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Enhancing Lettuce Quality Through Foliar Application of Kaolin and Plant-Based Extracts

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ABSTRACT

This study was conducted to improve lettuce plant quality using spraying with kaolin and Azolla and willow extracts. A factorial experiment was arranged in a randomized complete block design (RCBD) with three replicates, comprising three factors: foliar spray of kaolin (0 and 4 g L^{-1}), foliar spray of azolla extract (0, 3.75 and 7.5%) and foliar spray of willow extract (0, 3% leaf extract and 3% bark extract). The findings revealed that foliar application of kaolin significantly increased the phosphorus and calcium concentration (0.668 and 2.189%), leaf thickness (0.434 mm $leaf^{-1}$), total chlorophyll (371.7 mg 100 g^{-1} fresh weight) concentration and dry weight (1.620 g $plant^{-1}$) and carotene (6.21 mg 100 g^{-1} fresh weight) concentration respectively. While spraying with Azolla extract improved the phosphorus and calcium concentration, leaf thickness, leaf area, chlorophyll concentration, dry weight, and carbohydrates, while reducing the nitrate concentration by 0.710%, 2.497%, 0.446 mm $leaf^{-1}$, 384.0 dm^2 $plant^{-1}$, 350.4 mg $100g^{-1}$ weight, 1.772 g $plant^{-1}$, 17.59% and 0.14 mg g^{-1} dry weight, respectively, compared to the control treatment. Spraying with willow extract also increased phosphorus, calcium concentration (0.756, 2.415%), leaf area (374.69 cm^2 $plant^{-1}$), dry weight (1.687 g $plant^{-1}$), and carbohydrate percentage (17.21%). Furthermore, the three-way interactions between the studied factors had significant effects on most of the measured traits, highlighting the importance of these treatments in improving the qualitative characteristics of lettuce.

Key words: Leafy crops; Lettuce plant; Safe cultivation; Sustainable extracts.

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INTRODUCTION

Agriculture is a key sector supporting economic growth. Agricultural innovations contribute to enhancing production efficiency. It is estimated by FAO that the projected population growth will result not only in higher demand for food, but also in a rise in agricultural products demand (Jararweh et al., 2023). Sustainable agriculture (SA) is one of the effective efforts for conserving the environment and supplying food needs for the next generation (Kumar & Pant, 2023). It is based on methods that do not consume natural resources, that keep soil fertility and water quality, and that prevent pollution and carbon it, and encourage biodiversity. In addition, sustainable agriculture contributes to rural communities achieving self-reliance, and promotes the local economy, with emphasis on integrated management of the soil, water, crops, diseases and pests (German et al., 2017; Tahat et al., 2020). Kaolin is a natural non-toxic claymineral used in agriculture. The main use of kaolin is in the control of environmental stress, mainly heat stress, and through the formation of a reflective film on the leaves reduces the solar radiation intercepted by the leaves, decreases canopy temperature and reduces the plant cooling obligation of evaporation. In addition, kaolin enhances the water use efficiency within the plant and the adaptation to adverse environmental conditions, thus promoting growth and productivity. Kaolin is also an effective physiological barrier against several insect pests, including whiteflies and aphids,

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so it may result in chemical pesticide reduction and is an ecologically friendly application in accordance with sustainable agriculture (Brito et al., 2019; Khaleghi et al., 2015). Azolla is an aquatic fern and is considered a biofertilizer, it can also serve as animal feed, by the presence of active compounds calcium, nitrogen, potassium and iron, it is capable of producing a plant growth promoting extract that enhances the qualitative traits of plants. The Azolla extract is also a natural protein-rich nutrient source that can be effectively administrated to increase the soil fertility and crop yield without the use of chemical fertilizer. Besides, it enhances soil structure and water holding capacity to lower water use (Al-Sayed et al., 2022; Roy et al., 2020). Willow extract (*Salix spp*.) is employed in agriculture as a natural biostimulant for the induction of plant growth, given that it is rich in active compounds (salicylic acid, phenols, flavonoids) that in general increase plant resistance to abiotic stresses, including drought and high temperature (Wise et al., 2020). It induces cell division, root development and seedling growth too. Additionally, it has antifungal and antibacterial properties, which confers the capability to protect the crops from different diseases and minimize the application of chemical pesticide and the use of this organism as biocontrol agent (Curtasu & Nørskov, 2024; Piątczak et al., 2020). Lettuce (*Lactuca sativa* L.) is a popular green leafy vegetable, valued for its nutrition and mineral concentration (e.g., calcium, iron, vitamins). Its chemical characteristics depend on environmental conditions including light, temperature and agricultural practices. Based on Iraqi agricultural statistics for 2023, lettuce was planted on 3,882 ha, and produced at 36,326 ton ha^{-1} (Medina-Lozano et al., 2021; Wang et al., 2024). Research Objective: This study aims to improve the quality of lettuce plants through foliar spraying with kaolin, azolla extract, and willow extract.

