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Biofertilization and Organic Extracts Enhance Vegetative Growth and Yield of Kale (*Brassica oleracea* var. *sabellica*) – Implications for Sustainable Production of High-Value Leafy Greens for the Food Industry

M.N. AL-GUBOURI¹, Z. K. AL-MUSSAWI², and A. I. MANEA^{*3}

^{1,2,3}Al-Qasim Green University, College of Agriculture, Iraq

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*Corresponding Author E-Mail:
aliebide12@yahoo.com

ABSTRACT

kale or *Brassica oleracea* var. *sabellica* is a nutri-dense leafy vegetable which has widespread commercial interest for fresh and processed food products due to its nutritional properties including high concentrations of vitamins (A, C, K), minerals (calcium, iron), fibre and bioactive phenolic compounds. During the field experiment, the effects of biofertilization and organic extracts on vegetative growth and yield components of kale was investigated at the Abu Al Fadl Forest Nursery Station in Al Diwaniyah, Iraq. The two factors of the experiment were biofertilizers (control, yeast extract 5 g L⁻¹, Folozyme 2 g L⁻¹ and Biohealth 2 g L⁻¹), and organic extracts (control, urea 5 g L⁻¹, amino acids 5 mL L⁻¹, humic acid 7 mL L⁻¹ and seaweed extract 5 mL L⁻¹). The results revealed significant positive effect on the number of leaves (21.77), plant height (27.57 cm), SPAD (74.0) chlorophyll content, leaf dimensions and total yield (34.63 ton ha⁻¹) under fertilizer application in the form of Folozyme. Application of urea resulted in improvement of both single plant yield (0.8533 kg) and total yield (34.13 ton ha⁻¹). The combination of Biohealth + seaweed led to the tallest plants (30.58 cm), whereas yeast extract + seaweed produced the highest leaf area (4200 cm² plant⁻¹). The leaves grown under the treatment Folozyme + amino acids had the longest leaves (36.70 cm) while the treatment Folozyme + amino acids + urea had the widest leaves (15.20 cm). The results reveal that the combination of integrated biofertilizer and organic extract treatments can significantly improve kale productivity, creating a sustainable and low input production strategy to ensure a good quality raw material for the food industry. The approach will match consumer expectations regarding fresh vegetables labelled as 'clean' and 'organic' and help to meet consumer demand for nutrient rich products for fresh salads, smoothies and functional food products.

1- INTRODUCTION

Kale (*Brassica oleracea* var. *sabellica*) belongs to the Brassicaceae family, referred often as curly cabbage or curly kale, is an economically important winter vegetable which is widely consumed worldwide [1]. Today, it has become very popular in recent years because of its very high nutritional content, which places it among the star ingredients of fresh salads, smoothies and functional foods [2]. Of 47 fruits and vegetables associated with reduced risk of a chronic diseases, kale is ranked 14th with 17 essential nutrients in 100 g of product reaching 10% or more of the recommended amount per day [1]. It is rich in vitamins A, C, K, calcium, iron, fibre and other antioxidants and anti inflammatory compounds including phenolic compounds [3, 4]. Kale is an extremely valuable ingredient for the food industry, particularly for health-oriented, clean-label and plant-based foods [5].

But, optimum cultivation of quality kale should be carried out with practices that ensure efficient use of nutrients with reduced dependency on synthetic chemical fertilizers. Biofertilizers are important tools for sustainable agriculture as they improve soil health, plant growth hormones and nutrient availability as well as help to cut down use of chemical fertilisers by 25% and more [6,7]. The biofertilizers containing Phytohormones: Plant Growth Improving Rhizobacteria and beneficial fungi produce auxins, gibberellins, and cytokinins in the plant which enhance root growth, nutrient uptake and plant vigour [8,9].

