration of cancer bush plants compared to control. The MDA content was reduced by 48.75% compared to control. In addition, MSE treatment under DI (60% SWHC) further reduced the MDA content of cancer bush plants by 62.28% compared to control. Overall, plants that were well-irrigated (80% of SWHC) as well as those who were DI-stressed (60% of SWHC) had high MDA content whereas MSE significantly ($p < 0.001$) reduced MDA content of plants that were well-irrigated and those that were DI-stressed. The positive effect of MSE foliar application was more noticeable under DI than under ideal irrigation, with no or slight significant differences between well-irrigated plants and DI-stressed plants treated with MSE.

Antioxidants enzyme activities

Higher activities of SOD, CAT, POX and APX were observed in well-irrigated (80% of SWHC) MSE-treated plants, which were significantly ($p < 0.001$) increased by 14.53, 36.79, 95.68 and 29.58% in comparison to respective controls (Fig. 5). However, DI (60% of SWHC) significantly ($p < 0.001$) reduced all the above-mentioned antioxidant enzyme activities compared to well-irrigated (80% of SWHC) plants. The foliar application of MSE had a positive effect on SOD, CAT, POX and APX activities of DI-stressed plants which were significantly ($p < 0.001$) increased by 16.54, 21.33, 73.13 and 65.85%, respectively compared to respective controls. The positive effect of MSE treatment was evident under DI than under ideal irrigation, with no minor statistical differences between well-irrigated plants and DI-stressed plants treated with MSE.

Discussion

Drought is one of the main abiotic factors reducing plant production and yield worldwide (Desoky et al. 2021a, b). In the present study, foliar application of MSE effectively ($p < 0.001$) alleviated cancer bush growth inhibition caused by DI through enhancing growth and yield traits such as shoot and root fresh and dry weight, number of inflorescences, number of fruit, individual fruit mass per plant and overall yield per plant (Table 1) and root characteristics attributes including root length, diameter, volume, tips, crossings and forks (Fig. 1). These positive effects of MSE on plant tolerant to water stress could be due to the presence of phytohormones which are important endogenic modulators that enhance numerous physiological processes that promotes plant growth and development and enhanced plant resistance against drought stress (Zulfiqar et al. 2020; Arif et al. 2023). Similarly, Adb El-Mageed et al. (2017) reported that 3% MLE effectively improved plant growth and yield of squash plants grown under water stress. Hanafy (2017) stated that 30% MLE significantly increase plant growth of soybean (*Glycine max* L.) plants under drought stress.

