

APPLICATION OF SALICYLIC ACID AND MORINGA LEAF EXTRACT ON GROWTH, PHYSIOLOGICAL ATTRIBUTES, AND YIELD OF KALE

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ABSTRACT

Kale (*Brassica oleracea* var. *sabellica*) is an economically important leafy vegetable with high nutritional value, yet its year-round productivity under the semi-arid conditions of Peshawar remains limited. A two-factor field experiment was conducted in a randomized complete block design to evaluate the effects of foliar-applied salicylic acid (SA; 0, 100, 200, and 300 mg L⁻¹) and moringa leaf extract (MLE; 0, 200, 400, and 600 mg L⁻¹) on kale growth, physiology, root development, and yield. Salicylic acid at 200 mg L⁻¹ produced the highest plant height (27.10 cm), leaf chlorophyll content (53.40 SPAD), and yield (33.25 tons ha⁻¹), representing a 12% yield increase over the untreated control. Moringa leaf extract at 600 mg L⁻¹ yielded the greatest root volume (65.97 cm³) and yield (34.58 tons ha⁻¹). Both factors significantly influenced all measured traits; however, their interaction was non-significant, indicating that SA and MLE act through independent physiological pathways. These results demonstrate that targeted foliar application of SA at 200 mg L⁻¹ and MLE at 600 mg L⁻¹ can serve as practical biostimulant strategies to improve kale productivity under semi-arid field conditions.

Keywords: Salicylic Acid, Moringa Leaf Extract, biostimulants, Growth, Yield

INTRODUCTION

Kale (*Brassica oleracea* var. *sabellica*) is an economically important leafy vegetable valued for its high nutritional density, including vitamin C, antioxidants, and β-carotene, with well-documented benefits for cardiovascular and general health (Alam et al. 2020).

As a cool-season Brassicaceae crop, kale has gained commercial relevance in South Asian urban markets, yet its productivity remains constrained by environmental variability under semi-arid conditions.

Developing targeted, sustainable agronomic interventions is therefore essential to improve and stabilize kale yield and quality in such regions.

Salicylic acid (SA) is an endogenous signaling molecule that regulates plant growth, stress tolerance, and defense responses. Exogenous SA application influences stomatal conductance, ion uptake, chlorophyll biosynthesis, and ethylene metabolism, collectively enhancing vegetative growth and yield in various crops (El-Tayeb, 2005; Basit et al. 2018; Alam et al. 2020). SA also contributes to systemic acquired resistance and modulates abscisic acid-mediated stress responses, improving plant performance under adverse field conditions (Yalpani and Raskin, 1993; Ali et al. 2021). Despite these documented benefits, the optimal SA concentration and its dose-response effects in leafy Brassica crops under semi-arid conditions remain poorly characterised.

Plant-based biostimulants have emerged as sustainable alternatives to synthetic growth regulators for improving crop performance under climate-related stress. Extracts derived from *Moringa oleifera* leaves are of particular interest due to their high content of cytokinins, auxins, minerals, and antioxidant compounds (Gebashe et al. 2021). Foliar application

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of moringa leaf extract (MLE) has been shown to enhance growth and yield in several vegetable crops, including cabbage and tomato (Culver et al. 2012; Bari et al. 2024), with effects attributed largely to zeatin-class cytokinins that promote cell division and delay leaf senescence (Howladar, 2014). Nevertheless, the optimal MLE concentration for leafy Brassica crops in semi-arid field conditions remains undefined.

Despite growing evidence supporting the individual benefits of salicylic acid and moringa leaf extract, limited information exists on their comparative and interactive effects on kale growth and productivity under field conditions. In particular, the optimal application levels of these biostimulants for leafy Brassica crops in semi-arid environments have not been defined. The concentration ranges selected in the present study were based on dose-response data reported for Brassicaceae and allied leafy vegetables (Basit et al. 2018; Culver et al. 2012; Maswada et al. 2018). We hypothesized that foliar application of SA and MLE, individually, would enhance kale growth and yield, and that their combined application might produce additional improvements in morphological, physiological, and yield-related traits.

