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# Foliar Application of Moringa Seed Extract Alone or in Combination with Salicylic Acid Enhanced Growth, Bioactive, and Phytohormone Compositions of Cancer Bush Plants under Heat Stress

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***Abstract.*** **Cancer bush (*Lessertia frutescens* L.) is an important medicinal plant that is rich in health beneficial compounds. It is commonly used in traditional medicine and as an ornamental plant. Heat stress is the most threatening abiotic factor restricting plant growth, thus causing crop yield and economic losses worldwide. The application of plant-derived biostimulant is as an innovative and promising approach for improving plant growth and productivity. The study was aimed to investigate the effect of moringa (*Moringa oleifera* Lam.) seed extract (MSE; 5%) either alone or in combination with salicylic acid (SA; 40 mg/L) on the growth, bioactive, and phytohormone attributes of cancer plants subjected to heat stress (38 °C for 2 hours for 5 days). Plants that were not treated were used as control. Plant pots were arranged in a randomized complete block design (RCBD) for treatments (MSE, SA, and MSE + SA) at 7-day intervals during the experiment. Both MSE and MSE + SA foliar application effectively increased plant growth characteristics and total carotenoids contents, and reduced electrolyte leakage and had no symptoms of wilting compared with SA and control. Plants treated with MSE showed higher number of branches and concentrations of abscisic acid (ABA), jasmonic acid (JA), and indole-3-acetic acid (IAA), and lower superoxide and hydrogen peroxide compared with other treatments and control. Also, plants treated with MSE + SA showed higher total chlorophylls and glutathione concentrations compared with other treatments and control. Overall, the application of MSE either alone or in combination with SA enhanced plant growth and productivity of heat-stressed cancer bush plants.**

Cancer bush (*Lessertia frutescens* L. syn. *Sutherlandia frutescens* L.) is an economically important multipurpose medicinal plant indigenous to southern Africa (Chuang et al. 2014). It is widely distributed in Namibia, Botswana, and South Africa (Chuang et al. 2014; Hamdi et al. 2021). In South Africa, cancer bush is commonly found in the Western Cape, Eastern Cape, Northern Cape, KwaZulu-Natal, and Mpumalanga provinces (Colling et al. 2010; Hamdi et al. 2021). Cancer bush is a small, attractive, perennial woody shrub that grows up to 1 m long (Gibson 2011). The striking scarlet flowers have made it a popular ornamental plant (Gibson 2011; Masenya et al. 2022). It is also reported to be used by British botanists since the early 1990s due to its phytochemical properties (Haffajee 2002; Street and Prinsloo 2013). Although extremely bitter, cancer bush is widely used in traditional medicine in Africa to treat diseases such as cancer, diabetes, infections, and inflammation (Gibson 2011; Mncwangi et al. 2023). In modern medicine, cancer bush is well known as an adaptogenic tonic, and the commercial tablets are popular to counteract the muscle-wasting effects associated with HIV-AIDS in patients and to stimulate appetite (Omolaoye et al. 2021; Mncwangi et al. 2023). The plant's a.i., such as L-canavanine, D-pinitol, γ-amino-butyric acid, triterpenoid glucoside known as "SU1," or triterpenoid saponins and flavanol glycosides are reported to possess anti-inflammatory, antioxidant, anticonvulsant, anticancer, antidiabetic, antimutagenic, analgesic, and immunomodulatory properties (Omolaoye et al. 2021; Shaik et al. 2011). Although cancer bush is an important medicinal plant, its growth and productivity are mainly limited by climate change (Hamdi et al. 2021).

Climate change intensifies the abiotic stress that reduces plant production and yield due to frequent drought and flooding, extreme temperatures, high wind speeds, and altered intensity and spectrum of ultraviolet radiation (Liu et al. 2020; Zhang et al. 2023). Heat stress due to high temperature can negatively affect plant growth, development, and more severely the reproductive stages causing a decrease of crop yield (Fahad et al. 2017; Mirón et al. 2023). Seed germination may be inhibited due to high temperatures above 30 to 38 °C (Cetin et al. 2023; Prasad and Djanaguiraman 2014). High temperature stress causes water deficiency in plant tissues, which in turn leads to injury of cell membranes and reduction in rates of transpiration, protein synthesis, and ion uptake and transport (Ahanger et al. 2017; Farooq et al. 2023). In addition, heat stress leads to overproduction of reactive oxygen species (ROS) and inhabitation of photosynthetic enzymes (Mittler et al. 2022; Shaffique et al. 2022; Tamta and Patni 2023), which ultimately results in the loss of cellular organization, cell death, crop failure, and economic losses (Gray and Brady 2016; Srivastava et al. 2023).

SA is a synthetic plant growth regulator (PGR) that serves as a critical signal molecule mediating immunity and plant growth (Kulak et al. 2021; Sakhabutdinova et al. 2003). SA is the major solute involved in flower induction, general growth and development, various enzyme biosynthesis, stomata movements, membrane protections, and cell respiration (Hafez et al. 2019; Sharma et al. 2020). It is also a defense-related plant hormone that plays a key role in resistance to different microbial pathogens such as virus, bacteria, fungi, and oomycetes (Koo et al. 2020). One of the most prominent roles of SA is in stress tolerance of plants, where it acts as a signaling molecule that induces resistance (Sharma et al. 2020). Rady and Mohamed (2015), Zulfiqar et al. (2020), and Guo et al. (2022) reported that 1 mM, 50 $mg \cdot L^{-1}$ and 0.75 mM SA effectively improved growth and yield of common bean (*Phaseolus vulgaris* L.), gladiolus (*Gladiolus grandifloras* L.), and maize (*Zea mays* L.) plants under normal or environmental stress conditions such as salt and heat stress, respectively. Although SA is effective, its excessive application could be associated with water and air pollution (Kaya et al. 2023). The residues of synthetic PGRs in agricultural products could be detrimental to human health due to toxicity (Kaya et al. 2023; Kobayashi et al. 2020), thus the need for green synthesis of SA. Also, little is known about the effect of SA in combination with natural biostimulants on plant performance.

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Plant biostimulants such as moringa (*Moringa oleifera* Lam.) extracts are substances applied as seed soaking and/or foliar spray that positively modify plant growth and productivity with alterations in metabolic processes under normal or environmental stress conditions (Batool et al. 2019; Soliman et al. 2020). Moringa, a multipurpose tree from the Moringaceae family is native to several habitats in South America, Africa, and Asia (Benettayeb et al. 2022; Shakour et al. 2023). Moringa leaves are a rich source of macro and micronutrients, vitamins, antioxidants, phytohormones such as auxins, gibberellins (GAs), cytokinins (CKs) or zeatin, SA, and JA (Azeem et al. 2023; Shakour et al. 2023). These components make moringa a potential natural plant growth stimulant (Azeem et al. 2023; Zulfiqar et al. 2020).

In addition, the preparation of plant-derived biostimulants such as moringa leaf extract (MLE) in ethanol or water is both cost-efficient and eco-friendly and could be used as a natural growth promoter and/or stress-reducing agent for many plant species due to the presence of phytochemicals and phytohormones (Yap et al. 2021; Zulfiqar et al. 2020). Latif and Mohamed (2016) and Yap et al. (2021) reported that the application of MLE (5 to 10 g/L) as a foliar spray effectively improved plant growth and yield of common bean and milk thistle (*Silybum marianum* L.) under environmental stress such as heat and salinity stresses. However, little is known about the biostimulant potential of moringa seed extract (MSE) to enhance plant productivity under heat stress conditions. To the best of our knowledge, no findings have been reported on the effect of a combination of MSE and SA on the growth and productivity of medicinal plants under environmental stress conditions. The aim of this study was to evaluate the effect of MSE, SA, and their combination on growth, bioactive, and phytohormone attributes of cancer bush plants subjected to heat stress.

## Materials and Methods

*Plant material and growth conditions.* This research was carried out in the tunnel of the School of Science and Technology, Sefako Makgatho Health Sciences University (SMU), South Africa (lat. 25°37′8″ S, long. 28°1′22″E, elevation 1276 m), during winter–spring (first trial) and spring–summer (second trial) seasons of 2021. The 4-m tunnel was covered with a green photo-selective colored net (40% shading) [ChromatiNet™, Carports and Pergola Builders (Pty) Ltd., Pretoria, South Africa]. During this period, daily temperatures ranged from 12.18 to 29.98 °C, with an average of 21.08 ± 0.60 °C. Daily relative humidity averaged 43.01 ± 0.57% and ranged from 35.99% to 50.01%.

