INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2024, Vol. 3, NO. 2, 177-187
doi: 10.56612/ijaaeb.v1i1.96
https://eminentscientists.com/

Potential of natural plant extracts as biostimulants to alleviate salt stress-induced adverse effects on wheat

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Abstract
A field experiment was conducted to investigate the role of different plant extracts in alleviating salt stress-induced adverse effects on wheat plants. Two potential wheat cultivars, Galaxy-2013 and Faisalabad-2008, were sown in November 2020 in saline soil with soil electrical conductivity of 9.8 dS m^{-1}. At the booting and anthesis stages, the wheat plants were exogenously sprayed with three different types of plant extracts, i.e., moringa leaf extract (MLE, 3%), sorghum water extract (SWE, 2%), and brassica water extract (BWE, 2.5%). The MLE spray at the booting and anthesis stages resulted in a maximal increase in 1000-grain weight (34-43%), grain yield (25-29%), biological yield (39-40%) and harvest index (23-24%) of wheat over the control. However, exogenous application of all three plant extracts in both wheat cultivars increased plant height (44-64%), spike length (99-100%), number of spikelets (40-42%), and grains per spike (32-64%), as compared to the untreated plants grown under saline conditions. Similarly, chlorophyll (Chl) a, Chl b, total chlorophyll, and carotenoid contents were higher in the MLE-treated plants than those of the plants treated with the other extracts. Overall, MLE at the rate of 3% had the most significant growth promoting effect on both wheat cultivars under saline conditions. Cultivar Faisalabad-2008 fed with different plant extracts was superior to Galaxy-2013 in terms of performance in growth and physiological attributes under saline stress.

ARTICLE TYPE
Research Paper (RP)

SECTION
Plant Biology (PB)

HANDLING EDITOR
İbadullayeva, S.J. (PB)

ARTICLE HISTORY
Received: 9 Nov, 2023
Accepted: 15 Feb, 2024
Published: 04 Jul, 2024

KEYWORDS
Brassica water extract;
Cereal crops;
Chlorophyll contents;
Moringa leaf extract;
Sorghum water extract

Introduction

Various biotic and abiotic factors significantly influence crop development and production,

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TO CITE THIS ARTICLE: Baig, H.A.T., Khaliq, A., Rehmani, M.I.A., Shoukat, U., Sannan, M., Sanaullah, Baloch, H., Iqbal, S., Raza, S., Raza, M.H., Saddiq, M.S. (2024). Potential of natural plant extracts as biostimulants to alleviate salt stress-induced adverse effects on wheat. *International Journal of Applied and Experimental Biology* 3(2): 177-187.

contributing to decreased field crop yield (Lu et al., 2023; Monteiro et al., 2022). Of several abiotic stresses occurring in nature, salinity stress is a common and significant abiotic stress. For example, about 20% of the world's arable land is affected by salt stress and is steadily increasing due to climate change and human activity (Hasanuzzaman and Fujita, 2022). Salinization is particularly important in Pakistan as in most parts of the country underground saltwater is being widely and frequently used for irrigation, which causes the soil to accumulate high amounts of salts (Haj-Amor et al., 2022) .

The most important crop for ensuring Pakistan's food security is wheat, as it is the most staple food being widely utilized (Hafeez et al., 2021a). Among various crops, wheat is generally more sensitive to salinity, as salt stress hinders its growth and development, leading to reduced productivity or even complete crop failure under extreme salinity stress (Saddiq et al., 2021a, b). Salinity stress inhibits seedling emergence, plant development and various plant processes, causing compromised yield and quality of crops including wheat (Askari-Khorasgani et al., 2021; Yasir et al., 2021; Zahra et al., 2023). During various stages of growth and development, cultivars' capacity for salt tolerance may differ (Siddiqui et al., 2017). Large amounts of soluble salts like sodium chloride retard the germination, a very sensitive growth stage, through osmotic stress and specific ion toxicity (Kamran et al., 2021; Naz et al., 2021; Feghhenabi et al., 2022; Roy et al., 2022). Moreover, anthesis and grain filling periods have also been reported as very sensitive stages under certain environmental conditions, such as salinity (Teng et al., 2023). It has been stated that wheat grain yield is impacted by electrical conductivity (ECs) greater than 8 dS m^{-1}; 50% of yield is lowered at 10 dS m^{-1}; and 100% yield losses are recorded at 16–24 dS m^{-1} of salinity stress (Hussain et al., 2019).