To address this gap, the present study investigated the effects of foliar-applied salicylic acid and moringa leaf extract on kale growth, physiological performance, root development, and yield. The study was designed to identify effective application levels of each biostimulant and to assess their interactive influence on plant performance under field conditions. By elucidating the combined potential of salicylic acid and moringa leaf extract, this work aims to contribute to the development of environmentally sustainable strategies for enhancing kale productivity in semi-arid agro-climatic regions.

MATERIALS AND METHODS

Experimental Site

The field experiment evaluating the effects of salicylic acid concentrations and moringa leaf extract on kale performance was conducted at the Horticulture Research Farm, The University of Agriculture Peshawar, Pakistan. The site lies within a semi-arid agro-ecological zone and experiences a subtropical climate characterized by pronounced seasonal variability (Ali et al. 2024). Geographically, the experimental area is located at 34.01° N latitude and 71.35° E longitude, at an elevation of approximately 350 m above sea level in the Peshawar valley. The

region is situated roughly 1600 km north of the Indian Ocean and is exposed to maximum wind speeds reaching up to 35 km h⁻¹. Irrigation water for the research farm is supplied through the Warsak Canal originating from the River Kabul, while the Indus River also enters the city from the northwest and subsequently divides into multiple distributary channels (Alam et al. 2020).

The soil profile of the Peshawar valley consists primarily of alluvial deposits with a loamy to sandy loam texture. Surface soils are characterized by a pH of approximately 7.8–8.2, low organic matter content (< 1.0%), and an electrical conductivity below 0.5 dS m⁻¹, consistent with the alluvial soils typical of the valley (Basit et al. 2021). Climatic conditions in the region are extreme, with cold winters and prolonged hot summers (Basit et al. 2019). Average minimum temperatures during winter fall to approximately 5 °C, whereas summer temperatures frequently rise to around 45 °C. Rainfall distribution is uneven, with March being the wettest month and June the driest (Gilani et al. 2021; Sajid et al. 2020).

The experiment was arranged in a randomized complete block design with a factorial structure comprising sixteen treatment combinations derived from varying concentrations of salicylic acid and moringa leaf extract. Treatments were replicated three times, resulting in a total of forty-eight experimental units. Each experimental unit (plot) measured 4 m × 3 m (12 m²), accommodating 20 plants per plot at a spacing of 50 cm between rows and 40 cm between plants. Border plants were excluded from data recording to eliminate edge effects. Basal fertilizer (NPK 120:90:60 kg ha⁻¹) was applied at transplanting, and furrow irrigation was carried out every 10–12 days or as required based on soil moisture status. Foliar applications were carried out at the three- to four-leaf stage, approximately three weeks after transplanting.

Preparation of Moringa Leaf Extract and Salicylic Acid Solution

Fresh leaves of *Moringa oleifera* L., harvested at approximately 40 days of age, were collected from Malakander Farm. The leaves were air-dried under complete shade at room temperature for several days to ensure uniform moisture loss. Once fully dried, the leaves were ground into a fine powder using a mechanical grinder. Crude aqueous suspensions were prepared by dissolving defined quantities of the dry powder in one litre of distilled water to obtain concentrations of 0, 200, 400, and 600 mg L⁻¹ (w/v).

The suspensions were filtered through muslin cloth to remove coarse particulate matter and applied immediately after preparation. It should be noted that these concentrations reflect powder loading (w/v) rather than a quantified content of specific bioactive constituents; extraction efficiency was not determined, and future studies should quantify cytokinin or zeatin content per unit concentration to enable cross-study comparisons.

Salicylic acid was procured from the local market (Shaubha Bazar, Peshawar) and used to prepare foliar spray solutions. To ensure complete dissolution, salicylic acid powder was first dissolved in 5 mL of absolute ethanol ($\geq 99.5\%$) per 200 mg of SA, and the volume was subsequently made up to 1 L with distilled water to obtain a 200 mg L⁻¹ stock solution. The same dissolution procedure (5 mL ethanol per 200 mg SA equivalent) was followed proportionally to prepare solutions at 100 and 300 mg L⁻¹. All solutions were freshly prepared prior to each application to maintain chemical stability.