Cancer bush seedlings were purchased from Plant & Palm Kwekery nursery at Akasia, Pretoria, South Africa (lat. 25°39′50.6″ S, long. 28°08′01.1″ E, elevation 1300 m). The seedlings were then placed in the tunnel at SMU where they were irrigated three times a day with 200 mL tap water per plant for a period of 1 week. Afterward, only healthy seedlings were transplanted into individual plastic terracotta pots (40 cm in diameter and 50 cm depth), filled with 5 kg of culterra potting soil obtained from Builders Express, Pretoria, South Africa (lat. 25°40′28.49″ S, long. 28°6′31.22″ E, elevation 1305 m). This product was made from raw organic materials with COCO peat, forest products, water retentive agents, 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 being the main ingredients.

*Solutions.* Moringa seeds were harvested from the commercial orchards of Afrinest Moringa Farm in Tzaneen, Limpopo, South Africa (lat. 23°49′15.3″ S, long. 30°10′08.7″ E, elevation 719 m). The seeds were ground into fine powder and extracted using a method described by Khalofah et al. (2020) with slight modifications. The MSE was prepared by mixing 500 g of ground seeds with 5 L of 80% ethanol and left at room temperature for 24 h with occasional manual swirling. Then, the mixture was purified by filtering twice through Whatman no. 1 filter paper. Centrifugation (laboratory centrifuge-TD4C, Hermle Labortechnik, Germany) at 8000 $g_n$ for 15 min was then conducted for the supernatant. The supernatant was diluted with distilled water (v/v) to obtain the required concentration of 4% MSE to use as a foliar spray. Based on the preliminary study (data not shown) 40 mg/L SA solution was used in this study. Therefore, 4% MSE and 40 mg/L SA singularly or in combination (MSE + SA) were used as treatments. Tween-20 (0.01 mL/L) was added to the MSE and SA foliar sprays as a surfactant and spreading facilitator. The extracts were then used immediately or stored in the refrigerator at −20 °C for further use. Control plants were only sprayed with tap water.

*Experimental setup.* Both experiments were arranged in an RCBD with plant spacing of 40 cm and row spacing of 50 cm (Buthelezi et al. 2023). Plant pots were arranged for MSE, SA, and MSE + SA foliar spray application. Cancer bush plants not treated with the extracts were used as control. The three treatments and control were replicated 12 times, making a total of 48 pots per experiment. Two weeks after transplanting, plants were subjected to heat stress (38 °C). During morning hours (0900 HR to 1100 HR), pot plants were transferred to a laboratory artificial climate incubator [Plant Growth Chamber – LGP-250E, Labotec (PTY) LTD, Johannesburg, South Africa] for 2 h for 5 d and then transferred to the normal temperature at the tunnel. Cancer bush plants were sprayed with MSE, SA, and MSE + SA once a week and at 7-d intervals for 8 weeks after plants were subjected to heat stress. Plants were irrigated with an average of 3 L of tap water/plant/day during the experiment.

*Plant growth measurements.* At the end of the trial (8 weeks after plants were subjected to heat stress), plant height was determined using a measuring tape (Buthelezi et al. 2022) and the number of branches was counted. Aerial part dry weight was determined by drying samples using a digital oven (EcoTherm Digital Ovens-279, Labotec (PTY) LTD, Durban, South Africa) at 50 °C to constant weight (Yap et al. 2021).

*Determination of leaf pigment concentration.* Total chlorophyll and carotenoid concentrations were determined according to a method of Mahmood et al. (2022). Briefly, leaf discs of 0.2 g were homogenized in 50 mL 80% (v/v) acetone and centrifuged at 10,000 $g_n$ for 10 min. The absorbance was measured at 663, 645, and 470 nm using a spectrophotometer (ultraviolet-1700; Shimadzu, Milan, Italy). Pigment concentrations were expressed in milligrams of pigment per gram of tissue fresh weight ($mg \cdot g^{-1}$ FW).

*Determination of electrolyte leakage.* Electrolyte leakage (EL), taken as a parameter of damage and membrane integrity, was measured according to Zulfiqar et al. (2020) using a portable conductivity meter (HI98192, Shalom Laboratory Supplies CC, Johannesburg, South Africa). The EL values were obtained using three leaf samples/plant/treatment. The leaf samples were placed in a tube containing 10 mL boiling deionized water and the electrical conductivity ($EC_1$) was recorded. The contents were then heated at 55 °C for 30 min in a water bath (Biobase – WBS0001, Masiye Laboratories, Johannesburg, South Africa) and the electrical conductivity ($EC_2$) was recorded. The samples were then boiled at 95 °C for 15 min and the electrical conductivity ($EC_3$) was recorded. EL% was calculated according to Howladar (2014) using the following formula:

$$EL(\%) = \frac{EC_2 - EC_1}{EC_3} \times 100 \qquad [1]$$

*Determination of phytohormones.* ABA, JA, and IAA were determined in root exudates at harvest (8 weeks after plants were subjected to heat stress) according to Latif and Mohamed (2016), with minor alterations. A 50-mg amount of frozen samples was ground in cold 80% methanol followed by triple extraction with 500 µL methanol containing 0.1 ng/µL of each stable isotope-labeled internal standard ($^2H_6$-ABA, $^2H_2$-IAA and $^2$H6-JA). The extraction was performed in 2-mL cryotubes using a laboratory bead mill (ESW-1.0, Chongqing DEGOLD Machine Co., Ltd, Chongqing, China) with acceleration of 6.5 $m/s^2$ for 40 s. After centrifugation at 20,000 $g_n$ for 15 min at 0 °C, 20 µL of supernatant was transferred into a polypropylene tube, mixed with water to 5 mL and injected into the high-performance liquid chromatography (HPLC) system (LC-4500; Thermo Fisher Scientific Inc., Johannesburg, South Africa).

The HPLC separation and quantitation were performed at ambient temperature with a C18 column (5-µm particle size, L × I.D. 15 cm × 4.6 mm; Merck, Johannesburg, South Africa), using a methanol:water mixture, supplemented with 0.1% acetic acid, gradient at a flow rate of 300 $\mu L \cdot min^{-1}$ (Latif and Mohamed 2016). Results were processed using the Masslynx v4.1 software and the phytohormone contents were quantified with a standard curve prepared with commercial standards.

*Determination of hydrogen peroxide.* The hydrogen peroxide ($H_2O_2$) level in roots was assayed according to the method of Ahmed et al. (2021), with some modifications. Briefly, the samples were extracted into 5 mL of 0.1% (v:v) trichloroacetic acid solution and centrifugation at 12,000 $g_n$ for 10 min. In 1 mL of supernatant, 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) was added, followed by the addition of 1 mL of potassium iodide (pH 7.0) and the absorbance was recorded at 390 nm using a spectrophotometer (ultraviolet-1700; Shimadzu). The results of $H_2O_2$ concentration were expressed as $\mu mol \cdot g^{-1}$ FW.

*Determination of superoxide.* The superoxide ($O_2^{\bullet-}$) content was measured according to the method described by Sun et al. (2017), with minor modifications. Briefly, 0.1 g of fresh roots was extracted in 10 mM K-phosphate buffer (pH 7.8), 0.05% NBT, and 10 mM $NaN_3$ and then centrifuged at 15,000 $g_n$ at 5 °C for 10 min. A 2-mL amount of immersed solution was heated at 90 °C for 10 min and cooled rapidly. Optical density was measured colorimetrically at 580 nm and the $O_2^{\bullet-}$ content was expressed as $\mu mol \cdot g^{-1}$ FW.

*Determination of glutathione content.* Glutathione was determined according to Alharby et al. (2020), with some alterations. Briefly, 0.5 g of frozen plant tissue was homogenized in 1 mL of 3% trichloroacetic acid and centrifuged at 12,000 $g_n$ at 5 °C for 10 min. Subsequently, 500 μL of leachate was transferred to a tube containing 600 μL of 100 mg of phosphate regulator (pH 7.0) and 40 μL of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). After 3 min, the absorbance was then read at 412 nm against blank (distilled water) using a spectrophotometer (ultraviolet-1700; Shimadzu). The results were expressed as reduced glutathione (μmol GSH/g FW).