High levels of salinity may physiologically result in ionic imbalance in plant tissues due to increased chloride (Cl^-) and sodium (Na^+) concentrations which subsequently result in the reduction of nutrient uptake (Granaz et al., 2022). Bio-molecules, i.e., DNA, proteins, and lipids are severely damaged by the reactive oxygen species production (ROS) causing peroxidation, mutation, denaturation, and degradation of membrane lipids and other cellular ultra-structures resulting ultimately in cellular death (Sharma et al., 2012; Zahra et al., 2021). Under salinity, the ability of the plants to regulate the Na^+/K^+ ratio in the tissues has a significant impact on plant growth (Granaz et al., 2022). Excessive toxic salts taken up by crop plants diminish their growth through disturbing enzyme activities, turgor pressure, and photosynthesis (Shahid et al., 2022; Zahra et al., 2022).

There are several ways to mitigate the negative effects of salinity stress, such as the use of plant growth regulators (phytohormones, osmoprotectants, and natural plants extracts), cultural practices, fertilizers, natural and synthetic mulches, etc. (Iqbal et al., 2019; Shaukat et al., 2022). Among them, exogenous application of natural plant extracts is an eco-friendly and economical approach to overcoming salinity stress (Bajwa et al., 2020). For example, foliar application of MLE delayed crop maturity, increased the time for grain filling, and improved the yield of wheat by 10% (Yasmeen et al., 2013). The wheat plants fed with MLE were reported to have absorbed maximum amount of nitrogen (N), phosphorus (P), and potassium; also the treated plants showed improved quantity of photosynthetic pigments such as chlorophyll a and b, and carotenoids (Merwad et al., 2017). Sorghum [*Sorghum bicolor* (L.) Moench] is the most researched allelopathic crop (Farooq et al., 2017). For example, application of sorghum water extract (SWE) at a concentration of 5–10% to wheat plants at the anthesis stage enhanced water relations, membrane stability, and grain and biological yields (Bajwa et al., 2020). In another study with wheat, SWE improved grains per spike, spike length, and 1000-grain weight (Afzal et al., 2020). *Brassica* water extract (BWE) has been widely used to mitigate abiotic stresses like high temperature and salinity (Farooq et al., 2021), because it contains a vital growth regulator brassinolide that can affect different development processes such as growth, seed germination, senescence, and flowering (Sharma et al., 2018). Many other studies suggested a beneficial role for brassinolide in mitigating different abiotic stresses, like oxidative stress in plants caused by herbicides and heavy metals (Shahzad et al., 2018; Sharma et al., 2018).

Although a variety of allelochemicals as biostimulants are under use for achieving enhanced crop tolerance to abiotic stressors, no trial has been conducted so far to assess the impact of exogenously applied plant water extracts on regulation of growth and key physiological processes in wheat. In the current trial, it was assessed that up to what extent exogenous application of natural plant extracts could improve the salinity stress tolerance in wheat cultivars.

Materials and Methods

Field experiment site

An experiment was conducted in saline field conditions at the Agronomic Research Area (PARS Campus), University of Agriculture Faisalabad, during 2020-21. The weather data of the site is presented in **Table 1**. The soil had a loamy texture, electrical conductivity of 9.8 dS m^{-1}, organic matter 0.72%, total nitrogen 0.045 mg kg^{-1}, pH 8.9, available phosphorus 7.3 mg kg^{-1}, and available potassium 260 mg kg^{-1}.

Table 1. Weather data of experimental station during the wheat season 2020-2021

Months	RF	RH	Tmax	Tmin	Tave	Sun
November	1.4	63.3	27.9	10.8	19.3	6.7
December	1.7	79.6	21.6	6.8	14.2	6.4
January	49.6	83.9	21.0	5.8	12.0	5.2
February	0	74.6	25.3	9.4	17.3	7.7
March	40.8	67.0	30.0	16.0	23.0	7.9
April	11.2	54.6	34.6	18.7	26.7	8.5

RF = Rainfall (mm); RH = Mean daily relative humidity (%); Tmax = Mean daily maximum temperature (°C) ; Tmin = Mean daily minimum temperature (°C); Tave = Mean daily average temperature (°C); Sun = Mean sunshine (hours)
Source: Agricultural Meteorology Cell, University of Agriculture, Faisalabad, Pakistan.