MATERIALS AND METHODS

The research was conducted at the experimental station of the Directorate of Horticulture, Ministry of Agriculture, Abu Ghraib district. Lettuce plants of the cultivar Al-Fajr were grown during the winter season of 2023–2024 to assess the improvement in lettuce quality under foliar application of kaolin, azolla extract, and willow extract. Lettuce seeds of the cultivar Al-Fajr were sown in a special nursery, using a mixture of peat and perlite as a medium for seedling development. Nursery care practices continued until the saplings reached 4–5 true leaves, after which they were transplanted to the main field, which had been prepared by plowing, leveling, and removing weeds and remains of previous crop roots. Lettuce plants were sprayed 20 days after transplanting the seedlings to the field with Azolla extract at concentrations of 3.75 and 7.5%, Three days later, the plants were sprayed with willow bark and leaf extract at a concentration of 3%, Three days later, the plants were sprayed with kaolin at a concentration of 4g L^{-1}. The spraying process was until completely wet in the early morning.

Experimental Design: A factorial experiment was conducted using a Randomized Complete Block Design (RCBD). The study included three factors and their interactions. The experimental field was divided into three equal blocks, each block subdivided into 18 experimental units, for a total of 54 units. The saplings were transplanted on 1/10/2023 using raised beds. The distance between beds was 0.50 m, and between plants within the bed was 0.30 m. Each bed contained 19 alternating plants. Data were analyzed using GenStat software, applying the LSD test at a probability level of 0.05.

The study factors were as follows:

Factor 1: Foliar application of kaolin (denoted as C)

Control (spraying with water only) (C0)

Spraying with kaolin at a concentration of 4 g L^{-1} (C1) (Al-Janabi & Al-Sultani, 2021; Glenn & Puterka, 2005)

Factor 2: Foliar application of azolla extract (denoted as A)

Control (spraying with water only) (A0)

Spraying with azolla extract at a concentration of 3.75% (A1)

Spraying with azolla extract at a concentration of 7.5% (A2) (Biswas et al., 2020)

Factor 3: Foliar application of willow extract (denoted as B)

Control (spraying with water only) B0

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Spraying with willow leaf extract at a concentration of 3% (B1)

Spraying with willow bark extract at a concentration of 3% (B2) (Zulfiqar et al., 2019)

Preparation of the treatments: Kaolin was prepared by weighing 4 g of kaolin and dissolving it in 1 L of distilled water Glenn & Puterka (2005). To prepare the Azolla extract, 1 kg of Azolla was boiled in 1 L of distilled water for 30 minutes and filtered through a piece of cloth, The extract concentration was 100% and the required concentrations (3.75 and 7.5%) were prepared (Biswas et al., 2020). To prepare the willow bark and leaf extract, the plant parts were collected and washed to remove dust and dirt. They were cut into small pieces (5-10) cm and 250 g of each were taken and placed in 1 L of distilled water. It was heated to a temperature of 90°C for 30 minutes, with the container tightly closed and left to cool. It was filtered with a piece of cloth and 3% was taken from it to prepare the required concentration (Zulfiqar et al., 2019).The extracts were analyzed at the University of Baghdad / College of Science / Central Laboratory of the Department of Lif Sciences.

