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Comparative effectiveness of Moringa leaf extract, a natural biostimulant, with inorganic NPK fertilizer for regulating growth and the activities of enzymatic antioxidants in wheat (*Triticum aestivum* L.)

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Abstract
Bio-stimulants include a range of compounds effective enough to enhance certain physiological processes that promote crop ontogeny and development, so that the use of synthetic fertilizers can be greatly decreased. Moringa Leaf Extract (MLE) is a good source of fertilizers alterative to NPK and other macro- and micro-nutrients. MLE has the ability of promoting healthy and safe foods that are environment-friendly. Therefore, the use of safe natural bio-stimulants such as MLE to enhance the growth and productivity of food crops is indispensable these days. The present study was designed to examine the efficiency and effectiveness of MLE with respect to NPK fertilizers on two spring wheat varieties, Akbar-2019 and Ujala-2016. Pot experiments were conducted in the Botanical Garden of the University of Gujrat, Gujrat., Pakistan. The experiments were laid down in a completely randomized design (CRD) with four replications. Three levels of Moringa leaf extract (0%, 10%, and 20%) and three levels of NPK fertilizers (0%, 10%, and 20%) were applied after two weeks of seed germination. It was found that 10% and 20 % of both MLE and NPK enhanced the crop growth by increasing foliage growth, and antioxidative enzymes activities. MLE applied as 10% showed better results in terms of enhancing the crop growth as compared to NPK. MLE was also more effective than NPK in promoting the activities of key enzymatic antioxidants such as SOD, CAT and POD. Of the two varieties of wheat, Ujala-2016 had better response to MLE 10% in terms of growth and biochemical attributes measured in this study. It was concluded that MLE, being cost-effective and eco-friendly, can be applied as a potential source of fertilizers to improve growth and yield of wheat.

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Introduction

Food demand will increase due to an increase in the human population of the world from the current 7.7 billion to approximately 9.6 billion till the year 2050, due to which there is a high demand of food crop

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production world-over (Zulfiqar et al., 2020). Wheat (*Triticum aestivum* L.) is grown in many countries of the world as a staple food. Wheat is a chief source of food for an ever-growing world population, but despite adopting a multitude of strategies its production is not being increased significantly so as to meet the demands of human consumption. Although inorganic fertilizers have been in use for many decades for enriching fertilization for all types of crops, the rise in prices of the inorganic fertilizers has forced the farmers to adopt other means of fertilization (Kumar et al., 2022).

Interest in the use of Moringa Leaf Extract (MLE) as a bio-stimulant ranges from ecologists to researchers and scientists worldwide (Rady and Mohamed, 2015; Pusta and Macusi, 2024). MLE can be derived in different ways such as pressurized water extract, aqueous, and solvent extract. The Moringa leaf extract has the ability of promoting healthy and safe foods that are environment-friendly and nutritious. Therefore, the use of safe natural bio-stimulants such as MLE to enhance growth and harvest of food crops is indispensable these days (Elzaawely et al., 2017; Pusta and Macusi, 2024).

Moringa (*Moringa oleifera* L.) extract has been recognized as one of the best bio-stimulants that has a marked stimulatory effect on improving quantity and quality of various plants such as alfalfa, senna, clitoris and mung beans (Yasmeen et al., 2016; Yuniati et al., 2022). Moringa extract is enriched in K, Fe, Ca, amino acids, ascorbic acid and zeatin (Gopalakrishnan et al., 2016; Mashamaite et al., 2022). MLE extracts are ideal plant growth promoters for a variety of crops such as cotton, tomato and wheat, which increased yield by 20-35% (Yasmeen et al., 2013). Biswas et al. (2016) reported that the spray of MLE to maize enhanced plant growth and improved yield. In another study, application of Moringa leaf extract to soybean (*Glycine max* L.) improved the pod size and sugar content (Phiri and Mbewe, 2010). Likewise, the growth and yield of kidney bean (*Phaseolus vulgaris* L.) was reported to increase with the application of Moringa leaf extract.

In view of all these earlier-mentioned reports, it is naïve to expect that Moringa Leaf Extract, being an organic fertilizer, could be a potential alternative to inorganic fertilizers such as those of NPK. Thus, the primary objective of the present investigation was to examine the efficiency and effectiveness of MLE in comparison with inorganic NPK fertilizers in promoting growth of wheat.