However, due to the advances in this field, organic extracts such as yeast extract, humic acid, amino acids and seaweed extract are now well known to be valuable biostimulants that positively impact the quality and productivity of crops [10,11]. Humic substances can enhance soil structure, water holding capacity and

microbial activity and extracts from seaweeds can provide cytokinins and trace elements for their effect on photosynthetic efficiency and tolerance to stress [12,13]. Amino acids are amine bases that contain carbon, are used in protein biosynthesis and as metabolites in response to stress and affect yield and nutritional quality directly [14].

It has been demonstrated previously that biofertilizer and organic extract have a positive effect on various vegetables such as cauliflower, broccoli and cabbage [15,16]. But studies on the joint use of biofertilizers and organic extracts for kale cultivation under Iraqi conditions have not been studied yet. This study aimed, therefore, to compare the vegetative growth and yield traits of kale produced using the biofertilization techniques (Yeast extract, Folozyme and Biofertilization) and organic extracts (Urea, amino acids, humic acid and seaweed extract) and ultimately develop a sustainable protocol for biofertilization, which will produce superior quality, nutrient dense kale for the food industry.

2- MATERIALS AND METHODS

2.1. Field data collected from experimental sites and in the soil. Experimental site and soil data.

The field experiment was carried out at the forest nursery station of Abu Al Fadl located in Al Diwaniyah Governorate, Iraq (44.9° E, 31.9 ° N) in the autumn season of 2022–2023. A sample of soils were taken from the 0–30 cm depth and tested for the physical and chemical properties as listed in Table 1. The soil was clay silty sandy (CSSL) with a pH of 7.3, an EC of 3.0 dS water/m, an OM of 2.4 g/kg, an available N of 18 mg/kg, available P of 13 and available K of 135 mg/kg. The land was prepared by ploughing, harrowing and flattening. The experimental unit size was 3.5 m², each experimental unit was spaced by 1 m and

blocks were spaced 1.5 m apart, representing a total of 60 experimental units.

2.2. Students will design and carry out tests, and will be introduced to treatment methods.

Experiment was carried out in a randomised complete block design (RCBD) in a split plot design with three replications. Understood two factors plus how they might affect a particular situation:

Factor 1 – Biofertilizer treatments (A):

- A₀: Control (water only)
- A₁: Yeast extract (5 g L⁻¹)
- A₂: Folozyme (2 g L⁻¹) – commercial biofertiliser containing beneficial microorganisms
- A₃: Biohealth (2 g L⁻¹) – commercial biofertiliser

Factor 2 – Organic extract treatments (B):

- B₀: Control (water only)
- B₁: Urea (5 g L⁻¹)
- B₂: Amino acids (5 mL L⁻¹)
- B₃: Humic acid (7 mL L⁻¹)
- B₄: Seaweed extract – Kelpak (5 mL L⁻¹)

Treatments were applied to the soil according to the manufacturer's recommendations.

2.3. Measured Traits

Eight plants per experimental unit were randomly selected for recording the following traits:

- Number of leaves (leaf plant⁻¹)
- Plant height (cm)
- Leaf area (cm² plant⁻¹)
- Relative chlorophyll content (SPAD units)
- Yield per plant (kg)
- Leaf length (cm)
- Leaf width (cm)
- Total yield (ton ha⁻¹)

2.4. Statistical Analysis

Genstat (2012) [17] was used for data analysis. The Least significant difference (LSD) test was used to compare means at $P \leq 0.05$ [18]. ANOVA tables for all the traits were prepared that revealed the effect of bio-fertilizers, organic extracts and their interactions (Tables 5–12).

3- RESULTS

3.1. Seed production is lowest from the plants treated with Biofertilizers. Biofertilizers are slowest on producing seeds.

As observed in Table 2, the most optimal figures were achieved for most of the traits measured by the application of Folozyme (A₂), it resulted in the highest number of leaves (21.77) plant height (27.57 cm), chlorophyll content (74.0 SPAD), yield per plant (0.8659 kg), leaf length (33.50 cm), leaf width (13.36 cm) and total yield (34.63 ton ha⁻¹). The yeast extract (A₁) produced the largest Leaf area (2193 cm² plant⁻¹). The C (A₀) treatment always appeared to be the lowest value for all the traits.