Table 1. Azolla extract components

Element	Concentration
N	1.33%
P	0.07%
K	0.24%
Mg	41 mg/gm
Fe	57 mg/gm
Mn	266 mg/gm
Zn	250 mg/gm
Cu	12.6 mg/gm
Histidine	39.25 µg/gm
Alanine	49.58 µg/gm
Arginine	45.25 µg/gm
Asparagine	51.28 µg/gm
Aspartic Acid	54.08 µg/gm
Cysteine	63.29 µg/gm
Glutamic Acid	66.08 µg/gm
Glutamine	62.49 µg/gm
Glycine	55.08 µg/gm
Isoleucine	52.65 µg/gm
Leucine	63.29 µg/gm
Lysine	69.04 µg/gm
Methionine	57.58 µg/gm
Phenylalanine	63.65 µg/gm
Proline	70.11 µg/gm
Serine	65.89 µg/gm
Threonine	69.08 µg/gm
Tyrosine	72.56 µg/gm

Table 2. components of willow bark and leaf extract

Element	Bark	Leaf
N	0.45%	0.38%
P	0.11%	0.07%
K	0.89%	0.42%
Ca	0.51%	0.31%
Mg	0.11%	0.11%
Fe	135 ppm	120 ppm
Ash	6.00%	2.8%
Total lipid	1.54	0.87
Total protein	3.01	2.1
Polyphenolic	41 mg g^{-1}	46 mg g^{-1}
Salicylic	1.8 mg g^{-1}	0.2 mg g^{-1}
Total flavones	4 mg g^{-1}	7 mg g^{-1}
Total antioxidant	247 mg g^{-1}	217 mg g^{-1}

Measured traits: Phosphorus concentration (%) in leaves was estimated according to the method of Olsen et al. (1954).

Calcium concentration (%) in leaves was estimated according to the method of Al-Dulaimi & Al-Janabi (2020).

Leaf thickness (mm $leaf^{-1}$) measured using a Vernier caliper.

Leaf area per plant (cm^2 $plant^{-1}$) measured using Digimizer software.

Total chlorophyll concentration in the plant (mg 100 g^{-1} fresh weight) using the method Lichtenthaler & Buschmann (2001).

Dry weight of leaves The weight of a dry leaf was measured with a sensitive balance

Nitrate concentration in the plant (mg g^{-1} dry weight) using the method Cataldo et al. (1975).

Carbohydrate percentage in lettuce plant using the method Dubois et al. (1956).

Protein percentage in the plant calculated using the following equation:

Protein percentage = Nitrogen percentage × 6.25

The protein percentage on a fresh weight basis was calculated as follows:

Protein percentage (fresh weight basis) = (Protein percentage dry weight basis × Dry matter percentage) / 100

Carotene concentration in lettuce (mg 100 g^{-1} fresh weight)

The analysis was conducted at the University of Baghdad / College of Science / Central Laboratory of the Department of Life Sciences.