Treatments

The study used a Randomized Complete Block Design (RCBD) with five treatments and three replications. The treatments were T1 (Control, No Spray, NS), T2 (Water spray, WS), T3 (3.0% moringa leaf extract, MLE), T4 (2.5% brassica water extract, BWE), and T5 (2.0% sorghum water extract, SWE).

Seed sowing

Line sowing of two wheat cultivars, Glaxy-2013 and Faisalabad-2008, was done manually with a hand drill. Each plot was 3 m long and 1.35 m wide, with 6 rows spaced 22.5 cm apart. The seed rate per hectare was 125 kg ha^{-1}.

Preparation of plant extracts

Moringa leaf extract (MLE) was prepared from fresh, disease-free, and healthy moringa leaves obtained from mature moringa plants. Before the extraction process, plant material (mature leaves and tender branches) was thoroughly washed with distilled water and stored in a freezer for 12 h. The extraction was then carried out using a locally manufactured extraction machine (Yasmeen et al., 2013). Thereafter, the extract was sieved with a cheesecloth, and the filtrate was considered as a stock solution and then diluted with distilled water to get the appropriate concentrations.

Sorghum and brassica water extracts were prepared from the mature plants collected from the Agronomic Farm, University of Agriculture Faisalabad, Pakistan. The collected plants were cut into 2-3 cm pieces using a fodder cutter, air-dried, and kept for drying in shade for a few days. Following the procedure mentioned by Khaliq et al. (2013) , the chopped plant material was steeped in distilled water for 24 h at room temperature in a 1:10 (w/v) ratio. The extract was filtered with a sieve after 24 h. This was 10% sorghum and brassica extract, thereafter each extract was diluted to get desired concentrations as mentioned earlier.

Intercultural practices

Canal water was used to irrigate the crop and the crop was irrigated five (05) times in the growing season. All other protective measures were adopted according to the requirement and availability.

Crop harvesting and data collection

At full maturity, the crop was harvested. The grain yield was calculated at 14% moisture, whereas the straw yield was calculated using sun-dry conditions. Five hills were chosen at random from each plot and data on plant height, spike length, number of spikelets per spike, number of grains per spike, 1000-grain weight, biological yield, and grain yield were recorded.

Chlorophyll contents

Wheat fresh leaf samples (0.5 g each) were used to measure the amount of chlorophyll and carotenoids. Following Arnon (1967), levels of chlorophyll a, b, and carotenoids in the leaves were determined. This procedure involved extracting the pigments in 80% aqueous acetone and measured them in the same solvent. The filtrates were read for Chl a at 665 nm, Chl b at 648 nm, and carotenoids at 480 nm.

Enzymatic Antioxidants

The activity of catalase (CAT) was appraised following the procedure described by Dhindsa et al. (1981). The enzyme extract (50 µL) was reacted with I mL of the CAT reaction solution containing 50 mM phosphate buffer (pH 7.0) and 15 mM H_2O_2. Alteration in OD was read at 240 nm after 1 min. One unit of CAT activity was defined as a decrease in OD of 0.01 unit min^{-1}.

The activity of guaiacol peroxidase (POX) was appraised according to the protocol illustrated by

Pütter (1974). A proportion of (0.1 g) frozen leaf tissue was finely homogenized in 1 mL of potassium phosphate extraction buffer (300 mmol L^{-1}, pH 7.0), containing 1 mmol L^{-1} EDTA. After properly centrifugating the extract, 50 µL of the extract was treated with potassium phosphate buffer (300 mmol L^{-1}, pH 6.1) by adding 8.42 mmol L^{-1} guaiacol and 2.10 mmol L^{-1} H_2O_2. Conversion of guaiacol to tetraguaiacol was observed at 470 nm, and POD activity was worked out using the absorbance coefficient, 26,600 l M^{-1} cm^{-1}.For measuring the activity of superoxide dismutase (SOD) following the method illustrated elsewhere (Giannopolitis, Ries 1977), the leaf tissue was homogenized in 0.05 M phosphate buffer. The extract so obtained was treated with methionine (13 mM), nitroblue tetrazolium (75 µM), riboflavin (2 µM) and EDTA (0.1 mM). The amount of enzyme required to cause 50% inhibition of nitroblue tetrazolium reduction at 560 nm was considered as one unit of SOD activity.

Statistical analysis of data

The data collected for each variable was statistically evaluated using the analysis of variance (ANOVA) technique in a factorial randomized complete block design (RCBD). Data related to various factors were analyzed using the statistical package "Statisticx 8.1" and the mean data were compared through Tukey's Honestly Significant Difference (HSD) test.