Photosynthesis is one of the processes that are mostly affected by water stress (Tinte et al. 2022). The adaptation of plants to water stress can influence the enhancement of productivity and biological activities including photosynthesis and consequently, the growth and development of plants (Gupta et al. 2023). In our study, DI negatively affected photosynthetic parameters whereas MSE application effectively ($p < 0.001$) alleviated water stress effect above mentioned process (Fig. 2). Under DI stress, MSE increased the CO_2 net assimilation rate compared to non-treated plants (Fig. 2A), demonstrating the improved photosynthetic capacity after MSE treatment. This could be attributed to the maintenance of partially open stomata (Zulfiqar et al. 2020). Leaf stomata are the primary pores for regulating CO_2 and water exchange (Lawson and Matthews 2020), in which they typically open during the day to favour CO_2 diffusion when light is available for photosynthesis and close at night to restrict transpiration thus, reducing water loss (Kaiser et al. 2020). Under water stress, the reduced photosynthesis is predominantly prompted by stomatal closure which constraints CO_2 acquisition during the Calvin cycle (Claassens et al. 2020). In this study, the evident reduction of stomatal conductance in DI-stressed plants compared to MSE treated plants under DI further (Fig. 2B) confirmed the vital roles of CO_2 diffusion in enhancing plant photosynthesis (Lawson and Matthews 2020). Moreover, the increase of stomatal conductance in MSE treated plants under DI resulted in keeping stomata open, which allowed the continuous addition of CO_2 in the Calvin cycle, promoting a higher flow of Ca^{2+} and K^+ at stomatal level, crucial ions to protect ionic and osmotic stress, in addition to the enhancement of water rate and photosynthesis through photosynthetic efficiency (Van Oosten et al. 2017). Also, Li et al. (2021) stated that the higher stomatal conductance and improved photosynthesis are dependent on the low leaf temperature and faster water loss, which are also associated with the larger stomatal apertures under water stress conditions. Higher Ci and lower CE values were observed in DI stressed plants whereas the application of MSE on DI-stressed plants significantly ($p < 0.001$) reduced CI and increased CE (Fig. 2D,E). Zhang et al. (2016) stated that lower CE values linked with higher Ci reflect biochemical alterations in the photosynthesis machinery of plants and lower activity of the Ribulose bisphosphate carboxylase/oxygenase (Rubisco) enzyme. Rubisco is a vital enzyme in photosynthesis catalyzing CO_2 fixation (Kurepa and Smalle 2019). Therefore, MSE was effective in alleviating the negative impact of drought stress on photosystem by maintaining the stomatal control and gas exchange of cancer bush plants (Kurepa and Smalle 2019; Li et a. 2021). The DI significantly reduced transpiration rate and water use efficiency (Fig. 2C,F) in cancer bush plant which could be due to the fact that during water stress, stomatal closure results in reduced leaf conductance, photosynthesis and transpiration (Agliassa et al. 2021). However, the application of MSE effectively enhanced transpiration rate and water use efficiency (Fig. 2C,F) of DI-stressed plants by improving stomatal conductance (Fig. 2B). Also, due to the sensitive response of leaf conductance to decreased leaf water potential, the more conservative use of water leads to higher WUE in water-deficient plants, which may possibly be a mechanism for enhancing resource utilization (Mashilo et al. 2017; Wang et al. 2020). Furthermore, these observations may be due to the modified levels of phytohormones including gibberellins and abscisic acid in guard cells which enhance stomatal conductance to improve CO_2 diffusion and WUE (Fig. 2B,F) under water stress (Buthelezi et al. 2023). Our results are in agreement with Maswada et al. (2018) who demonstrated that MLE (1:30 w/v) improved stomatal condense and water use efficiency of maize (*Zea mays* L.) under water stress.

Figure 3 shows that DI-stressed plants had significantly ($p < 0.001$) reduced soluble protein and proline contents whereas the application of MSE effectively increased the contents of soluble protein and proline contents which might have enhanced plant tolerance to drought stress (Mahmood et al. 2017; Sujata et al. 2023). Proline, an amino acid, plays an important role in plants subjected to several environmental stress conditions (AlKahtani et al. 2021). In addition to functioning as an effective osmolyte, proline preserves turgidity under stress (Wang et al. 2020), improves the activities of antioxidant enzymes and facilitates the partial opening of the stomata, enabling photosynthesis to continue under water-deficit conditions (Meena et al. 2019; AlKahtani et al. 2021). Moreover, proteins play a central role in enhancing plant environmental stress including serving as the enzymes in metabolism pathway, the regulators and components of transcription and translation machinery and the components for plasma membrane, cell cytoskeleton, and intracellular compartments (Rasheed et al. 2020).

The MDA content is a commonly used parameter as a measure of lipid peroxidation in plant tissue that increases under oxidative stress (Meena et al. 2019). Low MDA levels were observed in plants that were treated with MSE while DI-stressed plants had higher content of MDA (Fig. 4), indicating severe ROS production and oxidative damage (Seymen 2021). However, the application of MSE significantly mitigated the oxidative damage in especially DI-stressed plants by favouring the activity of antioxidant enzymes, hence inhibiting accumulation of ROS (Sujata et al. 2023). The improvement of photosynthetic rates also contributed to the decrease of cell damage by consuming free electrons that would form free radicals (Meena et al. 2019). Moreover, oxidative damage is closely associated with the antioxidant defence mechanism, and plants with high stress tolerance exhibits higher activities of antioxidant enzymes (Rasheed et al. 2020; Gupta et al. 2023).