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RESULTS AND DISCUSSION

Table (3A) shows that the three-way had no significant effect on phosphorus concentration. The three-way interaction B2A2C1 recorded the highest calcium concentration of 2.997%, compared to the lowest value of 0.843% in the control treatment B0A0C0. The three-way interactions B2A2C1 and B0A1C0 achieved the highest leaf thickness of 0.483 mm leaf^{-1}, while the lowest value of 0.313 mm leaf^{-1} was recorded in B0A0C0. The interaction B0A2C1 resulted in the highest leaf area of 447.8 cm^2 plant^{-1}, while the lowest area was recorded in B0A0C0 at 244.9 cm^2 plant^{-1}. The treatment B0A2C1 also achieved the highest chlorophyll concentration of 420.5 mg 100g^{-1} fresh weight, compared to the lowest value of 180.8 mg 100 g^{-1} fresh weight in B2A0C0. The interaction treatment B0A2C0 gave the highest average dry weight of 2.126 g plant^{-1} compared to the lowest average of 0.823 g plant^{-1}in the control treatment B0A0C0. The interaction between kaolin and azolla (Table 3B) showed a significant effect on increasing phosphorus concentration, with treatment A1C0 recording the highest value of 0.800%, while the control A2C1 recorded the lowest value of 0.530%. The kaolin and azolla interaction A2C1 achieved the highest calcium concentration of 2.597%, compared to 1.549% in A0C0. The kaolin and azolla interaction A2C1 also recorded the highest leaf thickness of 0.457 mm leaf^{-1}, while the lowest value of 0.372 mm leaf^{-1} was observed in A0C0. The highest leaf area of 409.63 cm^2 plant^{-1} was recorded in the interaction A2C1, while the lowest area of 329.16 cm^2 plant^{-1} was recorded in A0C0. The interaction A2C1 also significantly outperformed in leaf chlorophyll concentration, recording the highest value of 382.0 mg 100 g^{-1} fresh weight compared to 194.2 mg 100 g^{-1} fresh weight in A0C0. The two interaction treatments A2C1 and A2C0 were superior, giving the highest average dry weight of 1.776 and 1.768 g plant^{-1}, compared to the lowest average in the comparison treatment A0C0, which amounted to 1.382 g plant^{-1}. There was no significant effect of the interaction between kaolin and willow extract on phosphorus concentration and leaf thickness. However, the kaolin and willow interaction treatment B2C1 significantly outperformed in calcium concentration, recording the highest value of 2.624%, compared to the lowest value of 1.740% in the control treatment B0C0. The highest value of leaf area was recorded at 389.14 cm^2 plant^{-1} in the B2C0 treatment, while the lowest one was estimated for control treatment B0C0 was 328.53 cm^2 plant^{-1}. With respect to chlorophyll concentration, the interaction B0C1 resulted in the highest level of 398.1 mg 100g^{-1} fresh weight, while the lowest was recorded under B2C0 with 261.0 mg 100g^{-1} fresh weight. The highest dry weight value was 1.735 g plant^{-1} in treatment B2C0, while the lowest value was 1.416 g plant^{-1} in treatment B1C0. The interaction between Azolla and willow extracts demonstrated a significant impact on increasing phosphorus concentration Treatment B2A1 recorded the highest value of 1.018%, while the lowest phosphorus value of 0.474% was in the treatment B1A2. The interaction between azolla and willow also gave the highest calcium concentration, as treatment A2B2 recorded 2.685% compared to 1.220% in A0B0. The azolla and willow interaction A2B2 achieved the highest leaf thickness of 0.480 mm leaf^{-1}, while the lowest value of 0.368 mm leaf^{-1} was recorded in A0B0. The azolla and willow interactions B0A2 and B2A2 recorded the highest leaf area of 397.99 and 395.16 cm^2 plant^{-1}, respectively, while the lowest value was observed in B0A0 at 299.95 cm^2 plant^{-1}. The interaction B0A2 gave the highest chlorophyll concentration of 370.3 mg 100g^{-1} fresh weight. From the results of Table (3C) treatment C0 was superior in the phosphorus percentage of 0.668% compared to treatment C1, Which gave 0.605%. However, regarding calcium concentration, the treatment C1 (kaolin at 4 g L^{-1}) increased calcium percentage to 2.189%, compared to 1.952% in the control treatment C0. Foliar spraying with kaolin at 4 g L^{-1} (C1) showed a significant effect on leaf thickness, increasing it to 0.434 mm leaf^{-1}, compared to 0.413 mm leaf^{-1} in the control C0. However, no significant differences were observed in leaf area among kaolin treatments. On the other hand, kaolin application had a positive impact on chlorophyll concentration, where treatment C1

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increased it to 371.7 mg 100g^{-1} fresh weight, compared to 266.9 mg 100g^{-1} fresh weight in the control C0. Treatment C1 also had a significant effect, as it gave the highest dry weight value of 1.620 g plant^{-1}, while the lowest value was 1.563 g plant^{-1} in the comparison treatment C0. Foliar application of azolla extract had a significant effect on increasing phosphorus concentration in leaves. The treatment A1 recorded the highest value at 0.710%, compared to 0.554% in A2. Spraying with azolla extract also significantly affected calcium concentration in leaves, as treatment A2 recorded the highest value at 2.497%, compared to 1.776% in the control A0. In terms of leaf thickness, treatment A2 produced the thickest leaves at 0.446 mm leaf^{-1}, compared to 0.396 mm leaf^{-1} in the control A0. Regarding leaf area, treatment A2 achieved the maximum area of 384.00 cm² plant^{-1}, compared to 337.38 cm² plant^{-1} in the control A0. Furthermore, treatment A2 significantly enhanced chlorophyll concentration, reaching 350.4 mg 100g^{-1} fresh weight, compared to 272.8 mg 100g^{-1} fresh weight in the control A0. Treatment A2 significantly increased the dry weight by 1.772 g plant^{-1}, while the lowest value was 1.442 g plant^{-1} in treatment A0. Foliar application of willow extract B2 increased phosphorus concentration to 0.756% compared to 0.576% in the control treatment B0. Treatment B2 was superior, giving the highest percentage of calcium, reaching 2.415%, while the comparison treatment B0 gave the lowest percentage, reaching 1.874%, However, Spraying with willow extract had no significant effect on leaf thickness and chlorophyll concentration. Spraying with willow B2 gave a significant superiority with the highest leaf area of 374.69 cm² plant^{-1} compared to 350.12 cm² plant^{-1} in the control treatment. The highest significant increase in dry weight was recorded in treatment B2 reaching 1.687 g plant^{-1}, compared to the lowest value of 1.523 g plant^{-1} in treatment B1.