Results

Influence of different plant extracts on growth components of wheat cultivars

Salt stress had a negative impact on wheat growth components, but foliar treatments with extracts of sorghum, moringa, and brassica were effective in reducing the salt-induced reduction in growth (**Figure 1**). The effects of the extracts on plant height, number of spikelets, spike length, and number of productive tillers were statistically significant. Application of all three extracts enhanced shoot length, spike length, the number of spikelets, and productive tillers of both wheat cultivars as compared to the controls (no spray) and water spray (**Figure 1**). At 2.5% BWE, plant height increased in Galaxy-2013 by 44-64%, spike length as 99-100%, number of spikelets 40-42%, and number of productive tillers 37-16% with respect to the control (no spray), while Faisalabad-2008 had lower values for all these measured variables (**Figure 1**).

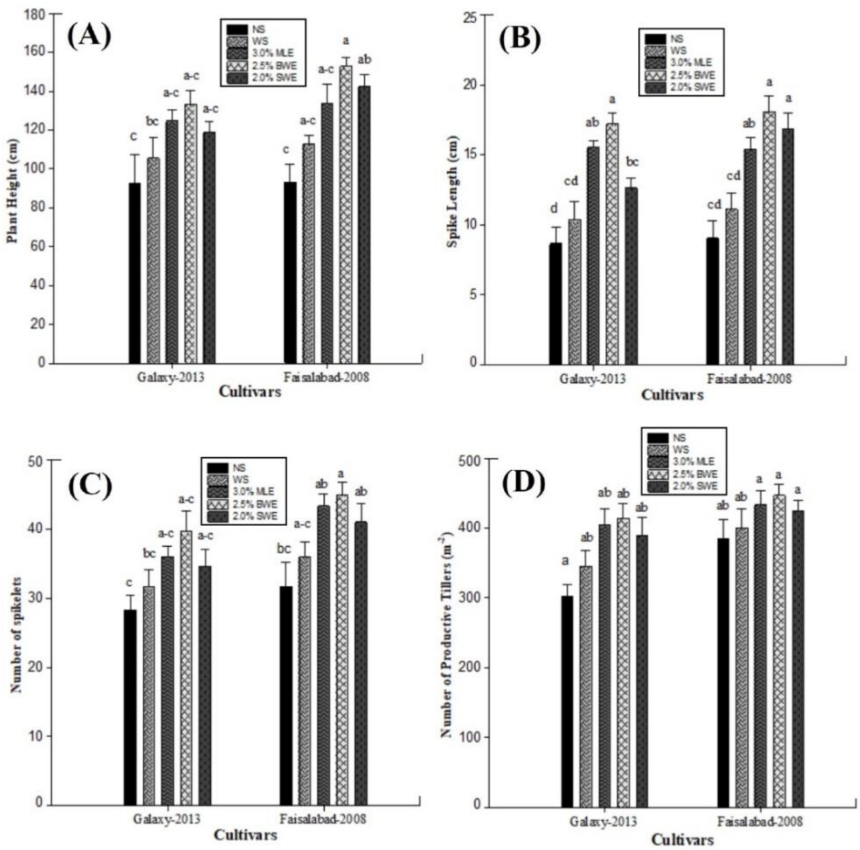

Figure 1. Effect of foliar application of different plant extracts [(NS, no spray), WS (water spray), MLE (moringa leaf extract), BWE (brassica water extract), SWE (sorghum water extract)] on plant height (A), spike length (B), number of spikelets/spike (C), and number of productive tillers (D) of two wheat cultivars under saline conditions.

Influence of different plant extracts on yield components

In comparison to the control (no spray) and water spray, the application of MLE, BWE, and SWE improved the number of grains per spike, 1000-grain weight, grain yield, biological yield, and harvest index in both wheat cultivars (**Figure 2**). In the treatment where water spray was used, the lowest values of 1000-grain weight, grain yield, biological yield, and harvest index were attained (**Figure 2**). Of the three extracts used, MLE had the highest stimulatory effect on grain yield, biological yield, harvest index, and 1000-grain weight in both wheat varieties (**Figure 1**). Galaxy-2013, a salinity-sensitive cultivar, responded less favorably to foliar treatments of 3.0% MLE, 2.5% BWE, and 2.0% SWE than did cv. Faisalabad-2008.