Time of Application and Studied Attributes

Foliar applications of moringa leaf extract and salicylic acid were performed three weeks after transplantation, when plants had reached the four-leaf stage. For all measurements, five plants were randomly selected from each experimental unit, excluding border plants, and used as biological replicates. Plant height was measured from the soil surface to the tip of the tallest leaf using a graduated measuring scale. The number of leaves per plant was determined by direct counting of fully expanded leaves. Leaf area was measured on the three largest fully expanded leaves per plant using a portable leaf area meter (LI-3000C, LI-COR Biosciences, USA), and the mean value was calculated per plant. Leaf chlorophyll content was assessed non-destructively from fully expanded leaves using a SPAD-502 chlorophyll meter (Konica Minolta, Japan), following the procedure described by Ullah et al. (2020), with readings averaged per plant.

Root traits were recorded at harvest after carefully uprooting the selected plants. Roots were gently washed under running water to remove adhering soil without damaging the root system. Root length was measured from the crown to the tip of the longest root using a measuring scale, while root diameter was determined at the basal region using a digital vernier caliper. Root volume was estimated using the water

displacement method, whereby roots were submerged in a graduated cylinder and the displaced water volume was recorded. Yield was calculated as the total fresh biomass of all marketable leaves harvested from each plot (excluding border plants) and converted to a per-hectare basis using the formula:

$$\text{Yield (tons ha}^{-1}\text{)} = \frac{\text{Plot yield (Kg)}}{\text{Plot area (m}^2\text{)}} \times 10000$$

Statistical Analysis

All data were analyzed using Statistix software (version 8.1; Tallahassee, FL, USA). Analysis of variance (ANOVA) was performed to assess treatment effects under the randomized complete block design, following established procedures (Gilani et al. 2018). F-values and associated p-values are reported for each trait in Table 1. When significant main effects were detected ($p \leq 0.05$), treatment means were compared using the least significant difference (LSD) test at the 1% and 5% probability levels. The LSD test was selected as appropriate given the balanced RCBD with equal replication and a moderate number of treatment levels (four per factor), which maintains adequate statistical power without undue inflation of Type I error (Ali et al. 2024).

RESULTS

Both salicylic acid and moringa leaf extract significantly affected all measured growth, physiological, and root traits, while their interaction was non-significant for every variable (Table 1). For plant height, SA at 200 mg L⁻¹ gave the maximum value (27.10 cm) versus 18.11 cm in the control (Fig. 1); MLE at 600 mg L⁻¹ yielded 26.88 cm versus 19.94 cm in the control (Fig. 3). All concentrations above the control differed significantly based on LSD values in Table 1.

Leaf number per plant followed the same trend: SA 200 mg L⁻¹ produced the most leaves (31.63) and SA 0 the fewest (26.44); MLE 600 mg L⁻¹ gave 31.94 leaves versus 26.24 in the control (Figs. 1, 3). Leaf area and chlorophyll content were similarly enhanced. SA at 200 mg L⁻¹ produced the greatest leaf area (127.96 cm²; control: 112.30 cm²) and the highest SPAD reading (53.40; control: 42.21) (Fig. 1). Under MLE, the 600 mg L⁻¹ treatment yielded the largest leaf area (126.53 cm²; control: 109.85 cm²) and the highest chlorophyll content (53.29 SPAD; control: 43.64 SPAD) (Fig. 3).