Materials and Methods

The experiments were conducted in the Botanical Garden, Department of Botany, University of Gujrat, Pakistan during 2022-23. Two wheat varieties, Akbar-2019 and Ujala-2016, were sown in plastic pots each filled with 7 kg sandy loam soil. Eight healthy seeds were sown in each pot. After 10 days of germination, thinning was done and 4 plants were kept in each pot. After 14 days of seed germination, following treatments were applied:

T0 = Control (water spray)
T1 = 10% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%)
T2 = 20% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%)
T3 = 10% solution of MLE powder
T4 = 20% solution of MLE powder

The experiment was laid down in a completely randomized design (CRD) with four replicates for each treatment. Data for various morphological, biochemical and physiological attributes were collected at the vegetative and grain filling stages.

Root and shoot fresh and dry weights (g) as well as root and shoot lengths were accurately recorded. Number of leaves per plant was counted to assess the foliage growth of plants. Leaf area (cm^2) was calculated manually by measuring leaf length and leaf width of flag leaf according to the formula described by Carleton and Foote (1965).

Leaf area (cm^2) = leaf length maximum x leaf width maximum x Correction factor (0.75)

Antioxidant enzyme activities

Wheat fresh leaves were crushed in a clean pestle & mortar by placing it on an ice bath for the determination of antioxidant enzyme activities and an aliquot of 5 mL of phosphate buffer was added and homogenized the mixture. Then, the mixture was centrifuged at 13000 rpm at 4 ^oC for 20 minutes and the supernatant was isolated for determining the enzyme activities. Superoxide dismutase activity (SOD) was determined using the method of Giannopolitis and Ries (1977). The enzyme extract solution (50 µL) contained 3 mL of 50 µM NBT, 1.3 µM riboflavin, 75 nM EDTA, 13 mM methionine, 50 mM phosphate buffer (pH 7.8). Then the test tubes containing the reaction mixtures were placed under light for 15 min.

The absorbance of the treated samples was read at 560 nm using a spectrophotometer. One unit of SOD was considered as the amount of enzyme repressed 50% of NBT photo-reduction. The peroxidase (POD) activity was determined using the method of Maehly and Chance (1954). The enzyme extract of 0.2 mL was reacted with 1.8 mL of 100 mM phosphate buffer (pH 7), 0.3 mL of 3 mM hydrogen peroxide and 0.1 mL of aqueous solution of 1% *p*-phenylenediamine. Then, the absorbance was documented at 485 nm using a spectrophotometer. One unit of POD was defined as variation in 1 unit of OD at 485 nm per minute. Catalase activity (CAT) was estimated according to Maehly and Chance (1954). The reaction mixture (3 mL) contained 0.2 mL of the enzyme extract, 2.6 mL of 50 mM potassium phosphate buffer (pH 7.2) and 0.2 mL of 15 mM H_2O_2. After 5 minutes, an aliquot of 2 mL of the titanium reagent was added to stop the reaction. The absorbance was read at 410 nm after centrifugation of the mixture for 10 minutes.

Statistical analysis

Data for all traits was subjected to analysis of variance (ANOVA) using Minitab Computer Program to work out the significance of different sources of mean squares. Tukey's test at the probability level 5% was applied to compare the mean values within each attribute.

Results

Morphological attributes

Analysis of variance showed that applications of MLE and NPK had highly significant ($P \leq 0.005$) effect on all morphological attributes measured, i.e., shoot and root fresh and dry weights, shoot and root lengths, number of leaves per plant and leaf area per plant (Fig. 1; Table 1). All growth attributes of both cultivars were improved with MLE and NPK applied at the vegetative and grain filling stages. Maximum foliage growth was observed at T_3 (10% solution of MLE powder) in Ujala-2016 at the grain filling stage (Fig. 2; Table 2). All the treatments of MLE and NPK improved the growth of both wheat cultivars as compared to that by the control treatment, but 10% applications of MLE and NPK applied at the grain filling stage resulted in higher growth of both cultivars (Figure 2a-h). Overall, the response of cv. Ujala-2016 was superior to Akbar -2019 with supplementation of MLE and NPK. Application of MLE and NPK had more significant effect when applied at the grain filling stage than their application at the vegetative in promoting growth of both wheat cultivars.

Activities of antioxidative enzymes

Foliar application of MLE and NPK at higher level (20%) displayed higher activities of all three enzymes in both wheat cultivars. However, 20% MLE applied as foliar spray was more effective than the same level of NPK in promoting the activities of all three enzymatic antioxidants (Fig. 3; Table 3). Overall, MLE application at all levels proved to be superior to NPK application in enhancing the POD activity in both wheat cultivars. Moreover, a progressive increase in POD activity was observed in both wheat varieties with increase in the level of MLE. The response of the two wheat cultivars differed with respect to a specific antioxidant enzyme with the supplementation of MLE or NPK.