The enzymatic antioxidants play a crucial role in detoxifying ROS (Afzal et al. 2020; Denaxa et al. 2020). In the present study, the application of MSE treatment effectively ($p < 0.001$) increased the activities of SOD, CAT, POX and APX in cancer bush plants under drought stress (Fig. 5), indicating that the use of MSE was successful in reducing oxidative damage by promoting the activity of antioxidant enzymes (Denaxa et al. 2020; Sujata et al. 2023). SOD acts in the first step of defence against the accumulation of ROS through $O^{2•-}$ dismutation in O_2 and H_2O_2 (Hanafy 2017; Desoky et al. 2021b). Other antioxidant enzymes such as CAT, POX, APX eliminate particularly H_2O_2 molecules (Khanet al. 2022; Gupta et al. 2023). The findings of this study correlated with Khan et al. (2021) and Ibrahim et al. (2023) who demonstrated that 2.5 and 3.0% MLE significantly enhanced the activities of SOD, CAT, POX and APX in rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) plants subjected to drought stress.

Conclusions

The present study demonstrated that drought stress significantly decreased growth and yield of cancer bush plants whereas the effectiveness of MSE treatment was more evident in DI-stress plants. MSE improved growth, yield and photosynthetic parameters of DI-stressed plants. Considering that photosynthesis is the major source of oxidative stress in plants, its maintenance reduces ROS production and decreasing lipid peroxidation in water stress plants as observed in this study. Also, the application of MSE effectively increased soluble proteins, proline and significantly decreased drought-caused oxidative toxicity or damage through the increased activities of antioxidant enzymes in DI-stressed plants. This study contributes to the present scientific background on the mode of action of especially plant derived biostimulants and provide a way forward for future studies to further explore diverse sources of biostimulants and its application on plants under various environmental stress conditions. It is recommended that MSE may be used as a cost-effective and environmentally friendly alternative for alleviating water stress and enhancing plant performance under drought stress. Future studies should explore various sources of plant-derived biostimulants and their role in improving plant productivity under different environmental stress conditions.

Declarations

Author contribution statement NMDB: Conceptualization, methodology, software, validation, formal analysis, investigation, re-sources, data curation, writing—original draft preparation, writing—review and editing, and visualization, project administration, funding acquisition. LLM: writing—review and editing. S.S.G: writing—review and editing.

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Data Availability All data are available in the manuscript file.

Conflicts of interest The authors declare no conflict of interest.

Consent for publication All authors have read and agreed to the published version of the manuscript.

Ethics approval No ethical permissions were required for this work, which involved no experimentation involving animals or human samples.