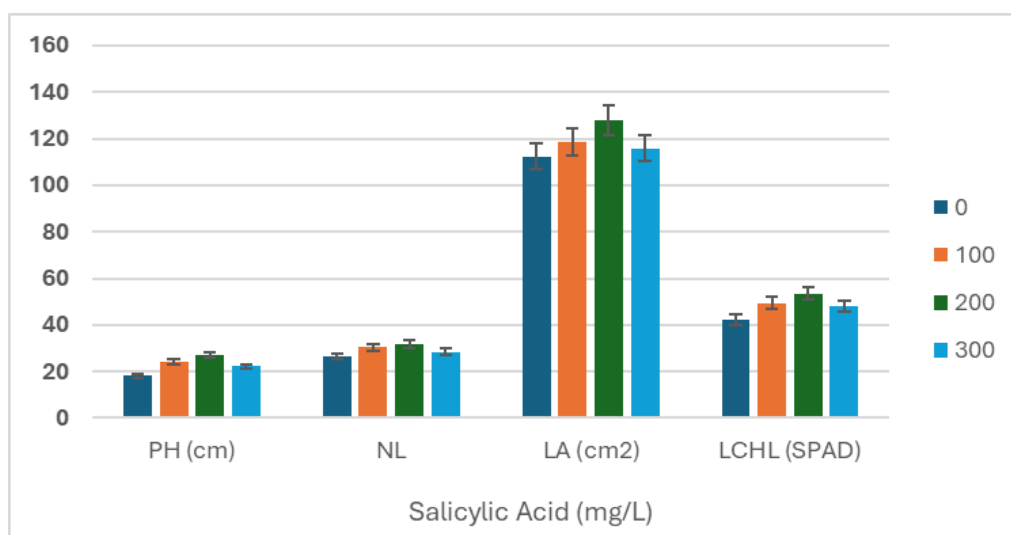


Figure 1. Influence of different concentrations of Salicylic acid on Plant height (PH), Number of leaves plant⁻¹ (NL), Leaf area (LA) and Leaf chlorophyll content (LCHL) of kale

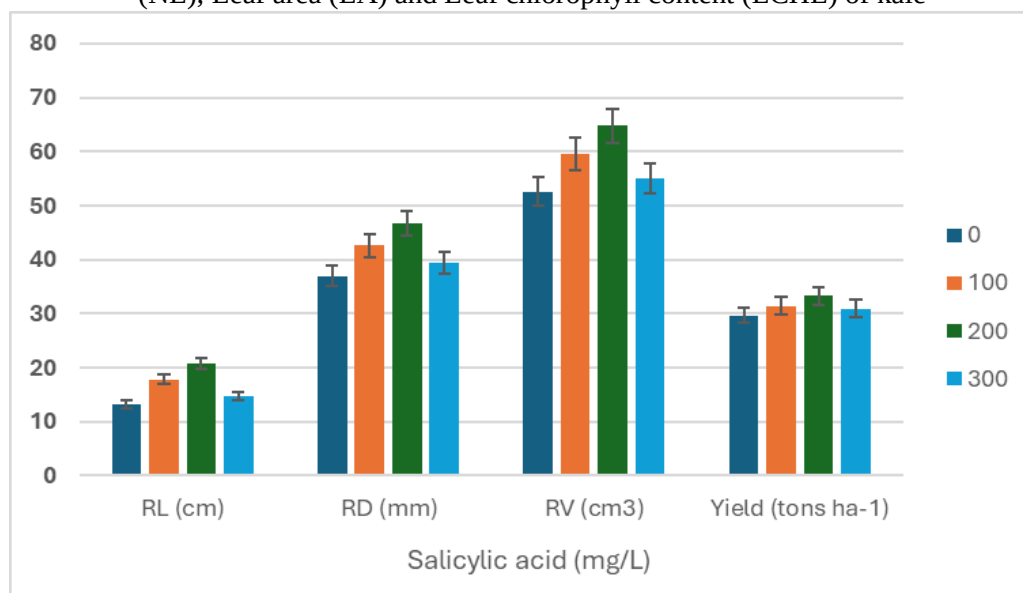


Figure 2. Influence of different concentrations of Salicylic Acid on Root length (RL), Root diameter (RD), Root volume (RV) and Yield (tons ha⁻¹) of kale

Root traits followed the same pattern of significant main effects with no significant interaction (Table 1). SA at 200 mg L⁻¹ produced the greatest root length (20.81 cm), diameter (46.75 mm), and volume (64.77 cm³) compared with controls (13.22 cm, 37.01 mm, and 52.61 cm³, respectively) (Fig. 2). MLE at 600 mg L⁻¹ achieved comparable improvements: root length 19.37 cm, diameter 47.72 mm, and volume 65.97 cm³, versus 13.80 cm, 34.49 mm, and 50.06 cm³ in the control (Fig. 4).

Yield was significantly influenced by salicylic acid and moringa leaf extract, while their interaction was non-significant. The highest yield (33.25 tons ha⁻¹) was recorded at 200 mg L⁻¹ salicylic acid, whereas the lowest (29.70 tons ha⁻¹) was observed in control plots (Fig. 2). In moringa leaf extract treatments, maximum yield (34.58 tons ha⁻¹) was obtained at 600 mg L⁻¹, while the minimum (28.12 tons ha⁻¹) occurred in the control (Fig. 4).