Table 1. Mean squares from ANOVAs for data for various morphological parameters of two varieties of wheat (*Triticum aestivum* L.) when subjected to different levels of MLE & NPK at the vegetative stage

Sou	df	SFW	RFW	SDW	RDW	RL	SL	LA	NLPP
V	1	0.47***	0.047***	0.11***	0.01***	27.27***	1008.6***	24.86***	49.61***
T	4	1.03***	0.086***	0.22***	0.02***	57.57***	1018.6***	72.42***	13.12***
VxT	4	0.01*	0.002 ***	0.001 ***	0.0005*	0.58**	44.23**	44.23**	0.56ns
Error	30	0.007	0.001	0.003	0.0002	0.59	9.593	1.98	0.81

Table 2. Mean squares from ANOVAs of data for various morphological parameters of two varieties of wheat (*Triticum aestivum* L.) when subjected to different levels of MLE & NPK at the grain filling stage

Sou	df	SFW	RFW	SDW	RDW	RL	SL	LA	NLPP
V	1	0.63***	0.18***	0.15***	0.046***	51.597***	230.45***	4.23 ***	7.23***
T	4	3.25 ***	0.34***	0.80***	0.087***	227.86***	701.14***	31.5***	11.37***
VxT	4	0.01*	0.009 **	0.003 ***	0.003***	0.42ns	1.80**	0.31**	0.10ns
Error	30	0.02	0.002	0.005	0.0005	2.18	2.47	0.46	0.61

Table 1 & 2: Sou: Sources; V: Variety; T: Treatment; SFW: Shoot fresh weight; RFW: Root fresh weight; SDW: Shoot dry weight; RDW: Root dry weight; RL: Root length; SL: Shoot length; LA: Leaf area; NLPP: Number of leaves per plant; *, **, *** = statistically significant at the 5%, 1%, and 0.1% respectively; ns, non-significant