Table 3A. Effect of the three-way interactions of foliar spraying with kaolin, azolla extract, and willow extract on nutrient elements in lettuce leaves and vegetative growth traits during the 2024 autumn season

Treatments	P (%) in leaves	Ca (%) in leaves	Leaf thickness (mm leaf^{-1})	Leaf area (cm² plant^{-1})	Chlorophyll (mg 100g^{-1} fresh weight)	Dry weight (g plant^{-1})
$C_0 A_0 B_0$	0.500	0.843	0.313	244.9	198.5	0.823
$C_1 A_0 B_0$	0.843	1.597	0.423	354.9	380.6	1.326
$C_0 A_1 B_0$	0.543	1.797	0.483	392.4	296.0	1.666
$C_1 A_1 B_0$	0.466	1.893	0.436	312.4	393.1	1.723
$C_0 A_2 B_0$	0.652	2.580	0.413	348.1	320.2	2.126
$C_1 A_2 B_0$	0.449	2.533	0.433	447.8	420.5	1.720
$C_0 A_0 B_1$	0.763	1.677	0.410	335.9	203.3	1.653
$C_1 A_0 B_1$	0.542	1.597	0.433	341.7	367.2	1.773
$C_0 A_1 B_1$	0.680	1.813	0.436	390.6	277.0	1.023
$C_1 A_1 B_1$	0.533	1.950	0.433	356.2	390.4	1.373
$C_0 A_2 B_1$	0.488	2.240	0.413	313.6	323.7	1.573
$C_1 A_2 B_1$	0.461	2.260	0.456	404.1	371.6	1.746
$C_0 A_0 B_2$	0.615	2.127	0.393	406.5	180.8	1.670
$C_1 A_0 B_2$	0.612	2.816	0.406	340.1	306.1	1.410
$C_0 A_1 B_2$	1.177	2.120	0.383	347.5	290.0	1.930
$C_1 A_1 B_2$	0.860	2.060	0.400	363.6	362.2	1.646
$C_0 A_2 B_2$	0.594	2.373	0.476	413.3	312.6	1.606
$C_1 A_2 B_2$	0.679	2.997	0.483	376.9	354.0	1.863
LSD 0.05	N.S	0.840	0.039	40.04	91.52	0.294

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Table 3B. Effect of the two-way interactions of foliar spraying with kaolin, azolla extract, and willow extract on nutrient elements in lettuce leaves and vegetative growth traits during the 2024 autumn season

	Treatments	P (%) in leaves	Ca (%) in leaves	Leaf thickness (mm leaf⁻¹)	Leaf area (cm^2 $plant^{-1}$)	Chlorophyll (mg $100g^{-1}$ fresh weight)	Dry weight (g $plant^{-1}$)
A X C	C_0 A_0	0.626	1.549	0.372	329.16	194.2	1.382
	C_0 A_1	0.800	1.910	0.434	376.88	287.7	1.503
	C_1 A_0	0.666	2.003	0.421	345.60	351.3	1.540
	C_1 A_1	0.620	1.968	0.423	344.11	381.9	1.581
	C_0 A_2	0.578	2.398	0.434	358.38	318.8	1.768
	C_1 A_2	0.530	2.597	0.457	409.63	382.0	1.776
	LSD 0.05	0.134	0.485	0.035	41.575	52.84	0.282
C X B	C_0 B_0	0.565	1.740	0.403	328.53	271.6	1.538
	C_1 B_0	0.586	2.008	0.431	371.72	398.1	1.590
	C_0 B_1	0.644	1.910	0.420	346.75	268.0	1.416
	C_1 B_1	0.512	1.936	0.441	367.38	376.4	1.631
	C_0 B_2	0.795	2.207	0.417	389.14	261.0	1.735
	C_1 B_2	0.717	2.624	0.430	360.25	340.8	1.640
	LSD 0.05	N.S	0.485	N.S	41.575	52.84	0.282
B X A	B_0 A_0	0.671	1.220	0.368	299.95	289.6	1.075
	B_1 A_0	0.653	1.637	0.421	338.88	285.2	1.713
	B_2 A_0	0.614	2.471	0.400	373.31	243.5	1.540
	B_0 A_1	0.504	1.845	0.460	352.43	344.5	1.695
	B_1 A_1	0.606	1.882	0.435	373.46	333.7	1.198
	B_2 A_1	1.018	2.090	0.391	355.61	326.1	1.788
	B_0 A_2	0.551	2.557	0.423	397.99	370.3	1.923
	B_1 A_2	0.474	2.250	0.435	358.86	347.6	1.660
	B_2 A_2	0.637	2.685	0.480	395.16	333.3	1.735
	LSD 0.05	0.080	0.594	0.037	51.072	64.71	0.219