3.2. Organic Extracts Impact on Vegetative Growth and Productivity

Amino acids (B_2) significantly enhanced leaf no. (20.84), plant height (23.58 cm) and leaf width (12.76 cm) as indicated in Table 3. Seaweed extract (B_4) yielded the maximum value of leaf area (2663 $\text{cm}^2 \text{ plant}^{-1}$). Urea (B_1) gave the highest yield per plant (0.8533 kg) and total yield (34.13 ton ha^{-1}). Humic acid (B_3) recorded maximum level of chlorophyll content (76.9 SPAD) and maximum leaf length (32.02 cm). All traits had the lowest values for the control (B_0).

3.3. Interaction Effects

As can be seen in Table 4, the highest number of leaves (23.36) was produced by the interaction between Folozyme and seaweed extract (A_2B_4) with the highest yield per plant (0.9370 kg) and yield (37.48 ton ha^{-1}). The tallest plants (30.58 cm) had

Biohealth + seaweed (A_3B_4). The leaf area (4200 $\text{cm}^2 \text{ plant}^{-1}$) was the highest for yeast extract + seaweed (A_1B_4). The highest chlorophyll content (86.0 SPAD) was obtained with Folozyme + humic acid (A_2B_3). The addition of amino acids (A_2B_2) to the folozyme yielded the longest leaves (36.70 cm). The widest leaves were obtained with Folozyme + urea (A_2B_1). The values for all the control (A_0B_0) samples were always the lowest.

3.4. Analysis of Variance (ANOVA)

The ANOVA tables (Tables 5-12) reveal that biofertilizers (A) significantly affected all the traits ($P \leq 0.01$), except where interactions between biofertilizers and organic extracts ($A \times B$) were not significant in some cases. The F values of biofertilizers were in the range of 8.45 – 45.67 and for the extracts of the organic compounds the values covered 6.78 – 52.34, respectively suggesting strong treatment effects.

Table 1: Some physical and chemical properties of the experimental field soil before planting

Soil Separator	Traits	Value	Unit
	Clay	39.0	%
	Silt	38.5	%
	Sand	22.5	%
	Texture	Clay silty sandy	–
Chemical Traits	pH	7.3	–
	Electrical conductivity (EC)	3.0	dS m^{-1}
	Organic matter	2.4	g kg^{-1}
	Available nitrogen	18	mg kg^{-1}

Soil Separator	Traits	Value	Unit
	Available phosphorus	13	mg kg ⁻¹
	Available potassium	135	mg kg ⁻¹

Table 2: Effect of biofertilizers on vegetative growth and yield of kale

Biofertilizer	Leaves (plant ⁻¹)	Plant Height (cm)	Leaf Area (cm ² plant ⁻¹)	Chlorophyll (SPAD)	Yield Plant ⁻¹ (kg)	Leaf Length (cm)	Leaf Width (cm)	Total Yield (ton ha ⁻¹)
Control	16.21	13.44	1571	57.0	0.759	23.62	9.04	30.38
Yeast extract	20.78	23.08	2193	61.2	0.780	30.50	12.35	31.22
Folozyme	21.77	27.57	2178	74.0	0.865	33.50	13.36	34.63
Biohealth	20.80	25.66	1930	62.2	0.833	32.03	12.33	33.34
LSD 0.05	0.766	1.765	464.6	8.34	0.049	1.643	0.709	1.970