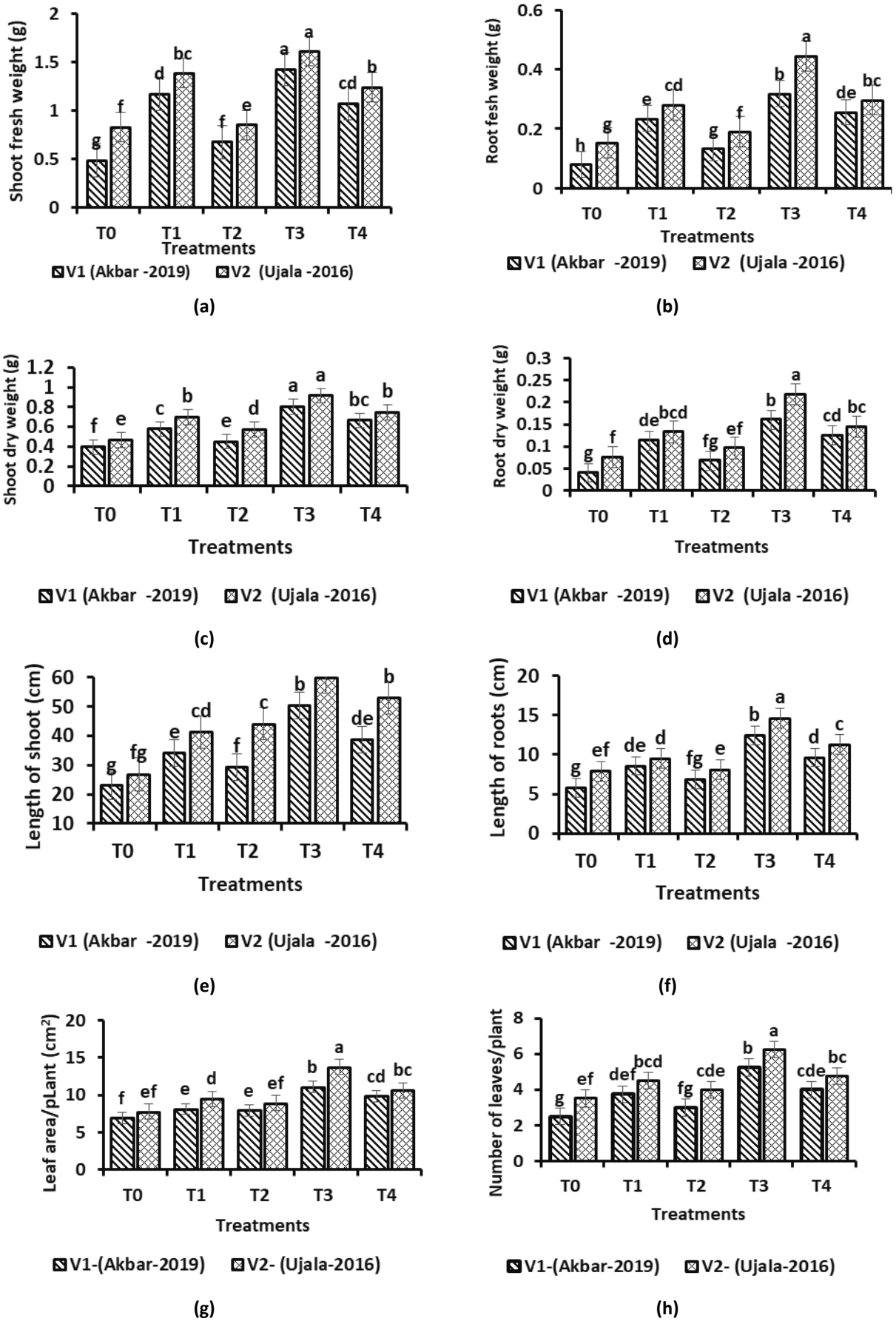

Figure 1 (a, b, c, d, e, f, g, h). Effect of different levels of NPK and MLE on shoot and root fresh and dry weights, length of root and shoot and number of leaves/plant of two wheat varieties. Where T0 = Control; T1= 10% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%); T2 = 20% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%); T3 = 10% MLE (10 g/100 mL water); T4= 20% MLE (20 g/100 mL water