*Visual wilting evaluation.* A visual evaluation of wilting of plant leaves was performed weekly during the experiment after plants were subjected to heat stress. The purpose of this assessment was to monitor the development of stress symptoms. To observe symptoms of leaf wilting, a 0 to 5 hedonic scale was used (Wang 2015); 1 = none, 2 = slight, 3 = moderate, 4 = moderately severe, and 5 = severe wilting. The results were expressed as the average score of the collected data.

*Statistical analysis.* Analysis of variance was used in our study to analyze the collected data under the RCBD. Means were compared using Duncan's multiple range test ($P < 0.001$) (GenStat®, 18.1 edition, VSN International, UK).

## Results

*Plant growth characteristics.* The application of MSE and SA or in combination effectively ($P < 0.001$) enhanced growth attributes of heat-stressed cancer bush plants compared with control (Table 1). Plants treated with MSE alone or in combination with SA had the highest plant height (52.31 and 51.01 cm) and aerial part dry weight (39.40 and 38.92 g) with no statistical differences compared with foliar application of SA (45.11 cm and 16.92 g) and control (39.07 cm and 13.45 g), respectively. In addition, plants treated with MSE had the highest number of branches (67.67) followed by MSE + SA (58.00) and SA (40.00) and control (30.66).

Table 1. Growth characteristics responses to either alone or combined foliar application of moringa seed extract (MSE) and salicylic acid (SA) in cancer bush plants subjected to heat stress.

| Treatments | Plant ht (cm) | Number of branches (n) | Aerial part dry wt (g) |
|---|---|---|---|
| Control | 39.07 ± 2.67 a | 30.67 ± 2.75 a | 13.45 ± 0.83 a |
| MSE | 52.31 ± 1.10 c | 67.67 ± 1.45 d | 39.40 ± 1.20 b |
| SA | 45.11 ± 2.06 b | 40.00 ± 1.15 b | 16.92 ± 1.09 a |
| MSE + SA | 51.01 ± 0.78 c | 58.00 ± 1.19 c | 38.94 ± 1.17 b |

MSE + SA = MSE combined with SA. Data presented as mean ± *SE*. Different letters among treatments for each attribute are significantly different ($P < 0.001$).

*Leaf pigments.* The highest ($P < 0.001$) total chlorophyll concentration was observed in plants treated with MSE + SA (2.31 $mg \cdot g^{-1}$ FW) followed by MSE (1.91 $mg \cdot g^{-1}$ FW) and SA (1.61 $mg \cdot g^{-1}$ FW) compared with untreated plants (1.38 $mg \cdot g^{-1}$ FW) (Fig. 1). The concentration of total carotenoids was significantly ($P < 0.001$) higher in plants treated with either MSE or MSE + SA (0.98 and 0.99 $mg \cdot g^{-1}$ FW, respectively) followed by SA (0.64 $mg \cdot g^{-1}$ FW) compared with control, which had the lowest concentration of total carotenoids (0.32 $mg \cdot g^{-1}$ FW) (Fig. 1).

*Electrolyte leakage.* Figure 2 shows that the foliar application of MSE, SA, and MSE + SA significantly ($P < 0.001$) reduced the EL values compared with untreated plants. Plants treated with MSE or MSE + SA had significantly ($P < 0.001$) lower EL values (8.17% and 8.14%, respectively) followed by SA (10.04%) compared with control, which had the highest values of EL (11.92%).

*Phytohormone attributes.* Treating heat-stressed cancer bush plants with MSE resulted in significantly ($P < 0.001$) higher concentration of ABA (16.14 $ng \cdot g^{-1}$ FW) followed by MSE + SA (14.11 $ng \cdot g^{-1}$ FW) and SA (11.22 $ng \cdot g^{-1}$ FW) compared with control (9.97 $ng \cdot g^{-1}$ FW) (Table 2). Also, the concentrations of JA and IAA were significantly ($P < 0.001$) higher in plants treated with MSE (12.91 and 9.21 $ng \cdot g^{-1}$ FW), followed by MSE + SA (10.49 and 7.11 $ng \cdot g^{-1}$ FW) and SA (8.92 and 6.01 $ng \cdot g^{-1}$ FW) in comparison with control (7.91 and 4.12 $ng \cdot g^{-1}$ FW), respectively (Table 2).

*ROS ($H_2O_2$ and $O_2^{\bullet-}$).* Heat-stressed cancer bush plants treated with the MSE foliar spray

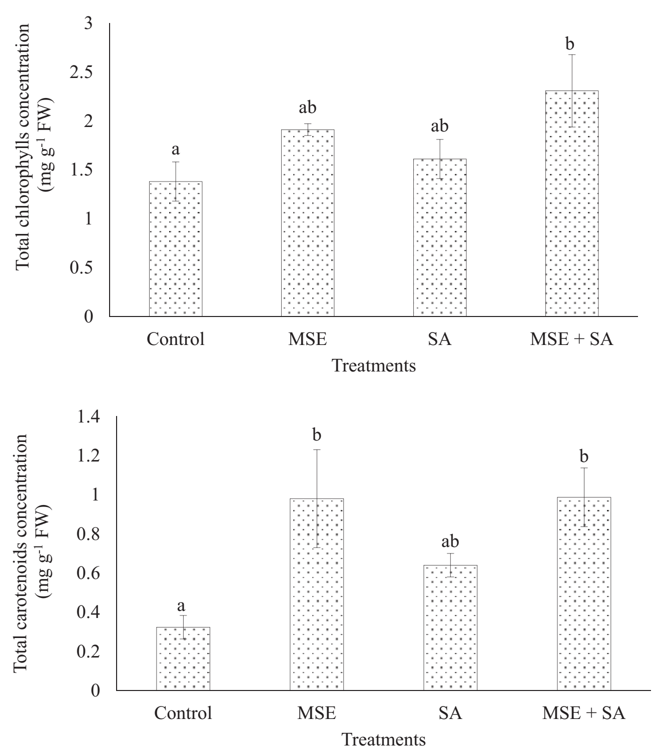


Fig. 1. Total chlorophyll and carotenoid concentrations of heat-stressed cancer bush plants treated with either separate or combined foliar application of moringa seed extract (MSE) and salicylic acid (SA). Means followed by different letters in each bar indicate a statistically significant difference ($P < 0.001$). MSE + SA = MSE combined with SA.

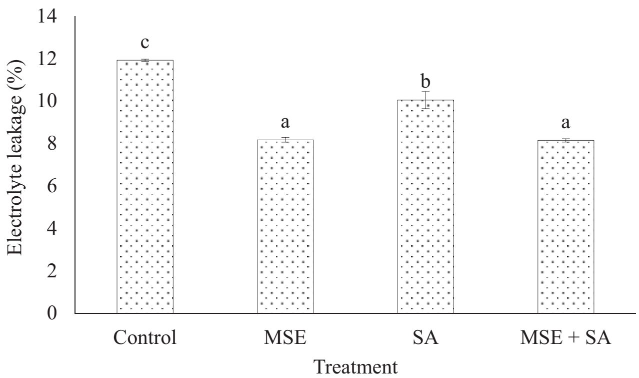


Fig. 2. Electrolyte leakage of heat-stressed cancer bush plants treated with either separate or combined foliar application of moringa seed extract (MSE) and salicylic acid (SA). Means followed by different letters in each bar indicate a statistically significant difference ($P < 0.001$). MSE + SA = MSE combined with SA.

had significantly ($P < 0.001$) reduced $H_2O_2$ (1.52 μmol·g$^{-1}$ FW) and $O_2^{\bullet-}$ (0.21 μmol·g$^{-1}$ FW) compared with other treatments (Fig. 3). This treatment had the maximum reduction of $H_2O_2$ and $O_2^{\bullet-}$ followed by MSE + SA (1.98 and 0.42 μmol·g$^{-1}$ FW) and SA (2.97 and 0.52 μmol·g$^{-1}$ FW) compared with control (4.09 and 061 μmol·g$^{-1}$ FW), respectively (Fig. 3).