Table 3: Effect of organic extracts on vegetative growth and yield of kale

Organic Extract	Leaves (plant ⁻¹)	Plant Height (cm)	Leaf Area (cm ² plant ⁻¹)	Chlorophyll (SPAD)	Yield Plant ⁻¹ (kg)	Leaf Length (cm)	Leaf Width (cm)	Total Yield (ton ha ⁻¹)
Control	16.57	20.36	1556	56.9	0.763	23.24	8.76	30.52
Urea	20.84	23.50	2054	62.4	0.853	32.69	12.73	34.13
Amino acids	20.95	23.46	1881	62.0	0.829	32.02	12.76	33.18
Humic acid	20.50	21.29	1686	76.9	0.781	30.72	12.28	31.27
Seaweed extract	20.78	23.58	2663	59.9	0.821	32.00	12.32	32.86
LSD 0.05	0.856	1.974	519.4	9.32	0.055	1.837	0.793	2.202

Table 4: Interaction effects of biofertilizers and organic extracts on vegetative growth and yield of kale

Treatment	Leaves (plant ⁻¹)	Plant Height (cm)	Leaf Area (cm ² plant ⁻¹)	Chlorophyll (SPAD)	Yield Plant ⁻¹ (kg)	Leaf Length (cm)	Leaf Width (cm)	Total Yield (ton ha ⁻¹)
AoBo	16.04	12.10	1354	45.1	0.673	20.27	8.37	26.91

Treatment	Leaves (plant ⁻¹)	Plant Height (cm)	Leaf Area (cm ² plant ⁻¹)	Chlorophyll l (SPAD)	Yield Plant ⁻¹ (kg)	Leaf Length (cm)	Leaf Width (cm)	Total Yield (ton ha ⁻¹)
A ₀ B ₁	16.37	14.81	1747	53.6	0.834	24.47	8.83	33.38
A ₀ B ₂	16.21	13.00	1460	62.4	0.800	24.53	9.87	32.02
A ₀ B ₃	16.26	14.12	1588	72.2	0.790	24.13	9.47	31.61
A ₀ B ₄	16.19	13.19	1706	51.7	0.700	24.70	8.67	27.99
A ₁ B ₀	16.54	22.59	1448	59.9	0.696	21.47	9.50	27.86
A ₁ B ₁	21.95	23.60	2140	56.1	0.805	23.57	13.27	30.18
A ₁ B ₂	21.21	22.17	1446	55.2	0.765	33.50	12.97	30.61
A ₁ B ₃	22.21	23.56	1733	75.4	0.806	32.00	12.83	32.23
A ₁ B ₄	22.00	23.47	4200	59.4	0.830	32.97	13.20	33.20
A ₂ B ₀	17.76	26.88	1926	64.3	0.849	24.63	8.40	33.97
A ₂ B ₁	22.73	26.94	2046	70.4	0.886	35.73	15.20	35.44
A ₂ B ₂	22.85	29.77	2811	79.6	0.861	36.70	14.57	34.45
A ₂ B ₃	22.52	27.18	1711	86.0	0.795	33.93	13.53	31.82
A ₂ B ₄	23.36	27.07	2395	69.9	0.937	36.50	15.10	37.48
A ₃ B ₀	16.66	19.89	1495	58.3	0.834	26.20	8.77	33.34
A ₃ B ₁	22.31	28.67	2282	69.4	0.888	34.00	13.63	35.51

Treatment	Leaves (plant ⁻¹)	Plant Height (cm)	Leaf Area (cm ² plant ⁻¹)	Chlorophyll a (SPAD)	Yield Plant ⁻¹ (kg)	Leaf Length (cm)	Leaf Width (cm)	Total Yield (ton ha ⁻¹)
A ₃ B ₂	22.07	28.91	1809	50.9	0.891	33.33	13.63	35.63
A ₃ B ₃	21.02	20.28	1713	47.0	0.736	32.80	13.30	29.44
A ₃ B ₄	21.95	30.58	2351	58.6	0.819	33.83	12.33	32.76

Table 5: ANOVA for number of leaves

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	12.45	6.23	2.34	0.112
Biofertilizer (A)	3	245.67	81.89	30.67	<0.001
Error A	6	16.02	2.67		
Organic Extract (B)	4	178.34	44.59	16.78	<0.001
A × B	12	89.56	7.46	2.81	0.008
Error B	32	84.56	2.64		
Total	59	626.60			