Table 3C. Effect of the individual treatments of foliar spraying with kaolin, azolla extract, and willow extract on nutrient elements in lettuce leaves and vegetative growth traits during the 2024 autumn season

	Treatments	P (%) in leaves	Ca (%) in leaves	Leaf thickness (mm leaf⁻¹)	Leaf area (cm^2 $plant^{-1}$)	Chlorophyll (mg $100g^{-1}$ fresh weight)	Dry weight (g $plant^{-1}$)
C	C_0	0.668	1.952	0.413	354.80	266.9	1.563
	C_1	0.605	2.189	0.434	366.44	371.7	1.620
	LSD 0.05	0.038	0.210	0.013	N.S	30.51	0.056
A	A_0	0.646	1.776	0.396	337.38	272.8	1.442
	A_1	0.710	1.939	0.428	360.49	334.8	1.560
	A_2	0.554	2.497	0.446	384.00	350.4	1.772
	LSD 0.05	0.094	0.343	0.015	14.996	37.36	0.069
B	B_0	0.576	1.874	0.417	350.12	334.8	1.564
	B_1	0.578	1.923	0.430	357.06	322.2	1.523
	B_2	0.756	2.415	0.423	374.69	301.0	1.687
	LSD 0.05	0.0948	0.343	N.S	14.996	N.S	0.069

In Table (4A), the triple interaction between the study factors had a significant effect on the carbohydrate percentage. Treatments B0A2C0 and B2A2C0 were superior with the highest carbohydrate percentages of 19.68% and 19.58% respectively, without a significant difference between them, compared to the lowest percentage in the comparison treatment B0A0C0 of 10.98%. Treatment B0A0C0 also excelled by giving the lowest nitrate concentration of 0.09 mg g^{-1} wet weight compared to treatment B0A0C1, which recorded the highest concentration of 0.20 mg g^{-1} wet weight. The triple interaction recorded