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Figures

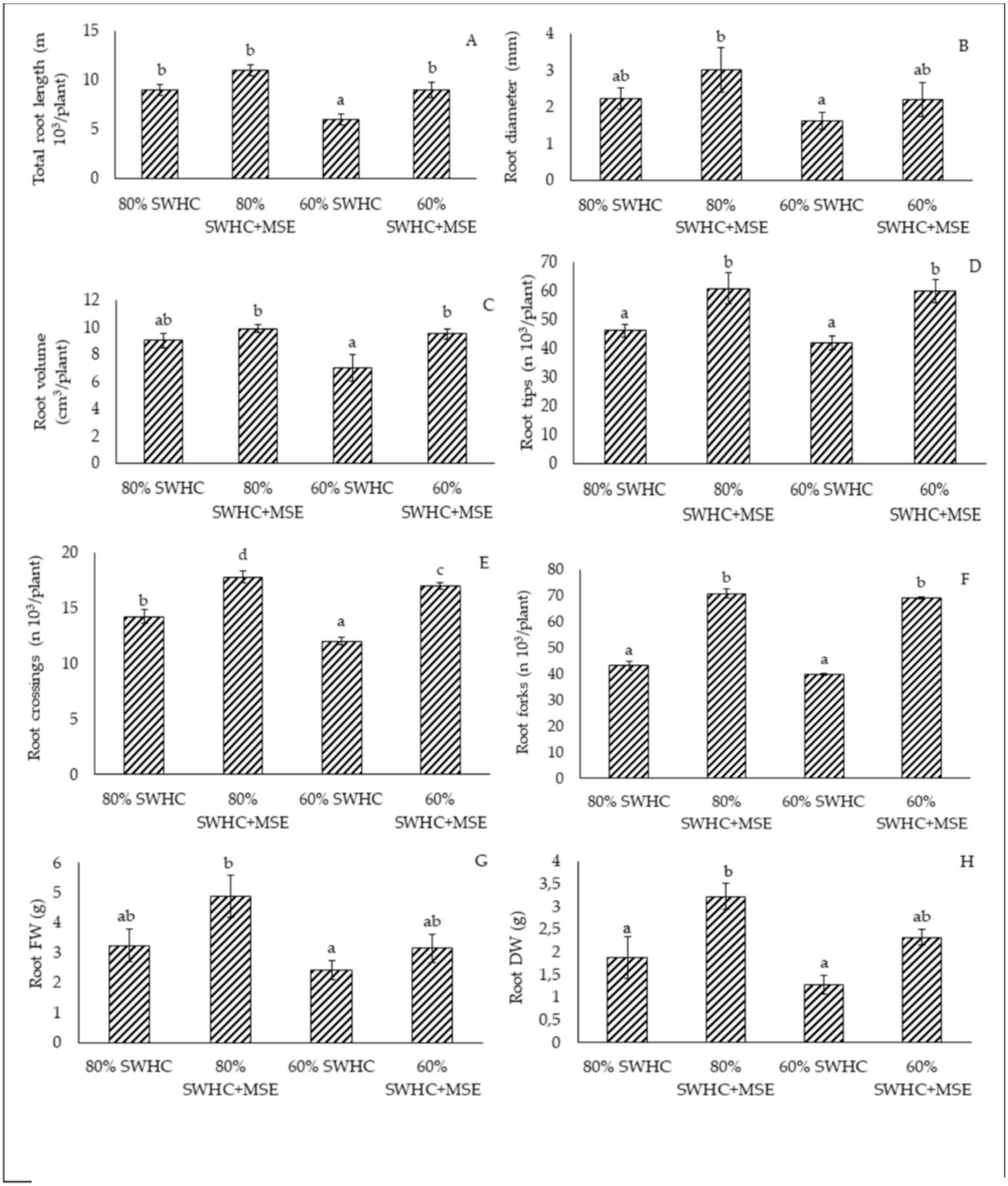

Figure 1

The effect of foliar moringa seed extracts (MSE) application on roots attributes; total root length (A), root diameter (B), root volume (C), root tips (D), root crossings (E), root forks (F), root fresh weight (G) and root dry weight (H) of cancer bush plants under drought stress. SWHC, soil water holding capacity. Vertical bars represent standard error (SE) of the mean value (n = 3). Different lowercase letters indicate significant differences ($p < 0.001$).