*Glutathione.* A significant ($P < 0.0001$) increase in glutathione was observed in heat-stressed cancer bush plants in response to all treatments (MSE, SA, and MSE + SA) compared with untreated plants (Table 2). The highest ($P < 0.001$) concentration of glutathione was noted in plants treated with MSE + SA (12.97 μmol GSH/g FW) compared with other treatments; MSE (10.99 μmol GSH/g FW) and SA (10.82 μmol GSH/g FW). Control had the lowest concentration of glutathione (8.08 μmol GSH/g FW) in comparison with treated plants.

*Wilting.* The MSE, SA, and MSE + SA treatments had a significant ($P < 0.001$) positive effect on wilting of cancer bush plants subjected to heat stress (Fig. 4). Plants treated with MSE and MSE + SA showed no symptoms of wilting, whereas plants sprayed with SA showed slight symptoms of wilting. On the other hand, control plants showed moderately severe symptoms of wilting compared with treated plants (Fig. 4).

## Discussion

Cancer bush is an important medicinal plant that is also used as an ornamental plant (Mncwangi et al. 2023). It is commonly harvested in the wild and commercially cultivated by a few farmers (Masenya et al. 2023). With the effect of global climate change, abiotic stresses such as high temperature, drought, saline-alkali, and flooding are the main factors restricting plant growth and development, leading to the loss of yield (Khan and Mehmood 2023). Natural biostimulants, which are more cost effective, safer, and environmentally friendly than common agrochemicals, can be innovative tools for enhancing the quality and yield of medicinal plants (Shahrajabian and Sun 2022). Moringa plant extracts, including leaves and seed extracts are a rich source of nutrients such as macro and micronutrients, vitamins, antioxidants, and phytohormones, including auxins, GAs, CKs, SA, and JA, which are responsible for promoting plant growth and development (Ahmed et al. 2021; Buthelezi et al. 2023). In addition, natural biostimulants enriched with natural or synthetic PGRs such as melatonin, SA, and ABA can promote plant resistance to environmental stresses (Abdel Megeed et al. 2021). Mostly, PGRs are applied at low concentrations and are involved in the hormonal homeostasis and/or signaling network, therefore triggering plant growth, development, metabolic processes, and responses to stress (Shah et al. 2023). Recently, there has been great interest in replacing synthetic agrochemicals with biostimulants.

The results of the current study showed that the application of MSE and MSE + SA significantly enhanced plant growth attributes. This could be attributed to the presence of bioactive compounds in MSE. MSE contains essential minerals, antioxidants, and phytohormones such as IAA, Gas, and CKs (Buthelezi et al. 2023). Phytohormones, such as IAA, GAs, and CKs including zeatin, promote cell division and expansion, plant growth, and yield (Ahmed et al. 2021; Shakour et al. 2023). This diverse composition of MSE indicates that this extract can be used as a potential plant biostimulant. Several studies highlighted the role of moringa plant extracts, particularly MLE, to improve plant growth and development in different crops (Ahmed et al. 2021; Buthelezi et al. 2023; Khalofah et al. 2020; Latif and Mohamed 2016; Mahmood et al. 2022; Rady and Mohamed 2015; Shakour et al. 2023; Yap et al. 2021; Zulfiqar et al. 2020), which is also evident from results of the current study.

Moreover, the increase in the growth attributes of heat-stressed cancer bush plants in response to SA treatment, especially in combination with MSE, could be attributed to the protective role of SA on membranes that might increase the tolerance of plants to heat stress (Kobayashi et al. 2020). SA is a naturalistic plant phenolic compound that contributes as a nonenzymatic antioxidant and an endogenous signaling molecule inducing stress tolerance against abiotic (heat, drought, heavy metal, and salt stress) and biotic (against pest and diseases) stresses in plants (Kaya et al. 2023; Kobayashi et al. 2020). In addition, SA interacts with other hormones in plants that are involved in the regulation of cell division and expansion, such as auxin, GA, and ethylene (ET), to modulate plant growth (Coşkun et al. 2023; Lin et al. 2023).

The increase of total chlorophyll and carotenoid contents in heat-stressed cancer bush plants by the foliar applications of MSE and/or MSE + SA could be attributed to that MSE is rich in phytochemicals such as antioxidants, particularly proline and ascorbic acid and phytohormones including CKs and GAs (Buthelezi et al. 2022, 2023). The MSE components also act to promote the biosynthesis of leaf photosynthetic pigments due to their high content of mineral nutrients and phytohormones, increasing leaf chlorophyll concentrations and consequently the enhanced photosynthesis process produces more assimilates including osmoprotectants (Mahmood et al. 2022; Zulfiqar et al. 2020). The increased total chlorophyll concentration in the leaves of heat-stressed cancer bush plants treated with SA and/or MSE + SA foliar spray could be related to the influence of SA on endogenous CK contents, which may further increase after MSE application (Buthelezi et al. 2023; Kaya et al. 2023). This is further supported by Fig. 1, which shows that total chlorophylls concentrations were higher in plants treated with MSE + SA followed by plants foliar sprayed with MSE compared with control. Also, SA-treated plants synthesize more CKs, which improves chloroplast differentiation and chlorophyll biosynthesis and prevents chlorophyll

Table 2. Phytohormone and glutathione concentrations responses to either alone or combined foliar application of moringa seed extract (MSE) and salicylic acid (SA) in cancer bush plants subjected to heat stress.

| Treatments | Abscisic acid (ng·g$^{-1}$ FW) | Jasmonic acid (ng·g$^{-1}$ FW) | Indole-3-acetic acid (ng·g$^{-1}$ FW) | Reduced glutathione (μmol GSH/g FW) |
|---|---|---|---|---|
| Control | 9.97 ± 0.61 a | 7.91 ± 0.53 a | 4.120 ± 0.42 a | 8.08 ± 0.52 a |
| MSE | 16.14 ± 0.66 c | 12.91 ± 0.52 c | 9.210 ± 0.44 d | 10.99 ± 0.43 b |
| SA | 11.22 ± 0.44 a | 8.92 ± 0.44 a | 6.010 ± 0.54 b | 10.82 ± 0.26 b |
| MSE + SA | 14.11 ± 0.46 b | 10.49 ± 0.76 b | 7.110 ± 0.39 c | 12.97 ± 0.39 c |

FW = fresh weight; MSE + SA = moringa seed extract combined with salicylic acid. Data presented as mean ± *SE*. Different letters among treatments for each attribute are significantly different ($P < 0.001$).

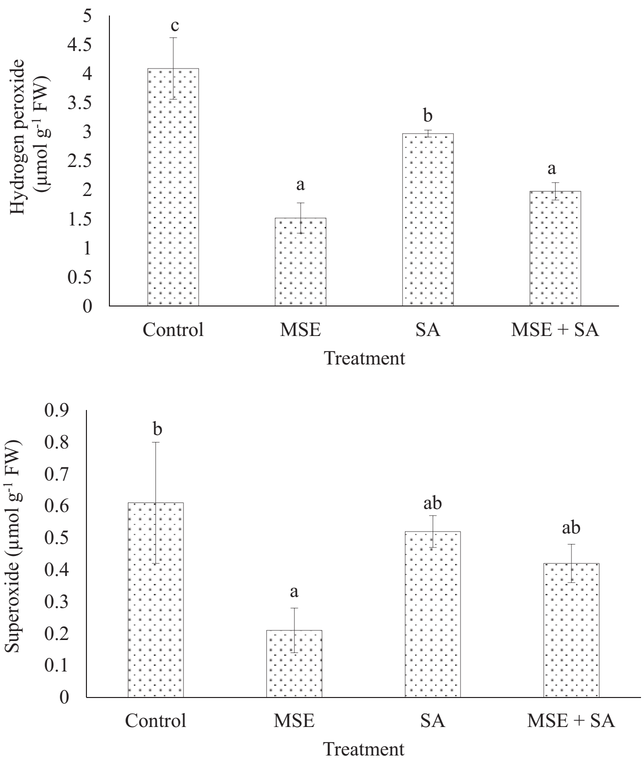


Fig. 3. Hydrogen peroxide and superoxide of heat-stressed cancer bush plants treated with either separate or combined foliar application of moringa seed extract (MSE) alone and salicylic acid (SA). Means followed by different letters in each bar indicate a statistically significant difference ($P < 0.001$). MSE + SA = MSE combined with SA.

degradation (Coşkun et al. 2023; Goncharuk and Zagoskina 2023).