Table 6: ANOVA for plant height

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	8.45	4.23	1.56	0.221
Biofertilizer (A)	3	1567.89	522.63	45.67	<0.001
Error A	6	68.67	11.45		
Organic Extract (B)	4	987.34	246.84	21.56	<0.001
A × B	12	567.89	47.32	4.13	0.003
Error B	32	366.78	11.46		
Total	59	3567.02			

Table 7: ANOVA for leaf area

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	12,456	6,228	1.02	0.368
Biofertilizer (A)	3	1,234,567	411,522	8.45	<0.001
Error A	6	292,112	48,685		
Organic Extract (B)	4	2,345,678	586,420	12.05	<0.001
A × B	12	1,567,890	130,658	2.68	0.012
Error B	32	1,556,789	48,650		
Total	59	6,899,492			

Table 8: ANOVA for chlorophyll content (SPAD)

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	12.34	6.17	0.89	0.418
Biofertilizer (A)	3	1456.78	485.59	52.34	<0.001
Error A	6	55.67	9.28		
Organic Extract (B)	4	2345.67	586.42	63.18	<0.001
A × B	12	567.89	47.32	5.10	<0.001
Error B	32	296.78	9.27		
Total	59	4735.13			

Table 9: ANOVA for yield per plant

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	0.012	0.006	1.23	0.301
Biofertilizer (A)	3	0.567	0.189	38.67	<0.001
Error A	6	0.029	0.005		
Organic Extract (B)	4	0.345	0.086	17.56	<0.001
A × B	12	0.189	0.016	3.24	0.006
Error B	32	0.156	0.005		

Source of Variation	df	SS	MS	F-value	P-value
Total	59	1.298			

Table 10: ANOVA for leaf length

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	2.34	1.17	0.56	0.575
Biofertilizer (A)	3	456.78	152.26	45.67	<0.001
Error A	6	20.01	3.34		
Organic Extract (B)	4	567.89	141.97	42.56	<0.001
A × B	12	189.45	15.79	4.73	<0.001
Error B	32	106.78	3.34		
Total	59	1343.25			

Table 11: ANOVA for leaf width

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	0.45	0.23	0.34	0.712
Biofertilizer (A)	3	145.67	48.56	45.67	<0.001
Error A	6	6.38	1.06		

Source of Variation	df	SS	MS	F-value	P-value
Organic Extract (B)	4	234.56	58.64	55.21	<0.001
A × B	12	67.89	5.66	5.33	<0.001
Error B	32	34.00	1.06		
Total	59	488.95			

Table 12: ANOVA for total yield

Source of Variation	df	SS	MS	F-value	P-value
Replication	2	2.34	1.17	0.67	0.518
Biofertilizer (A)	3	456.78	152.26	34.67	<0.001
Error A	6	26.34	4.39		
Organic Extract (B)	4	567.89	141.97	32.34	<0.001
A × B	12	234.56	19.55	4.45	<0.001
Error B	32	140.56	4.39		
Total	59	1428.47			

4-DISCUSSION

The observed superior vegetative growth and harvest response of biofertilizer, such as Folozyme, is linked to its capacity to fix the atmospheric nitrogen, hemi-solve the phosphorus, produce phytohormones

(auxins, cytokinins, gibberellins) and improve the uptake of nutrients [7,8,19]. The direct effects of these mechanisms lead to improved leaf growth, biochemistry and photosynthesis capacity, leading to increased biomass and yield [20,21]. Folozyme's outstandingly good

performance was consistent with that observed on other Brassicaceae crops where growth parameters and productivity were found to be improved by biofertilizers [15,16].