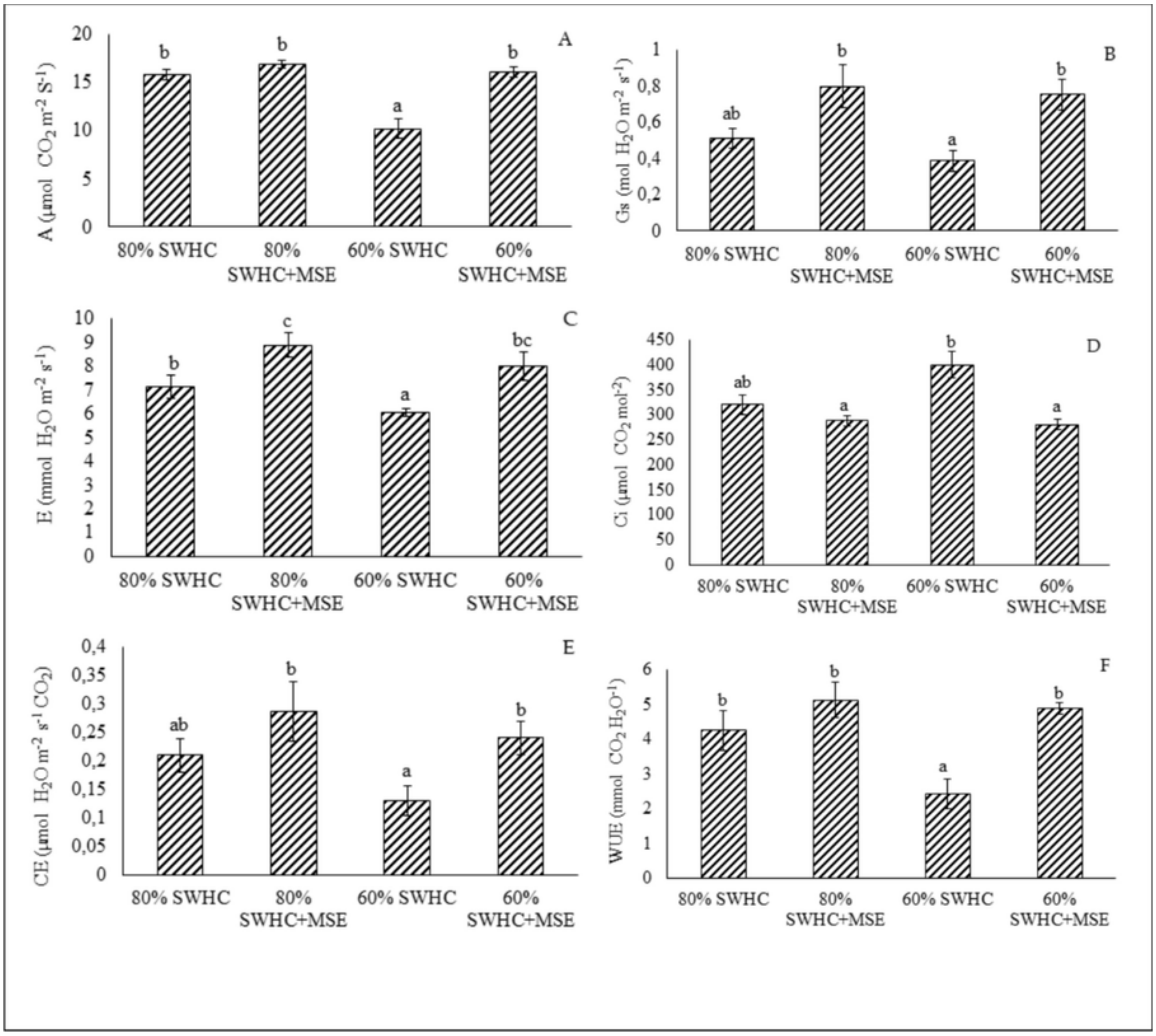

Figure 2

The effect of foliar moringa seed extracts (MSE) application on photosynthetic parameters; A (A), Gs (B), E (C), Ci (D), CE (E) and WUE (F) of cancer bush plants under drought stress. SWHC, soil water holding capacity; A, CO2 net assimilation rate; Gs, stomatal conductance; E, transpiratory rate, Ci, intercellular CO2 concentration, CE, carboxylation efficiency and WUE (Water use efficiency). Vertical bars represent standard error (SE) of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($p < 0.001$).