Influence of different plant extracts on photosynthetic pigments

The photosynthetic pigments of both wheat cultivars were significantly affected by salinity stress and foliar application of different plant extracts (**Figure 3**). In comparison to the control (no spray) and water spray, the application of MLE, BWE, and SWE increased the levels of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in both wheat cultivars (**Figure 3**). As compared to the control (no spray), application of 3.0% MLE enhanced the amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids (91-48%) in both wheat cultivars (**Figure 3**). The treatment in which water spray was administered, produced the lowest values of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids (**Figure 3**). According to the findings, MLE was more effective than the other extracts in increasing total chlorophyll, chlorophyll a, and b, as well as carotenoids in both wheat varieties. In contrast to cv. Faisalabad-2008, Galaxy-2013, a salinity-sensitive cultivar, responded less effectively to foliar sprays of different plant extracts applied.

Influence of different plant extracts on enzymatic antioxidants

The activities of the antioxidant enzymes, catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), were considerably impacted by salinity stress (**Figure 4**). In both wheat cultivars, the effects of extracts on the antioxidant enzymes were significant at $P < 0.05$. (**Figure4**). When compared

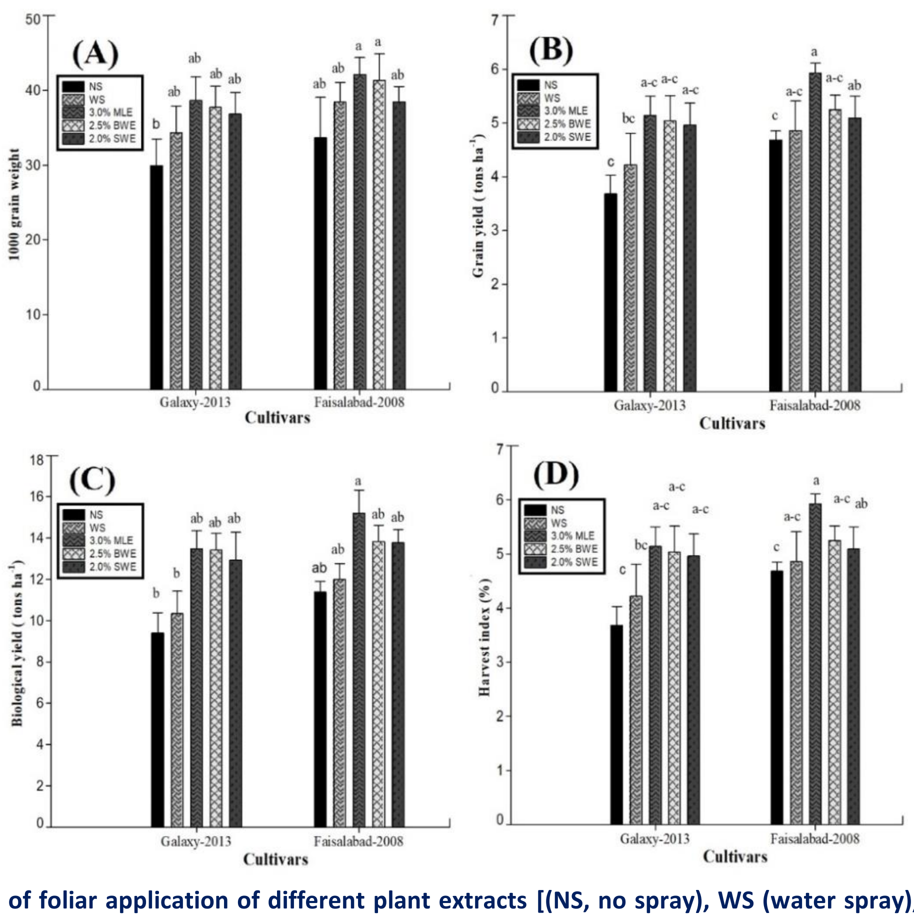

Figure 2. Effect of foliar application of different plant extracts [(NS, no spray), WS (water spray), MLE (moringa leaf extract), BWE (brassica water extract), SWE (sorghum water extract)] on 1000-grain weight (A), grain yield (B), biological yield (C), and harvest index (D) of two wheat cultivars grown under saline conditions.

with the control (no spray) and water spray, the application of MLE, BWE, and SWE increased the activities of SOD, POD, and CAT of both wheat cultivars (Fig. 4). In comparison to the control (no spray), 3.0% MLE application raised POD by 105% in Galaxy-2013, and in Faisalabad-2008 by 60%, and CAT by 37% in Galaxy-2013 and 38% in Faisalabad-2008, respectively (Fig. 4). However, in contrast, the application of 2.5% BWE raised SOD by 42% in Galaxy-2013 and 21% in Faisalabad-2008, respectively (Fig. 4). The results showed that MLE was more effective than the other extracts used in elevating the activities of POD and CAT in both wheat cultivars. However, in contrast, the activity of SOD was increased much with the foliar application of BWE. Overall, Galaxy-2013, being a salinity sensitive cultivar, compared to Faisalabad-2008, was less responsive to foliar applications of different plant extracts.