The enhanced total chlorophyll content in heat-stressed plants by MSE and/or SA foliar spray might have increased plant growth (Table 1) and productivity because photosynthesis and biomass production are controlled directly by the amount of chlorophyll present in plants (Taffouo et al. 2017). Chlorophyll harvests and converts the energy of absorbed photons to the energy of chemical bonds resulting from photosynthesis, thus enhancing plant growth (Croft et al. 2020). In plants, carotenoids are essential for photosynthesis and photoprotection and play critical roles as light harvesting pigments and structural components of photosystems (Dhami and Cazzonelli 2020). In addition, carotenoids can act as signaling molecules in response to environmental and developmental cues or serve as regulators of plant growth (Rocha Júnior et al. 2023; Sherin et al. 2022). This is further supported by the results of the current study, which shows that plants treated with MSE or MSE + SA had higher total carotenoid contents (Fig. 1), which may have improved plant growth attributes (Table 1). Our results are similar to those of Rady and Mohamed (2015), who stated that the application of 1 mM SA and 3% MLE significantly enhanced the total chlorophyll (1.76 and 1.77 $mg \cdot g^{-1}$ FW) and carotenoid (0.45 and 0.44 $mg \cdot g^{-1}$ FW) concentrations of common bean plants grown on a saline soil (EC = 6.23–6.28 $dS \cdot m^{-1}$) compared with control (1.70 and 0.43 $mg \cdot g^{-1}$ FW), respectively. Also, Batool et al. (2020) stated that foliar application of 3% MLE improved leaf chlorophyll a (51%) and b (61%), and total chlorophyll contents (54%) of moringa seedlings compared with control.

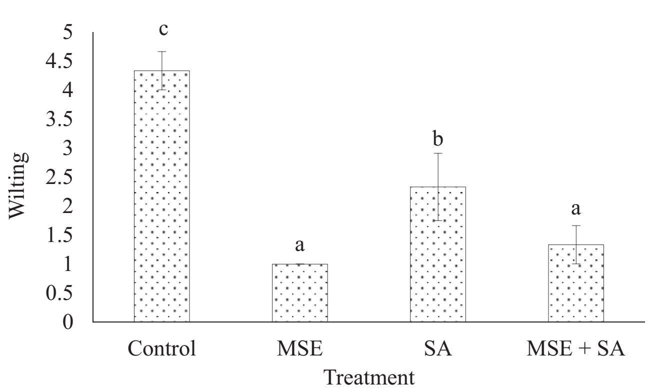


Fig. 4. Wilting of heat-stressed cancer bush plants treated with either separate or combined foliar application of moringa seed extract (MSE) and salicylic acid (SA). Means followed by different letters in each bar indicate a statistically significant difference ($P < 0.001$). MSE + SA = MSE combined with SA.

The EL from plant tissues is commonly used as a parameter to evaluate cell integrity and as an indicator of plant stress tolerance (Rady and Mohamed 2015). Because membrane damage often results in an increased leakage of cytosolic constituents to the apoplastic space, higher values of EL imply lower membrane stability (Sujata Goyal et al. 2023). In our study, the treatments with MSE either alone and/or in combination with SA showed the best results under heat stress by reducing the accumulation of EL in cancer bush leaves (Fig. 2). These results suggest that MSE components such as essential mineral nutrients and phytohormones (Buthelezi et al. 2022, 2023) could have easily translocated through leaf stomata to active parts such as biosynthesizing and/or meristematic cells to provide them the ability to overcome the stress conditions (Buthelezi et al. 2023; Shakour et al. 2023). Mineral nutrients enable crop plants to adapt to environmental stress conditions through signaling pathways that affect the adaptive responses of plants to environmental stresses and/or expression and regulation of stress-induced genes that contribute to stress tolerance (Mahmood et al. 2022; Yap et al. 2021). Also, the application of MSE with SA induces phytohormone biosynthesis that further maintains integrity of cellular membranes under environmental stress conditions such as drought and heat stress, which are considered an integral part of the heat stress tolerance mechanism (Batool et al. 2020; Coşkun et al. 2023; Shakour et al. 2023). Our results are similar to those of Rady and Mohamed (2015), who reported that the application of 1 mM SA and 3% MLE effectively reduced EL (8.04 and 8.08%, respectively) compared with control (9.68%).

The higher concentrations of ABA, JA, and IAA were observed in heat-stressed cancer bush plants treated with MSE, which could be due to the presence of bioactive compounds and phytohormones in MSE (Buthelezi et al. 2022, 2023). Phytohormones promote many plant-related physiological processes and signaling networks in plants to modify plant responses to environmental stressors (Iqbal et al. 2023; Singh et al. 2023). ABA is synthesized under environmental stress conditions and triggers an adaptive response by activating a group of genes responsible for stress resistance (Iqbal et al. 2023; Ma et al. 2020). It is involved in the regulation of many aspects of plant performance, including seed germination, embryo maturation, leaf senescence, stomatal aperture, and tolerance to environmental stress (Pal et al. 2023; Singh et al. 2023).

JA is a plant-signaling molecule closely associated with plant resistance to abiotic stress and is usually involved in physiological and molecular responses such as the activation of the antioxidant system, accumulation of amino acids and soluble sugars, and regulation of stomatal opening and closing (Hewedy et al. 2023; Iqbal et al. 2023). IAA regulates growth and developmental processes such as cell division and elongation, tissue differentiation, and apical dominance, and promotes stem and lateral root growth, fruit development, and tolerance to pathogens (Singh et al. 2023; Sosnowski et al. 2023). This is in accordance with our results in which heat-stressed plants treated with MSE had enhanced plant growth attributes (Table 1) and leaf pigments (Fig. 1) and reduced EL (Fig. 2). Moreover, a study by Buthelezi et al. (2023) showed that this extract is rich in phytohormones such as IAA, GA, and CK, which promotes plant growth, productivity, and tolerance to environmental stresses (Khalofah et al. 2020; Shakour et al. 2023).

The water loss caused by drought can induce excessive production of ROS such as $O_2^{\bullet -}$, $^1O_2$, and $H_2O_2$, which results in membrane lipid peroxidation, protein denaturing, and cell death (Hamedeh et al. 2022; Kesawat et al. 2023). The application of MSE effectively mitigated the oxidative damage as a result of the decreased ROS production; $H_2O_2$ and $O_2^{\bullet -}$ (Fig. 3), which could be due to either the presence of antioxidants or direct ROS scavenging capacity of peptides and amino acids in MSE (Buthelezi et al. 2022, 2023). Also, plants treated with MSE + SA showed lower values of $H_2O_2$ and $O_2^{\bullet -}$ compared with control, which also could be attributed to SA being an important signal molecule that can activate different defense mechanism in plants, thus enhancing plants' adaptability to abiotic stress conditions (Kaya et al. 2023).

The higher concentration of glutathione observed in heat-stressed cancer bush plants treated with MSE + SA could be because MSE is rich in some antioxidants, proline, and ascorbic acid and phytohormones (Buthelezi et al. 2022, 2023). Also, SA is reported to produce osmolytes such as proline and antioxidants (Coşkun et al. 2023). In addition to crucial roles in defense system and as enzyme cofactors, these components, particularly antioxidants, directly or indirectly scavenge ROS and/or control ROS production and influence plant growth and development by modifying processes from mitosis and cell elongation to senescence and plant death (Altaf et al. 2022; Goncharuk and Zagoskina 2023).