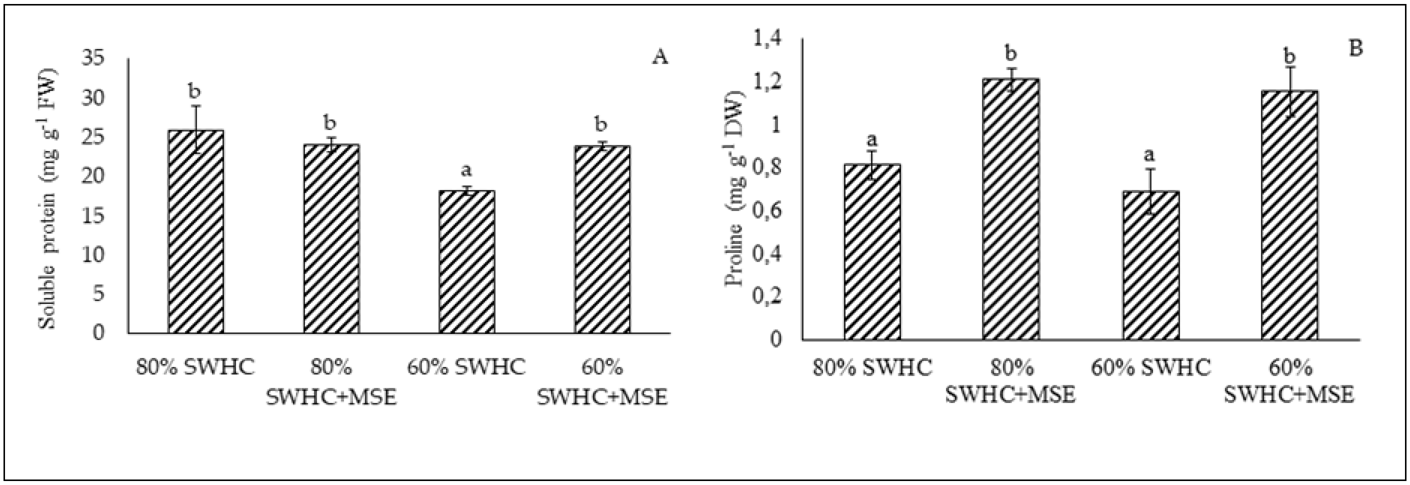

Figure 3

The effect of foliar moringa seed extracts (MSE) application on soluble proteins and proline of cancer bush plants under drought stress. SWHC, soil water holding capacity. Vertical bars represent standard error (SE) of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($p < 0.001$).

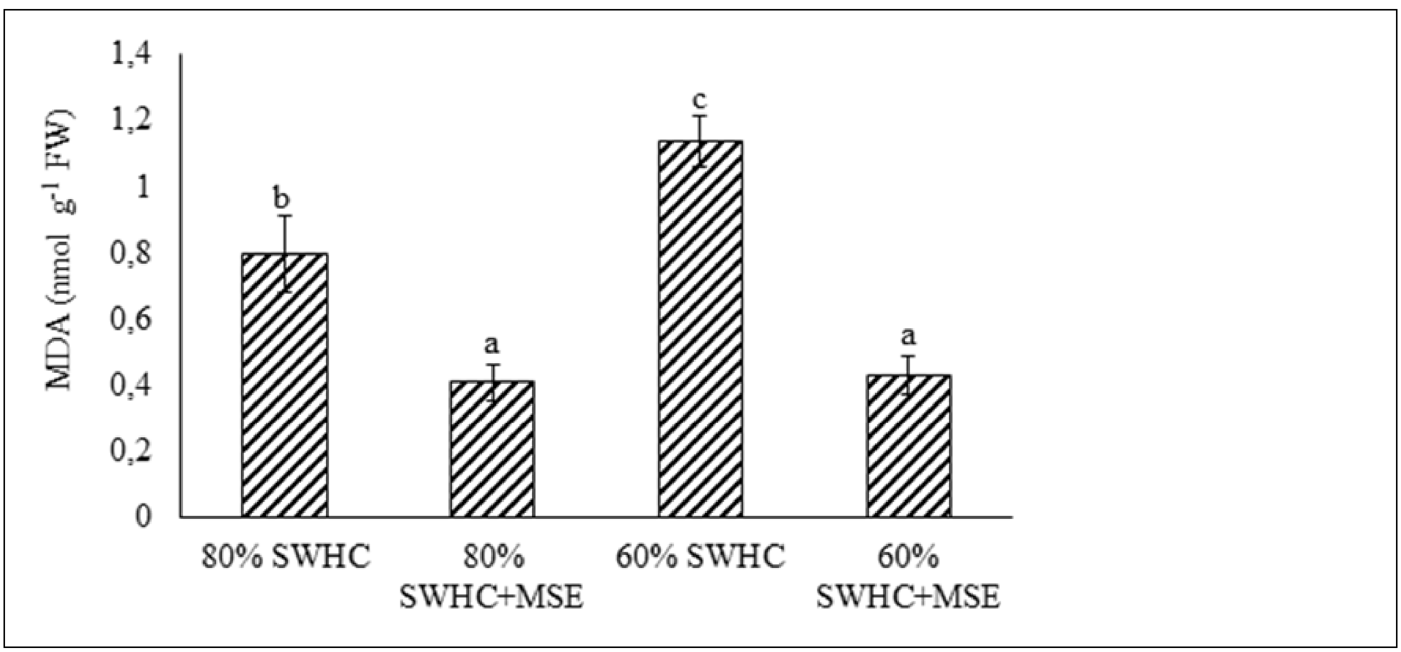

Figure 4

The effect of foliar moringa seed extracts (MSE) application on malondialdehyde (MDA) of cancer bush plants under drought stress. SWHC, soil water holding capacity. Vertical bars represent standard error (SE) of the mean value ($n = 3$). Different lowercase letters indicate significant differences ($p < 0.001$).