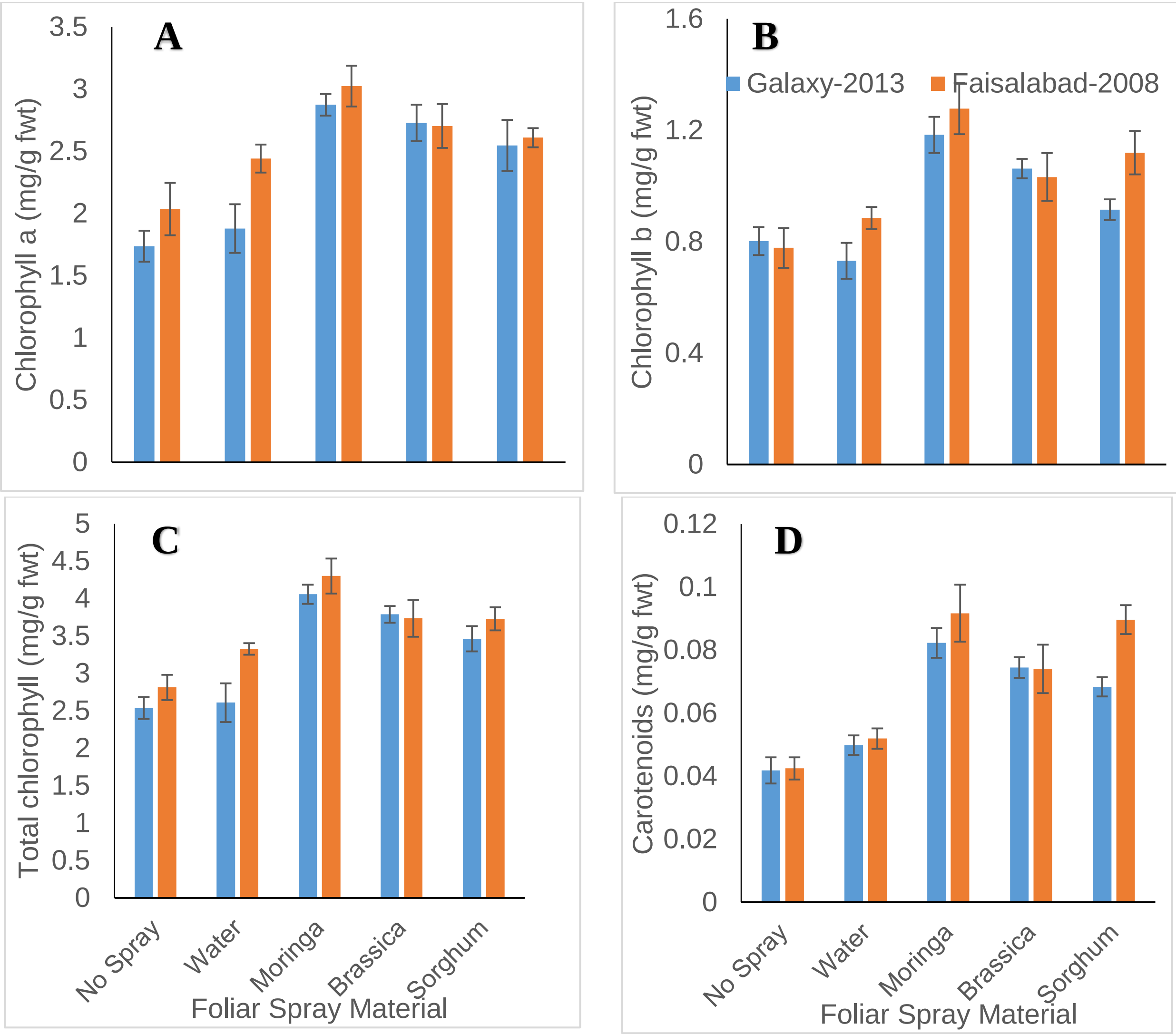

Figure 3. Effect of foliar application of different plant extracts [(NS, no spray), WS (water spray), MLE (moringa leaf extract), BWE (brassica water extract), SWE (sorghum water extract)] on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) of two wheat cultivars under saline conditions.