Moreover, glutathione acts as an antioxidant in many ways. It can react chemically with $O_2^{\bullet -}$, $OH^{\bullet}$, and $H_2O_2$, and, therefore, can function directly as a free radical scavenger (Madhu et al. 2023). This is further supported by the results of the current study, which shows that heat-stressed cancer bush plants treated with MSE or MSE + SA had higher glutathione concentrations and lower levels of $O_2^{\bullet -}$ and $H_2O_2$ compared with control. Abd El Mageed et al. (2023) reported that the enzyme glutathione reductase maintains the glutathione pool in the reduced state that in turn reduces dehydroascorbate to ascorbate, which is a primary antioxidant and direct scavenger of ROS (Hasanuzzaman et al. 2019). It was also reported that increased glutathione reductase expression enhances the tolerance to oxidative stress (Abd El Mageed et al. 2023; Madhu et al. 2023). Our results are similar to those of Khalofah et al. (2020), who reported that glutathione content was significantly higher in cadmium stressed garden cress (*Lepidium sativum* L.) plants treated with 6% MLE (159.42 U/mg protein) compared with 2% and 4% MLE (151.62 and 156.25 U/mg protein) and control (148.51 U/mg protein). This could be because the antioxidant glutathione plays an important role in the regulation of plant growth, development, and responses to abiotic stresses (Madhu et al. 2023).

Heat-stressed cancer bush plants treated with MSE and MSE + SA showed no symptoms of wilting, which could be attributed to the presence of phytohormones, particularly CKs in MSE (Buthelezi et al. 2023; Shakour et al. 2023). The presence of CKs in MSE (Buthelezi et al. 2022, 2023) prevents premature leaf senescence and maintains higher leaf area for photosynthetic activity and higher chlorophyll concentration in plant leaves (Iqbal et al. 2023; Zulfiqar et al. 2020). SA treatments enhance synthesis of CKs in plants, which promotes chlorophyll biosynthesis or prevents chlorophyll degradation in leaves (Kaya et al. 2023; Kobayashi et al. 2020). This is further supported by the results of this study, which showed that heat-stressed cancer bush plants treated with either MSE or MSE + SA had higher total chlorophyll contents compared with control (Fig. 1). This might have delayed wilting or leaf senescence, which is accompanied by various changes in cell structure, physiological metabolism, and gene expressions (Madhu et al. 2023). In addition, the phytohormones and bioactive compounds present in MSE might have played an important role in genetic modification, which can delay leaf aging, maintain photosynthesis for a long time, and sustain leaf activity, thereby increasing yield (Buthelezi et al. 2023; Zulfiqar et al. 2020).

## Conclusions

The current study demonstrated that the negative effect of heat-induced stress on the growth and productivity of cancer bush plants could be alleviated by the foliar application of MSE and/or in combination with SA, which could protect the plants against injuries by heat stress. The application of MSE alone or in combination with SA effectively improved plant growth, total chlorophyll and carotenoid concentrations, phytohormones (ABA, JA, and IAA), glutathione and reduced EL and ROS (superoxide and hydrogen peroxide) compared with untreated plants. In addition, plants treated with MSE and MSE + SA showed no symptoms of wilting, whereas SA and control showed slight and moderate severe symptoms of wilting, respectively. Overall, MSE and MSE + SA mitigated the adverse effects of heat stress on cancer bush plants by improving plant growth attributes, and biochemical and phytohormone compositions of plants. Therefore, the results of the current study demonstrated that MSE either alone or in combination with SA can be used as an environmentally friendly plant biostimulant to promote sustainable cultivation of medicinal plants. These positive results open the possibility for future research based on improving the concentration and mode of application of MSE either alone or in combination with different plant-derived extracts under various environmental conditions.

## References Cited


Abd El Mageed TA, Semida W, Hemida KA, Gyushi MA, Rady MM, Abdelkhalik A, Merah O, Brestic M, Mohamed HI, El Sabagh A, Abdelhamid MT. 2023. Glutathione-mediated changes in productivity, photosynthetic efficiency, osmolytes, and antioxidant capacity of common beans (*Phaseolus vulgaris*) grown under water deficit. Peer J. 11:1–23. https://doi.org/10.7717/peerj.15343.

Abdel Megeed TM, Gharib HS, Hafez EM, El-Sayed A. 2021. Effect of some plant growth regulators and biostimulants on the productivity of Sakha108 rice plant (*Oryza sativa* L.) under different water stress conditions. Appl Ecol Environ Res. 19:2859–2878.

Ahanger MA, Akram NA, Ashraf M, Alyemeni MN, Wijaya L, Ahmad P. 2017. Plant responses to environmental stresses—from gene to biotechnology. AoB Plants. 9(4):1–17. https://doi.org/10.1093/aobpla/plx025.

Ahmed T, Abou Elezz A, Khalid MF. 2021. Hydropriming with moringa leaf extract mitigates salt stress in wheat seedlings. Agriculture. 11(12):1–13. https://doi.org/10.3390/agriculture11121254.

Alharby HF, Alzahrani YM, Rady MM. 2020. Seeds pretreatment with zeatins or maize grain-derived organic biostimulant improved hormonal contents, polyamine gene expression, and salinity and drought tolerance of wheat. Int J Agric Biol. 24(4):714–724.

Altaf MA, Shahid R, Ren MX, Naz S, Altaf MM, Khan LU, Tiwari RK, Lal MK, Shahid MA, Kumar R, Nawaz MA. 2022. Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. Antioxidants. 11(2):309. https://doi.org/10.3390/antiox11020309.

Azeem M, Pirjan K, Qasim M, Mahmood A, Javed T, Muhammad H, Yang S, Dong R, Ali B, Rahimi M. 2023. Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. Sci Rep. 13(1):1–17. https://doi.org/10.1038/s41598-023-29954-6.

Batool S, Khan S, Basra SM, Hussain M, Saddiq MS, Iqbal S, Irshad S, Hafeez MB. 2019. Impact of natural and synthetic plant stimulants on moringa seedlings grown under low-temperature conditions. Int Lett Nat Sci. (76):50–59.

Batool S, Khan S, Basra SMA. 2020. Foliar application of moringa leaf extract improves the growth of moringa seedlings in winter. S Afr J Bot. 129:347–353. https://doi.org/10.1016/j.sajb.2019.08.040.

Benettayeb A, Usman M, Tinashe CC, Adam T, Haddou B. 2022. A critical review with emphasis on recent pieces of evidence of *Moringa* *oleifera* biosorption in water and wastewater treatment. Environ Sci Pollut Res. 29(32): 48185–48209. https://doi.org/10.1007/s11356-022-19938-w.

Buthelezi NMD, Gololo SS, Mugivhisa LL. 2022. An assessment of moringa (*Moringa oleifera* L.) seed extract on crop water productivity and physico-biochemical properties of cancer bush (*Sutherlandia frutescens* L.) under deficit irrigation. Horticulturae. 8(10):1–14. https://doi.org/10.3390/horticulturae8100938.

Buthelezi NMD, Ntuli NR, Mugivhisa LL, Gololo SS. 2023. *Moringa oleifera* Lam. Seed extracts improve the growth, essential minerals, and phytochemical constituents of *Lessertia frutescens* L. Horticulturae. 9(8):1–12. https://doi.org/10.3390/horticulturae9080886.

Cetin M, Sevik H, Koc I, Cetin IZ. 2023. The change in biocomfort zones in the area of Muğla province in near future due to the global climate change scenarios. J Therm Biol. 112:1–12. https://doi.org/10.1016/j.jtherbio.2022.103434.

Chuang DY, Cui J, Simonyi A, Engel VA, Chen S, Fritsche KL, Thomas AL, Applequist WL, Folk WR, Lubahn DB, Sun AY. 2014. Dietary Sutherlandia and elderberry mitigate cerebral ischemia-induced neuronal damage and attenuate p47phox and phospho-ERK1/2 expression in microglial cells. ASN Neuro. 6(6):1–15. https://doi.org/10.1177/1759091414554946.

Colling J, Stander MA, Makunga NP. 2010. Nitrogen supply and abiotic stress influence canavanine synthesis and the productivity of in vitro regenerated *Sutherlandia frutescens* microshoots. J Plant Physiol. 167(17):1521–1524. https://doi.org/10.1016/j.jplph.2010.05.018.

Coşkun F, Alptekin Y, Demir S. 2023. Effects of arbuscular mycorrhizal fungi and salicylic acid on plant growth and the activity of antioxidative enzymes against wilt disease caused by *Verticillium dahliae* in pepper. Eur J Plant Pathol. 165(1):163–177.

Croft H, Chen JM, Wang R, Mo G, Luo S, Luo X, He L, Gonsamo A, Arabian J, Zhang Y, Simic-Milas A. 2020. The global distribution of leaf chlorophyll content. Remote Sens Environ. 236:1–15. https://doi.org/10.1016/j.rse.2019.111479.