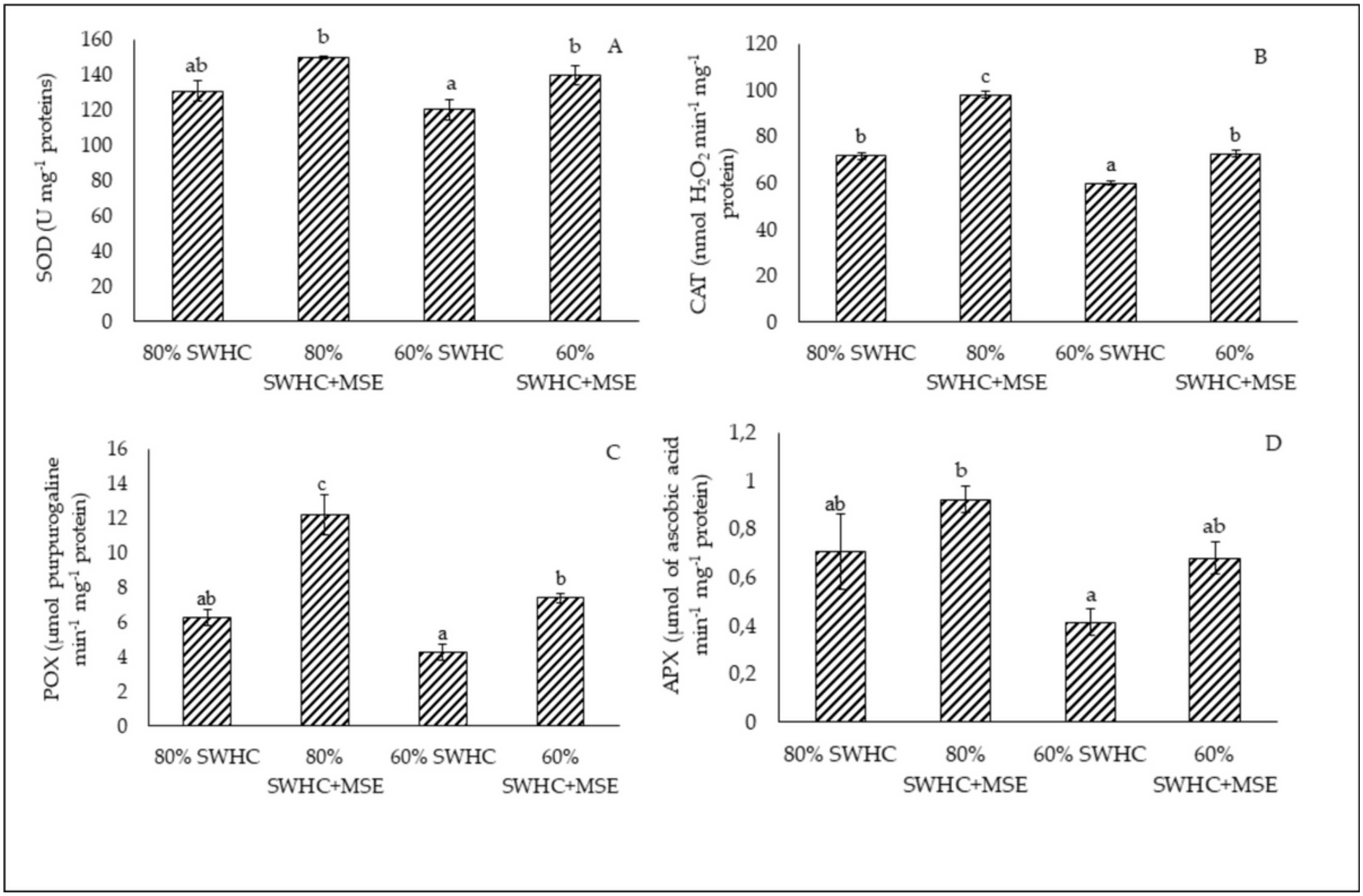

Figure 5

The effect of foliar moringa seed extracts (MSE) application on photosynthetic parameters; SOD (A), CAT (B), POX (C) and APX (D) of cancer bush plants under drought stress. SWHC, soil water holding capacity; SOD, Superoxide dismutase; CAT, Catalase, POX, Peroxidase; APX, Ascorbate peroxidase. Vertical bars represent standard error (SE) of the mean value (*n*= 3). Different lowercase letters indicate significant differences ($p < 0.001$).