Discussion

The most common allelochemicals found in plant extracts include organic acids, phenols and terpenoids. Among organic acids, particularly phenolic acids are the most essential allelochemicals (Qasem and Issa, 2018). Many studies have found that lower concentrations of phenolic acids can promote plant growth, whereas high concentrations inhibit it (Afzal et al., 2020). The current study showed that different plant extracts most likely enriched with earlier-mentioned biomolecules, had different growth-regulating effects on the wheat varieties. The control of various physiological events may be responsible for these growth-promoting effects (Bajwa et al., 2020).

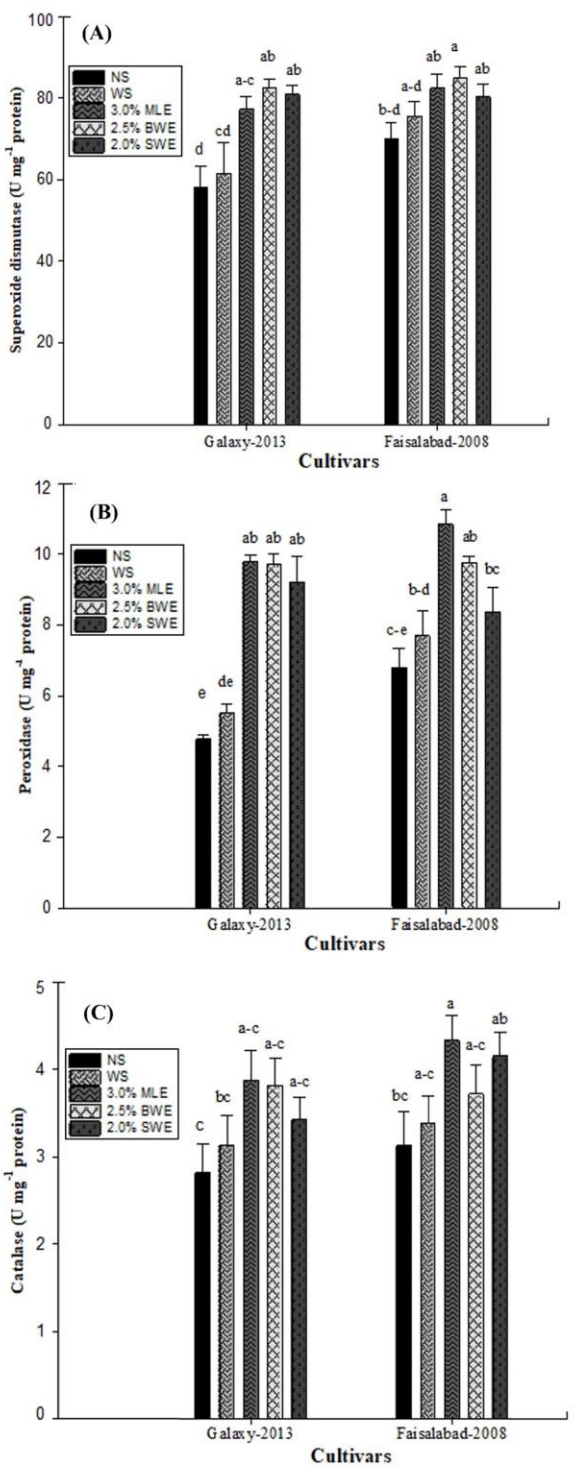

Figure 4. Effect of foliar application of different plant extracts [(NS (no spray), WS (water spray), MLE (moringa leaf extract), BWE (brassica water extract), SWE (sorghum water extract)] on SOD (A), POD (B) and CAT (C) of two wheat cultivars grown under saline conditions.