Dhami N, Cazzonelli CI. 2020. Environmental impacts on carotenoid metabolism in leaves. Plant Growth Regul. 92(3):455–477. https://doi.org/10.1007/s10725-020-00661-w.

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Sau S, Ihsan, M.Z. 2017. Crop production under drought and heat stress: Plant responses and management options. Front Plant Sci. 8:1–15. https://doi.org/10.3389/fpls.2017.01147.

Farooq A, Farooq N, Akbar H, Hassan ZU, Gheewala SH. 2023. A critical review of climate change impact at a global scale on cereal crop production. Agronomy. 13(1):162. https://doi.org/10.3390/agronomy13010162.

Gibson D. 2011. Ambiguities in the making of an African medicine: Clinical trials of *Sutherlandia frutescens* (L.) R. Br (*Lessertia frutescens*). Afr Sociol Rev. 15(1):124–137.

Goncharuk EA, Zagoskina NV. 2023. Heavy metals, their phytotoxicity, and the role of phenolic antioxidants in plant stress responses with focus on cadmium. Molecules. 28(9):1–28. https://doi.org/10.3390/molecules28093921.

Gray SB, Brady SM. 2016. Plant developmental responses to climate change. Dev Biol. 419(1):64–77. https://doi.org/10.1016/j.ydbio.2016.07.023.

Guo J, Wang Z, Qu L, Hu Y, Lu D. 2022. Transcriptomic and alternative splicing analyses provide insights into the roles of exogenous salicylic acid ameliorating waxy maize seedling growth under heat stress. BMC Plant Biol. 22(1):1–13. https://doi.org/10.1186/s12870-022-03822-3.

Hafez E, Omara AED, Ahmed A. 2019. The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. Agronomy. 9(9):1–16. https://doi.org/10.3390/agronomy9090524.

Haffajee F. 2002. Home-grown healing. New Int. 349:20–21.

Hamdi Y, Abdeljaoued-Tej I, Zatchi AA, Abdelhak S, Boubaker S, Brown JS, Benkahla A. 2021. Cancer in Africa: The untold story. Front Oncol. 11:1–19. https://doi.org/10.3389/fonc.2021.650117.

Hamedeh H, Antoni S, Cocciaglia L, Ciccolini V. 2022. Molecular and physiological effects of magnesium–polyphenolic compound as biostimulant in drought stress mitigation in tomato. Plants. 11(5):586. https://doi.org/10.3390/plants11050586.

Hasanuzzaman M, Bhuyan MB, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M. 2019. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants. 8(9):384. https://doi.org/10.3390/antiox8090384.

Hewedy OA, Elsheery NI, Karkour AM, Elhamouly N, Arafa RA, Mahmoud GAE, Dawood MFA, Hussein WE, Mansour A, Amin DH, Allakhverdiev SI. 2023. Jasmonic acid regulates plant development and orchestrates stress response during tough times. Environ Exp Bot. 208:1–14. https://doi.org/10.1016/j.envexpbot.2023.105260.

Howladar SM. 2014. A novel *Moringa oleifera* leaf extract can mitigate the stress effects of salinity and cadmium in bean (*Phaseolus vulgaris* L.) plants. Ecotoxicol Environ Saf. 100:69–75. https://doi.org/10.1016/j.ecoenv.2013.11.022.

Iqbal A, Iqbal MA, Akram I, Saleem MA, Abbas RN, Alqahtani MD, Ahmed R, Rahim J. 2023. Phytohormones promote the growth, pigment biosynthesis and productivity of green gram [*Vigna radiata* (L.) R. Wilczek]. Sustainability. 15(12):9548. https://doi.org/10.3390/su15129548.

Kaya C, Ugurlar F, Ashraf M, Ahmad P. 2023. Salicylic acid interacts with other plant growth regulators and signal molecules in response to stressful environments in plants. Plant Physiol Biochem. 196:431–443. https://doi.org/10.1016/j.plaphy.2023.02.006.

Kesawat MS, Satheesh N, Kherawat BS, Kumar A, Kim HU, Chung SM, Kumar M. 2023. Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—Current perspectives and future directions. Plants. 12(4):864. https://doi.org/10.3390/plants12040864.

Khalofah A, Bokhari NA, Migdadi HM, Alwahibi MS. 2020. Antioxidant responses and the role of *Moringa oleifera* leaf extract for mitigation of cadmium stressed *Lepidium sativum* L. S Afr J Bot. 129:341–346. https://doi.org/10.1016/j.sajb.2019.08.041.

Khan N, Mehmood A. 2023. Revisiting climate change impacts on plant growth and its mitigation with plant growth promoting rhizobacteria. S Afr J Bot. 160:586–601. https://doi.org/10.1016/j.sajb.2023.07.051.

Kobayashi Y, Fukuzawa N, Hyodo A, Kim H, Mashiyama S, Ogihara T, Yoshioka H, Matsuura H, Masuta C, Matsumura T, Takeshita M. 2020. Role of salicylic acid glucosyltransferase in balancing growth and defence for optimum plant fitness. Mol Plant Pathol. 21(3):429–442. https://doi.org/10.1111/mpp.12906.

Koo YM, Heo AY, Choi HW. 2020. Salicylic acid as a safe plant protector and growth regulator. Plant Pathol J. 36(1):1–10. https://doi.org/10.5423/PPJ.RW.12.2019.0295.

Kulak M, Jorrín-Novo JV, Romero-Rodriguez MC, Yildirim ED, Gul F, Karaman S. 2021. Seed priming with salicylic acid on plant growth and essential oil composition in basil (*Ocimum basilicum* L.) plants grown under water stress conditions. Ind Crops Prod. 161:1–11. https://doi.org/10.1016/j.indcrop.2020.113235.

Latif HH, Mohamed HI. 2016. Exogenous applications of moringa leaf extract effect on retrotransposon, ultrastructural and biochemical contents of common bean plants under environmental stresses. S Afr J Bot. 106:221–231. https://doi.org/10.1016/j.sajb.2016.07.010.

Lin X, Huang J, Yang EL. 2023. The role of salicylic acid on plant growth and longevity. ScienceOpen Preprints. 1–24.

Liu M, Xu X, Jiang Y, Huang Q, Huo Z, Liu L, Huang G. 2020. Responses of crop growth and water productivity to climate change and agricultural water-saving in arid region. Sci Total Environ. 703:1–12. https://doi.org/10.1016/j.scitotenv.2019.134621.

Ma Y, Dias MC, Freitas H. 2020. Drought and salinity stress responses and microbe-induced tolerance in plants. Front Plant Sci. 11:1–18. https://doi.org/10.3389/fpls.2020.591911.

Madhu AS, Kaur A, Tyagi S, Upadhyay SK. 2023. Glutathione peroxidases in plants: Innumerable role in abiotic stress tolerance and plant development. J Plant Growth Regul. 42(2):598–613. https://doi.org/10.1007/s00344-022-10601-9.

Mahmood S, Ahmad W, Ali Z, Eed EM, Khalifa AS, Naeem M, Bibi A, Tahir A, Waqas K, Wahid A. 2022. Exploring the potential of moringa leaf extract for mitigation of cadmium stress in *Triticum aestivum* L. Appl Sci. 12(16): 8199. https://doi.org/10.3390/app12168199.

Masenya TA, Mashela PW, Pofu KM. 2022. Efficacy of rhizobia strains on growth and chemical composition of cancer bush (*Sutherlandia frutescens*). Acta Agric Scand. 72(1):358–363. https://doi.org/10.1080/09064710.2021.2003852.

Masenya TA, Mabila SW, Hlophe T, Letsoalo ML. 2023. Vesicular arbuscular mycorrhizal influence on growth of cancer bush (*Sutherlandia frutescens*) and alleviation of saline stress. Res Crops. 24(1):179–184. https://doi.org/10.31830/2348-7542.2023.roc-894.

Mirón IJ, Linares C, Díaz J. 2023. The influence of climate change on food production and food safety. Environ Res. 216:1–6. https://doi.org/10.1016/j.envres.2022.114674.

Mittler R, Zandalinas SI, Fichman Y, Van Breusegem F. 2022. Reactive oxygen species signalling in plant stress responses. Nat Rev Mol Cell Biol. 23(10):663–679. https://doi.org/10.1038/s41580-022-00499-2.