Table 1. Influence of Salicylic acid and Moringa leaf extract on the Plant height (PH), No. of leaves (NOL), Leaf Area (LA), Leaf chlorophyll content (LCHL), Root length (RL), Root diameter (RD), Root volume (RV), and Yield of kale

Salicylic acid (SA) (mg L ⁻¹)	PH (cm)	NOL	LA (cm ²)	LCHL (SPAD)	RL (cm)	RD (mm)	RV (cm ³)	Yield (tons ha ⁻¹)
0 (Control)	18.11	26.44	112.3	42.21	13.22	37.01	52.61	29.7
100	24.38	30.29	118.55	49.39	17.83	42.58	59.6	31.48
200	27.1	31.63	127.96	53.4	20.81	46.75	64.77	33.25
300	22.14	28.35	115.93	47.89	14.7	39.37	55.08	30.96
Moringa leaf extract (M) (mg L ⁻¹)								
0 (Control)	19.94	26.24	109.85	43.64	13.8	34.49	50.06	28.12
200	21.38	28.26	117.51	46.28	15.48	39.31	53.79	29.89
400	23.54	30.28	120.85	49.67	17.92	44.19	62.24	32.79
600	26.88	31.94	126.53	53.29	19.37	47.72	65.97	34.58
LSD	1.13	1.02	3.45	1.32	0.6	1.48	1.37	1.76
Interaction SA x M	NS	NS	NS	NS	NS	NS	NS	NS

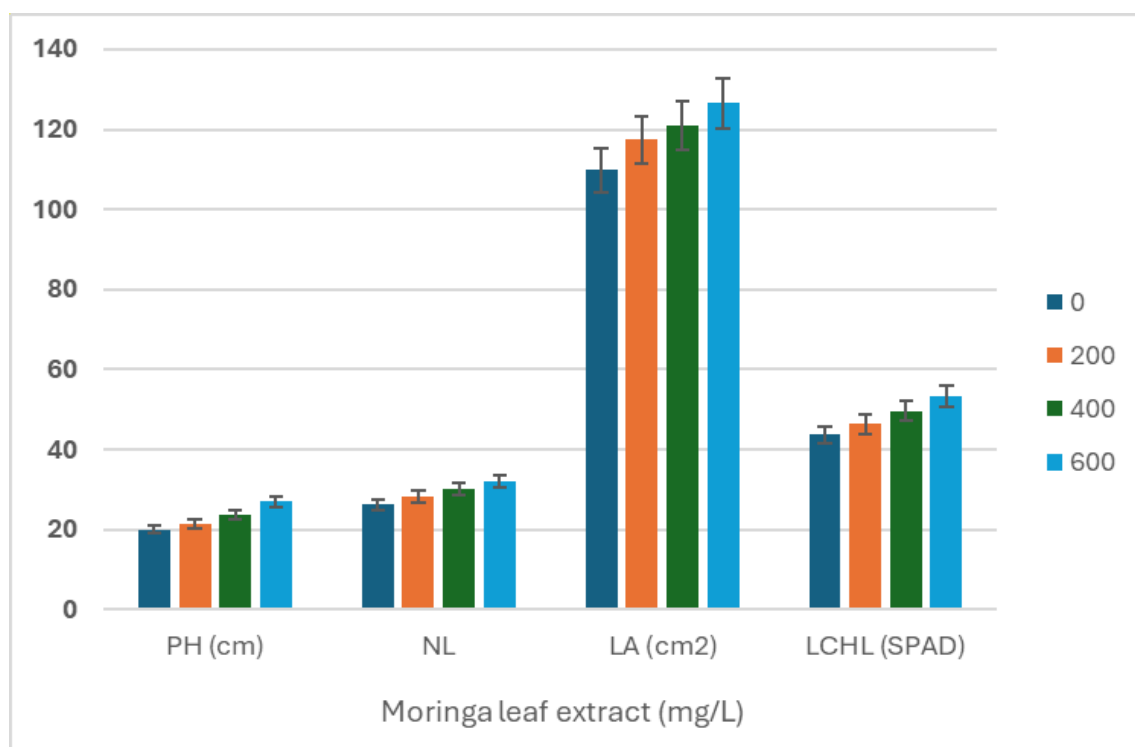


Figure 3. Influence of different concentrations of Moringa Leaf Extract (mg/l) on Plant height (PH), Number of leaves plant⁻¹ (NL), Leaf area (LA) and Leaf chlorophyll content (LCHL) of kale