Of various natural plant extracts used in the current study, MLE was found to be a very efficient and economical growth enhancer since it is enriched with cytokinins, macro-micronutrients, and antioxidants (Yasmeen et al., 2013), although the growth-promoting effects of other plant extracts (BWE and SWE) are also evident in terms of data for different growth and physiological parameters recorded in the current study. BWE is known to contain a vital growth regulator, brassinolide, which can effectively promote growth and key metallic processes in different plants (Sharma et al., 2018). Likewise, SWE has been reported to also contain a myriad of metabolites such as alkaloids, flavonoids and phenolics which have a substantial role in upregulating plant defense system (Mandal et al., 2010). The foliar application of MLE significantly increased growth-related attributes such as grain yield, biological yield, 1000-grain weight, and harvest index (Figure 2) in salt stressed plants of the two wheat cultivars. Merwad et al. (2020) also advocated that foliar-applied MLE can improve plant resistance against abiotic stresses and increase crop yield due to accelerated growth. Similar findings were shown while using MLE as a foliar treatment to the wheat crop (Yasmeen et al., 2013; Jhilik et al., 2017; Riaz et al. 2022).

Under salinity stress, chlorophyll a, b, carotenoids, and other photosynthetic pigments were found to be significantly reduced in both wheat cultivars. A similar salt-induced adverse effect on photosynthetic pigments has also been documented in faba bean (Ragab et al., 2022). The foliar application of MLE significantly enhanced photosynthetic pigments like as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in both wheat cultivars under saline stress as have been earlier reported elsewhere in the same crop (Riaz et al., 2022). Merwad et al. (2017) also reported similar results in terms of enhanced crop growth, chlorophyll a, chlorophyll b, carotenoids, and yield of wheat with foliar applied MLE. Ogbuehi and Agbim (2017) also found increased levels of chlorophyll content in soybean leaves fed with MLE. MLE may contain a variety of biomolecules such as auxins, abscisic acid (ABA) and cytokinins , ascorbate, Ca, K, Fe, amino acids and some other growth hormones (Faisal et al., 2020), which can significantly influence plant growth and synthesis of photosynthetic pigments. Moreover, foliar application of MLE is believed to prevent premature leaf senescence, and accelerate cell division and cell enlargement resulting in larger leaf area having more photosynthetic pigments (Farooq et al., 2023).

MLE is a natural source of antioxidants mainly such as phenolics, which involves scavenging of free radicals produced in plants under salt stress (Riaz et al., 2022). Additionally, MLE is also a natural source of adenine and zeatin derivatives (Faisal et al., 2020), which belong to the growth hormone group cytokinins (Jahanzaib et al., 2022). Zeatins trigger the antioxidant level of different enzymes and decrease the production of ROS. In the present investigation, the activities of SOD, POD and CAT increased significantly in the plants of both wheat cultivars fed with different types of natural plant extracts. These findings corroborate with those of Desoky et al. (2018) who also reported that application of MLE effectively improved the activities of SOD, POD, and CAT in salt stressed sorghum plants.. Earlier, Yasmeen et al. (2013) reported that fresh MLE possesses antioxidants in a considerable amount that can effectively regulate the key physiological processes in salt stressed wheat plants. Next to MLE, BWE also showed beneficial effects in terms of promoting growth and key metabolic processes including the antioxidant system in both wheat cultivars. Since BWE consists of a considerable amount of brassinolide, a plant growth promoter, so promotion of plant growth due to this vital hormone is expected as earlier reported elsewhere (Bajwa et al., 2020; Hafeez et al., 2021b).

Conclusion

Foliar application of 3.0% fresh MLE, 2.5% BWE, and 2.0% SWE played an effective role in enhancing growth, yield, photosynthetic pigments and the activities of antioxidant enzymes under saline stress of both wheat cultivars examined in this study. Thus, plant extracts being natural products are easily available throughout all seasons, and are an inexpensive source which can be easily used to alleviate the yield-limiting impacts of salt stress on the wheat crop as well as other such potential crops.

Author(s), Editor(s) and Publisher's declarations

Acknowledgement
The authors gratefully acknowledge the lab and field staff of Department of Agronomy, University of Agriculture Faisalabad, for their technical support throughout the whole experimentation.

Conflict of interest
The authors declare no conflict of interest.

Source of funding
Declared none

Contribution of authors
Conceptualized and designed the study: HAB, SI, AK. Conducted field experiments and wrote up the first draft of the manuscript: HAB, US. Analyzed the data: HAB, MSS, MIAR, SR. Reviewed and edited the manuscript: All authors have read, reviewed and agreed to publish the current version of the manuscript in IJAaEB.

Ethical approval
This study does not involve human/animal subjects, so no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors contributed in designing and writing the entire article. All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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Declaration of Generative AI and AI-assisted technologies in the writing process

It is declared that we the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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