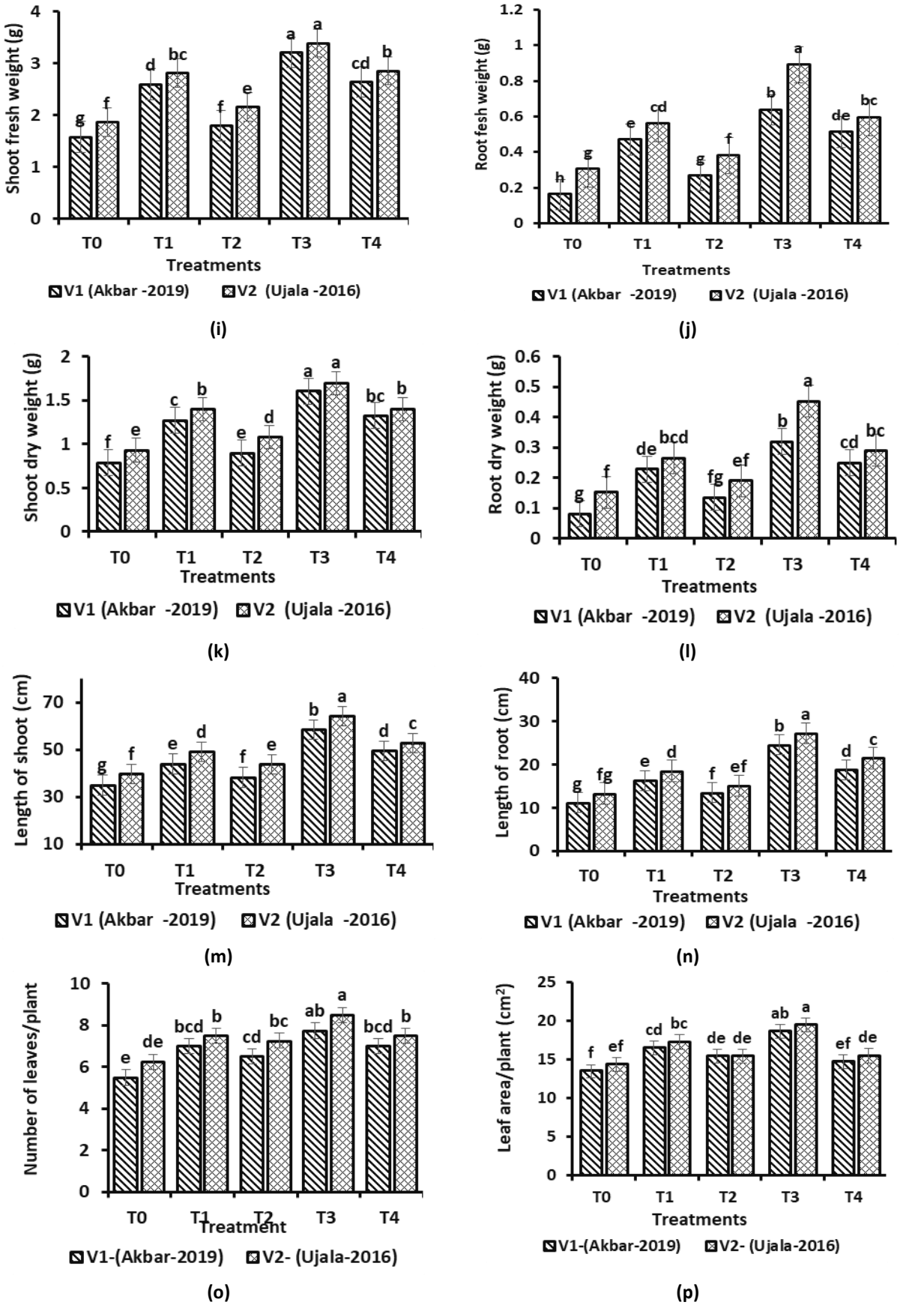

Figure 2 (I, j, k, l, m, n, o, p). Effect of different levels of NPK and MLE on shoot and root fresh and dry weights, length of root and shoot and number of leaves/plant of two wheat varieties. Where T0 = Control; T1= 10% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%); T2 = 20% NPK as foliar spray (N 20%, P_2O_5 20% & K_2O 20%); T3 = 10% MLE (10 g/100 mL water); T4= 20% MLE (20 g/100 mL water