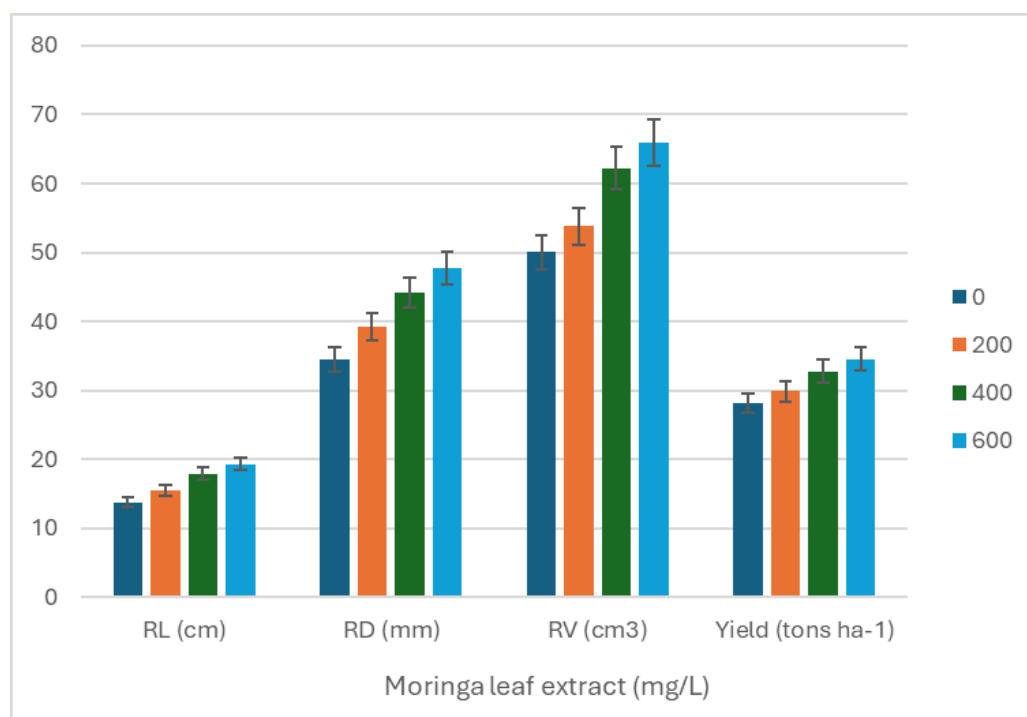


Figure 4. Influence of different concentrations of Moringa Leaf Extract (mg/l) on Root length (RL), Root diameter (RD), Root volume (RV) and Yield (tons ha⁻¹) of kale

DISCUSSION

The present study demonstrates that foliar application of salicylic acid and moringa leaf extract independently enhanced growth, physiological performance, root development, and yield of kale, while their interaction effects remained non-significant. The non-significant SA × MLE interaction indicates that the two biostimulants act through largely independent physiological pathways and their combined application does not produce additive or synergistic benefits beyond what each achieves independently. The observed responses directly address the study objectives of identifying optimal concentrations of SA and MLE and evaluating their individual roles in enhancing kale productivity under semi-arid field conditions.

Salicylic acid consistently promoted vegetative growth traits, including plant height, leaf number, leaf area, and chlorophyll content, with peak responses at 200 mg L⁻¹ and a decline at 300 mg L⁻¹ (e.g., yield declining from 33.25 to 30.96 tons ha⁻¹). This hormetic dose-response pattern is consistent with the known dual role of SA: at low-to-moderate concentrations, SA activates antioxidant enzymes, modulates hormonal balance, and upregulates photosynthetic pigment biosynthesis, collectively promoting growth and yield; at elevated concentrations, however, SA can inhibit catalase

activity and disrupt cellular redox homeostasis, causing oxidative stress and impaired growth (Ashraf et al. 2010). SA-mediated regulation of stomatal function and ethylene biosynthesis likely contributed to sustained leaf development and delayed senescence at the optimal dose, thereby increasing assimilatory capacity (El-Tayeb, 2005; Alam et al. 2020). These mechanisms collectively explain the superior vegetative performance and yield observed at 200 mg L⁻¹, in line with previous reports (Bagautdinova et al. 2022; Ibrahim et al. 2019).