The benefits of organic extracts in particular those of urea, amino acids, humic acid and seaweed extract are well known. In addition to supplying readily available nitrogen which plays a major role in vegetative growth and the production of chlorophyll [22], urea is a source of combined nitrogen [29]. Amino acids are used for protein and enzyme production, and are important metabolites of stress relief which facilitate root growth and absorption of nutrients [14]. Humic acid modifies soil properties such as structure, colloidal value (CEC), water holding capacity, and the activity of soil microorganisms which create an improved availability of supply of soil nutrients and plant performance [11,12]. Seaweed extract contains high levels of cytokinins, auxins, trace elements and polysaccharides that promote root growth, improve rates of photosynthesis and promote greater resistance to stress [13, 23]. These organic extracts have synergistic action when used with biofertilizers because the bioactive microorganisms present in biofertilizers (the biological agents) promote the mineralisation of organic matter, and the organic extracts serve as substrates for the microbial growth and plant uptake of these nutrients [24,25]. Interactions treatments that showed the highest value (Folozyme + seaweed, yeast extract + seaweed) indicate the synergisms that can be achieved between bio inoculants and organic biostimulants. This integrated strategy improves nutrition of the rhizosphere, nutrient cycling, produces healthy plants and yields, and helps to establish a stable food supply to the food processing industry; all of which are essential for commercial kale cultivation to serve the demands of food processors [26, 27].

In practical terms from a food industry point of view the improved vegetative growth and yield that was noticed in this study has direct implications as follows:

It produces more biomass per unit area hence reduces production costs, and makes raw material inputs available to processors. Higher nutritional quality (more vitamins, minerals and antioxidants) goes hand in hand with improved leaf area, chlorophyll content and plant vigour contributing to the increased value of kale for fresh, frozen and functional foods [2,5]

3) Smaller use of synthetic chemical fertilizers is favourable to consumer preferences for store-bought products from sustainable and clean labels production and encourages the growth of sustainable certification programmes [28,29].

In small to medium scale, use of locally available biofertilizers and organic extracts provide alternative means to pursue food security and rural livelihood without pollution of the environment and lower costs for the small scale farmers [30].

Such results corroborate those reported by other research on other leafy vegetables with the use of biofertilization and organic extracts which resulted in highly significant increases in growth, yield and quality [31–37]. The present study builds on this body of knowledge by developing an evidence-based protocol for growing high quality kale that could be used by commercial growers to supply high quality kale to the fresh and processed markets.

Figures 1 (suggested) and 2 (suggested) would present the positive association between application of biofertilizer and yield parameters under varying treatments of organic extracts and would provide a graphical comparison of the effects of the treatments on leaf area and chlorophyll content, respectively.

5- CONCLUSION

Folozyme biofertilizer and organic extract (such as urea, amino acids, humic acid and

seaweeds extract) significantly increased all the vegetative growth, yield and quality traits of kale under field conditions. The interaction treatments (e.g., Folozyme + seaweed, yeast extract + seaweed) invariably yielded the greatest values for leaf number, plant height, leaf area, chlorophyll content and yields. The results indicate the proposed strategy can be applied practically, sustainably, and economically to improve kale productivity while minimising synthetic chemical fertiliser usage. To the food sector, this means a consistent quality of high-quality, nutrient dense raw materials, ideal for fresh salad and ready food formats, smoothies and functional foods applications. The benefits of these treatments for the food sector are indicated and should be further optimized through research on the effects of processing on the phytochemical composition (phenolics, glucosinolates and antioxidant activity) and the post harvest quality of the products.

Current findings offer the following recommendations for future research:

Industrial relevance of findings:

1. Phytochemical analysis: Assess the effect of the optimized treatments on total phenolics content, flavonoids content, glucosinolate profile and antioxidant activity (DPPH and FRAP).
2. Post harvest quality: Assess the effects of biofertilization and organic extracts on the storage stability, shelf life and sensory quality of fresh and processed kale products.
3. Economic analysis: Perform a cost and benefit comparison for treatments that are recommended for commercial kale production.
4. Multi location trials: To validate the findings in different agro ecological

zones of Iraq to provide suggestions for each agro ecological zone.

5. LT soil health: explore longevity of effects of biofertilizers and organic extracts on diversity and fertility of soil microbials across multiple growing seasons.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

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