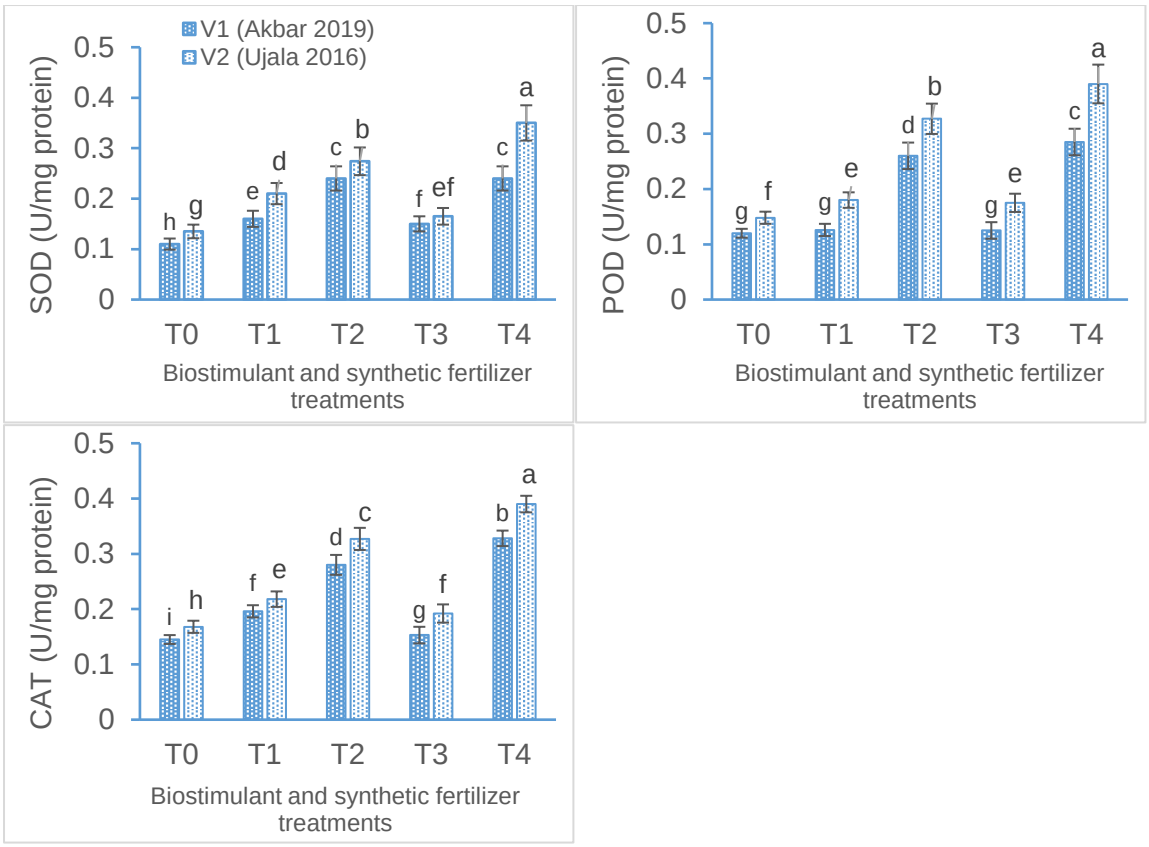

Figure 3. Effect of different levels of MLE and NPK on the activities of enzymatic antioxidants of wheat

Table 3. Mean squares from ANOVAs of data for key antioxidative enzymes of two varieties of wheat (*Triticum aestivum* L.) when subjected to different levels of MLE & NPK at the grain filling stage

Sources	df	SOD	POD	CAT
Variety (V)	1	0.01022***	0.03316***	0.00581 ***
Treatments (T)	4	0.03213***	0.06155***	0.04488***
V × T	4	0.00093***	0.00113***	0.00137***
Error	30	0.000096	0.00012	0.000061

*, **, *** = statistically significant at the 5%, 1%, and 0.1% respectively; ns, non-significant

Discussion

Recently moringa leaf extract (MLE) is receiving a considerable ground by plant scientists due to being a potential plant growth promoter (Hafeez et al., 2022; Khan et al., 2023). Correspondingly, in the current study, MLE enhanced the growth attributes, and activities of key antioxidative enzymes such as SOD, POD and CAT. These results are analogous to a previous study wherein Hassan et al. (2020) have shown that foliar application of MLE enhanced growth parameters in cut roses. Our results demonstrate that when wheat plants of both wheat cultivars were foliar-sprayed with MLE, they showed improved growth in terms of enhanced shoot and root fresh and dry weights; such MLE-induced growth promotion may have been due to the fact that MLE contains zeatin and adequate amount of required nutrients which might have promoted growth parameters of wheat plants by stimulating cell division and water uptake (Khan et al., 2021). The positive effects of MLE on wheat growth as observed in our study may have been also due to the complex composition of moringa leaf extract containing a variety of inorganic nutrients and biomolecules such as amino acids, phenolics, sugars, vitamins, flavonoids, antioxidant enzymes, ascorbic acid, thiamine, nicotinic acid, riboflavin, ascorbic acid, and phytohormones such as IAA, zeatin, and GA_3 (Islam et al., 2021; Mashamaite et al., 2022).

Although foliar-applied MLE and NPK at higher level (20%) gave rise higher activities of all three enzymes in both wheat cultivars, 20% MLE had been found to be more effective than the same level of NPK in elevating the activities of all three enzymatic antioxidants. Overall, foliar-applied MLE in varying concentrations proved to be superior to NPK in upraising the POD activity in both wheat cultivars. The two wheat cultivars differed significantly in terms of their enzymatic antioxidant response to the application of MLE or NPK. The results of the present study are analogous to those of Hafeez et al. (2022) in safflower treated with MLE. In another study with rice, Khan et al. (2023) have reported enhanced activities of SOD, CAT, and APX with foliar-applied MLE. Likewise, in pea Noreen et al. (2024) have reported enhancement in

the activities of SOD, CAT and APX in salt stressed plants fed with MLE either as a foliar spray or pre-sowing seed treatment.

Conclusion

Although both MLE and NPK enhanced the crop growth and the activities of key enzymatic antioxidants (SOD, CAT, POD), MLE was found to be more effective than NPK. It was concluded that MLE can be used as a substitute of NPK fertilizer. It is much cost-effective and affordable by the farming community.

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

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No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

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Contribution of authors
Conceptualization and designing of the study: ZAR, KH, KN. Conduction of experiment and collection of data: ZAR, NF, NA. Analytical work: ZAR, KH, KN, NF, NA, II. Helped to prepare figures and tables: ZAR, NF, NA, II. Statistical analysis of data: ZAR, NF, NA, II. Written first draft of the manuscript: ZAR, KH, KN, NF, NA, II. Final draft reviewed and read by all authors.

Ethical approval
This study does not involve human/animal subjects, and thus 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/eliminate 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 this article. All contributors have critically read this manuscript and agreed to publish in IJAaEB.

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