Mncwangi N, Viljoen A, Mulaudzi N, Fouche G. 2023. *Lessertia frutescens*, p 321–344. In: Viljoen A, Fouche G, Vermaak I, Sandasi M, Combrinck S (eds). South African herbal pharmacopeia. Academic Press, London, UK. https://doi.org/10.1016/B978-0-323-99794-2.00008-8.

Omolaoye TS, Windvogel SL, Du Plessis SS. 2021. The effect of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) and sutherlandia (*Lessertia frutescens*) on testicular insulin signalling in streptozotocin-induced

diabetes in Wistar rats. Diabetes Metab Syndr Obes. 2021:1267–1280. https://doi.org/10.2147/DMSO.S285025.
Pal P, Ansari SA, Jalil SU, Ansari MI. 2023. Regulatory role of phytohormones in plant growth and development, p 1–13. In: Khan MIR, Poor P, Singh A (eds). Plant hormones in crop improvement. Academic Press, London, UK. https://doi.org/10.1016/B978-0-323-91886-2.00016-1.
Prasad PV, Djanaguiraman M. 2014. Response of floret fertility and individual grain weight of wheat to high temperature stress: Sensitive stages and thresholds for temperature and duration. Funct Plant Biol. 41(12):1261–1269. https://doi.org/10.1071/FP14061.
Rady MM, Mohamed GF. 2015. Modulation of salt stress effects on the growth, physio-chemical attributes and yields of *Phaseolus vulgaris* L. Plants by the combined application of salicylic acid and *Moringa oleifera* leaf extract. Sci Hortic. 193:105–113. https://doi.org/10.1016/j.scienta.2015.07.003.
Rocha Júnior DS, Barbosa ACO, Batista IA, Camillo LR, Lopes NS, Costa MG. 2023. Impact of moderate water deficit at the fruit development stage of tomato (*Solanum lycopersicum* L.): Effects on plant growth, physiology, fruit yield and quality and expression of carotenoid biosynthesis genes. Acta Physiol Plant. 45(5):1–14. https://doi.org/10.1007/s11738-023-03549-0.
Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM. 2003. Salicylic acid prevents the damaging action of stress factors on wheat plants. Bulg J Plant Physiol. 21: 314–319.
Shaffique S, Khan MA, Wani SH, Pande A, Imran M, Kang SM, Rahim W, Khan SA, Bhatta D, Kwon EH, Lee IJ. 2022. A review on the role of endophytes and plant growth promoting rhizobacteria in mitigating heat stress in plants. Microorganisms. 10(7):1286. https://doi.org/10.3390/microorganisms10071286.
Shah SH, Islam S, Alamri S, Parrey ZA, Mohammad F, Kalaji HM. 2023. Plant growth regulators mediated changes in the growth, photosynthesis, nutrient acquisition and productivity of mustard. Agriculture. 13(3):570. https://doi.org/10.3390/agriculture13030570.
Shahrajabian MH, Sun W. 2022. Sustainable approaches to boost yield and chemical constituents of aromatic and medicinal plants by application of biostimulants. Pat Food Nutr Agric. 13(2):72–92. https://doi.org/10.2174/2772574X13666221004151822.
Shaik S, Singh N, Nicholas A. 2011. HPLC and GC analyses of in vitro-grown leaves of the cancer bush *Lessertia* (*Sutherlandia*) *frutescens* L. reveal higher yields of bioactive compounds. Plant Cell Tiss Organ Cult. 105:431–438. https://doi.org/10.1007/s11240-010-9884-4.
Shakour ZTA, Radwa H, Elshamy AI, El Gendy AENG, Wessjohann LA, Farag MA. 2023. Dissection of *Moringa oleifera* leaf metabolome in context of its different extracts, origin and in relationship to its biological effects as analysed using molecular networking and chemometrics. Food Chem. 399:133948. https://doi.org/10.1016/j.foodchem.2022.133948.
Sharma A, Sidhu GPS, Araniti F, Bali AS, Shahzad B, Tripathi DK, Brestic M, Skalicky M, Landi M. 2020. The role of salicylic acid in plants exposed to heavy metals. Molecules. 25(3):540. https://doi.org/10.3390/molecules25030540.
Sherin G, Aswathi KR, Puthur JT. 2022. Photosynthetic functions in plants subjected to stresses are positively influenced by priming. Plant Stress. 4:1–12. https://doi.org/10.1016/j.stress.2022.100079.
Singh P, Singh RK, Li HB, Guo DJ, Sharma A, Verma KK, Solanki MK, Upadhyay SK, Lakshmanan P, Yang LT, Li YR. 2023. Nitrogen fixation and phytohormone stimulation of sugarcane plant through plant growth promoting diazotrophic *Pseudomonas*. Biotechnol Genet Eng Rev. [advance online publication]. https://doi.org/10.1080/02648725.2023.2177814.
Soliman WS, Zakria Y, Abdel-Rahman SSA, Salaheldin S. 2020. Effect of salicylic acid, moringa leaves extract and seaweed extract on growth, yield and quality of roselle, *Hibiscus sabdariffa* L. under Aswan conditions. SVU-Int J Agric Sci. 2(2):476–483. https://doi.org/10.21608/svuijas.2020.52563.1061.
Sosnowski J, Truba M, Vasileva V. 2023. The impact of auxin and cytokinin on the growth and development of selected crops. Agriculture. 13(3): 724. https://doi.org/10.3390/agriculture13030724.
Srivastava K, Singh S, Singh A, Jain T, Datta R, Kohli A. 2023. Effect of temperature (cold and hot) stress on medicinal plants, p 153–168. In: Azamal H, Iqbal M (eds). Medicinal plants: Their response to abiotic stress. Springer Nature, Singapore.
Street RA, Prinsloo G, 2013. Commercially important medicinal plants of South Africa: A review. J Chem. 2013:1–16. https://doi.org/10.1155/2013/205048.
Sujata Goyal V, Baliyan V, Avtar R, Mehrotra S. 2023. Alleviating drought stress in *Brassica juncea* (L.) Czern & Coss. by foliar application of biostimulants—orthosilicic acid and seaweed extract. Appl Biochem Biotechnol. 195(1): 693–721.
Sun C, Liu L, Zhou W, Lu L, Jin C, Lin X. 2017. Aluminum induces distinct changes in the metabolism of reactive oxygen and nitrogen species in the roots of two wheat genotypes with different aluminum resistance. J Agric Food Chem. 65(43):9419–9427. https://doi.org/10.1021/acs.jafc.7b03386.
Taffouo VD, Nouck AE, Nyemene KP, Tonfack B, Meguekam TL, Youmbi E. 2017. Effects of salt stress on plant growth, nutrient partitioning, chlorophyll content, leaf relative water content, accumulation of osmolytes and antioxidant compounds in pepper (*Capsicum annuum* L.) cultivars. Not Bot Horti Agrobot Cluj Napoca. 45(2):481–490.
Tamta P, Patni B. 2023. Exploring the role of high-temperature stress on medicinal plants: A review. J Stress Physiol Biochem. 19(3):16–23.
Wang S. 2015. Sensory and GC profiles of roasted peanuts: Their relationships to consumer acceptability and changes during short storage (Master's Thesis). University of Georgia, Athens, GA, USA.
Yap YK, El-Sherif F, Habib ES, Khattab S. 2021. *Moringa oleifera* leaf extract enhanced growth, yield, and silybin content while mitigating salt-induced adverse effects on the growth of Silybum marianum. Agronomy. 11(12):2500. https://doi.org/10.3390/agronomy11122500.
Zhang Z, Li Y, Xinguo C, Wang Y, Niu B, Li Liu D, He J, Pulatov B, Hassan I, Meng Q. 2023. Impact of climate change and planting date shifts on growth and yields of double cropping rice in southeastern China in future. Agric Syst. 205:1–12. https://doi.org/10.1016/j.agsy.2022.103581.
Zulfiqar F, Younis A, Finnegan PM, Ferrante A. 2020. Comparison of soaking corms with moringa leaf extract alone or in combination with synthetic plant growth regulators on the growth, physiology and vase life of sword lily. Plants. 9(11):1590. https://doi.org/10.3390/plants9111590.