The progressive enhancement of above- and belowground traits with increasing MLE concentration, peaking at 600 mg L⁻¹, reflects a dose-dependent cytokinin effect. Zeatin present in MLE promotes cell division, delays leaf senescence, and upregulates chlorophyll biosynthesis (Howladar, 2014), mechanistically accounting for the observed gains in leaf number, area, and SPAD values. The concomitant improvement in root length, diameter, and volume indicates stimulated root meristem activity, likely facilitated by the auxin and mineral fractions of MLE (Fathy and Farid, 2000). The 23% yield advantage of MLE 600 mg L⁻¹ over the control is a practically meaningful outcome: it suggests that even a crude aqueous suspension of moringa leaf powder at 600 mg L⁻¹ (w/v) can deliver sufficient bioactive compounds to substantially improve leafy

vegetable productivity, consistent with reports in cabbage and potato (Culver et al. 2012; Mbuyisa et al. 2023).

The enhancement of root system architecture under both SA and MLE treatments highlights the importance of belowground traits in mediating overall plant performance. Notably, root traits declined at SA 300 mg L⁻¹ relative to SA 200 mg L⁻¹, mirroring the shoot response and confirming that supra-optimal SA concentrations create oxidative stress that impairs meristematic activity (Ashraf et al. 2010). A more extensive root system increases water and nutrient uptake efficiency, particularly under semi-arid conditions, thereby supporting sustained shoot growth and higher yield. SA may indirectly promote root development by improving photosynthate allocation and stress tolerance, whereas MLE likely acts through direct nutrient supplementation and hormonal stimulation. The improvement in root traits observed in this study is consistent with earlier findings reporting enhanced root growth following application of organic extracts and growth regulators (Safdar et al. 2022; Massimi et al. 2023).

Yield enhancement under SA and MLE application reflects an integrated outcome of improved photosynthetic capacity, prolonged leaf functionality, and enhanced root uptake potential. Greater leaf area and chlorophyll content increase assimilate production, while deeper and wider root systems facilitate more efficient water and nutrient acquisition, ultimately driving higher biomass accumulation. The 12% yield increase under SA 200 mg L⁻¹ and 23% increase under MLE 600 mg L⁻¹ over the untreated control are agronomically significant under the semi-arid production conditions of the Peshawar valley, where yield gaps are largely attributable to suboptimal nutrient and hormone management. Practically, both biostimulants are low-cost and locally accessible, making them viable options for smallholder kale growers in the region.

Overall, this study provides mechanistic evidence that salicylic acid and moringa leaf extract independently enhance kale performance through hormonal signaling, photosynthetic regulation, antioxidant activity, and improved root development. The identification of effective application levels of SA and MLE fulfills the study objectives and highlights their potential as sustainable, environmentally friendly alternatives to synthetic growth promoters for improving kale production under semi-arid agro-climatic conditions.

CONCLUSION

Foliar application of salicylic acid at 200 mg L⁻¹ and moringa leaf extract at 600 mg L⁻¹ significantly improved growth, physiological performance, root development, and yield of kale under semi-arid field conditions in the Peshawar valley. Salicylic acid at 200 mg L⁻¹ enhanced yield by approximately 12% over the untreated control, while MLE at 600 mg L⁻¹ increased yield by 23%, likely through cytokinin-mediated cell division, antioxidant activity, and improved root nutrient uptake. These findings recommend SA at 200 mg L⁻¹ and MLE at 600 mg L⁻¹ as cost-effective, environmentally benign foliar treatments for kale producers in semi-arid agro-ecological zones. Field validation across multiple seasons and diverse soil types is needed before broader adoption can be advocated.

AUTHOR'S CONTRIBUTIONS

I.A. conceived the idea; M.J. performed the experiment; A.S. provided technical assistance during the experiment; M.S.K. prepared the initial draft of the manuscript; M.S.K. prepared the final draft of the manuscript; S.I. and S.J. helped with data collection; and H.A. provided technical expertise and guidance; I.A. proofread the paper.

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