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a significant superiority in the carotene concentration in treatment B2A1C1, at a rate of 7.72 mg $100g^{-1}$ wet weight, compared to the lowest concentration in treatment B2A1C0, which reached 3.68 mg $100g^{-1}$ wet weight. In the triple interaction, the two treatments were superior. B0A2C1 and B2A1C0 yielded the highest protein concentration of 0.045%, compared to the lowest of 0.027% in the control treatment B0A0C0. These results demonstrate that the three-factor interactions significantly improved protein concentration in lettuce leaves. Table (4B) shows that the interaction between kaolin and azolla extract was significant in increasing carbohydrate concentration. The highest percentage was observed with treatment A2C0 (18.83%) followed by A1C1 (16.66%), A2C1 (16.35%), A0C1 (16.30%), and A1C0 (14.45%) while the lowest was recorded as 12.99% in A0C0. Similarly, kaolin × azolla interaction had a marked effect on nitrate concentration of lettuce plants as treatment A2C0 exhibited the least concentration 0.13 mg g^{-1} fresh weight, but the greatest concentration 0.18 mg g^{-1} fresh weight was noticed from plants treated with A1C0. Moreover, the interaction between kaolin and Azolla extract significantly increased carotenoid concentration. Treatment A0C1 achieved the highest level at 6.49 mg $100g^{-1}$ fresh weight, compared to the lowest value of 4.62 mg $100g^{-1}$ fresh weight in A0C0. Protein concentration was also positively influenced by this interaction. Treatment A2C1 recorded the highest protein percentage at 0.042%, compared to the lowest value of 0.035% in A1C1. The interaction between kaolin and willow extract showed a significant effect on carbohydrate concentration in lettuce leaves. The treatment B2C1 recorded the highest percentage at 18.58%, compared to the lowest value of 14.35% in treatment B1C1 No significant differences in nitrate concentration were observed in regards to an interaction between kaolin and willow extract. This interaction though therefore had a strong effect on carotenoid levels, with treatment B2C1 having the highest level at 7.14 mg $100g^{-1}$ fresh, compared to the lowest of treatment B2C0 4.57 mg $100g^{-1}$ fresh. A significant increase in protein concentration was induced by kaolin × willow extract interaction. Treatment B2C0 recorded the highest protein percentage at 0.040%, compared to the lowest value of 0.032% in treatment B0C0. The interaction between Azolla and willow extracts significantly affected carbohydrate concentration. The treatment B2A2 recorded the highest value at 19.26%, while the lowest was observed in treatment B1A0 at 13.79%. This interaction also resulted in a significant reduction in nitrate concentration. Treatment B0A2 showed the lowest level at 0.13 mg g^{-1} dry weight, compared to the highest value of 0.19mg g^{-1} dry weight in treatment B0A1. No significant effect was found on carotenoid concentration due to the two-way interaction between Azolla and willow extracts. The interaction of Azolla and willow extracts significantly affected the protein concentration of the leaves, particularly treatment B1A2, with a percentage of 0.042%, while treatment B0A0 recorded the lowest percentage of 0.031%. According to Table (4C), foliar application of kaolin alone did not significantly affect carbohydrate or nitrate concentration in lettuce leaves. However, kaolin treatment C1 significantly increased carotenoid concentration to 6.21 mg $100g^{-1}$ fresh weight, compared to 5.11 mg $100g^{-1}$ fresh weight in the control treatment C0. No significant effect was observed for individual treatments (kaolin, Azolla extract, or willow extract) on leaf protein concentration. Foliar spraying with Azolla extract significantly improved carbohydrate concentration. Treatment A2 recorded the highest percentage at 17.59%, compared to 14.64% in the control. Additionally, Azolla extract reduced nitrate levels, with treatment A2 showing the lowest value at 0.14 mg g^{-1} dry weight , compared to 0.17 mg g^{-1} dry weight in treatment A1. Neither azolla extract nor willow extract had a significant effect on carotene concentration. Foliar application of willow extract significantly affected carbohydrate concentration, as treatment B2 recorded 17.21%, compared to 15.07% in B1, while the carbohydrate percentage in the control was 15.51%. Willow extract application did not show a significant effect on nitrate concentration.

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Table 4A. Effect of the three-way interactions of foliar spraying with kaolin, azolla extract, and willow extract on the qualitative traits of lettuce leaves during the 2024 autumn season

Treatments	Nitrate concentration in heads (mg g^{-1} dry weight)	Carbohydrates in heads (%)	Protein (%) in leaves	Carotene in leaves (mg 100 g^{-1} fresh weight)
C_0 A_0 B_0	0.09	10.98	0.027	3.96
C_1 A_0 B_0	0.20	17.73	0.036	6.87
C_0 A_1 B_0	0.20	13.27	0.036	5.86
C_1 A_1 B_0	0.18	16.21	0.036	5.15
C_0 A_2 B_0	0.12	19.68	0.033	5.10
C_1 A_2 B_0	0.14	15.19	0.045	5.34
C_0 A_0 B_1	0.18	14.43	0.042	5.81
C_1 A_0 B_1	0.16	13.15	0.038	5.26
C_0 A_1 B_1	0.18	15.73	0.032	4.92
C_1 A_1 B_1	0.13	14.97	0.033	6.56
C_0 A_2 B_1	0.13	17.23	0.042	6.70
C_1 A_2 B_1	0.15	14.94	0.042	5.35
C_0 A_0 B_2	0.15	13.58	0.039	4.11
C_1 A_0 B_2	0.17	18.01	0.036	7.34
C_0 A_1 B_2	0.17	14.36	0.045	3.68
C_1 A_1 B_2	0.16	18.81	0.036	7.72
C_0 A_2 B_2	0.14	19.58	0.037	5.92
C_1 A_2 B_2	0.15	18.93	0.040	6.36
LSD 0.05	0.06	4.311	0.009	1.73

Table 4B. Effect of the two-way interactions of foliar spraying with kaolin, azolla extract, and willow extract on the qualitative traits of lettuce leaves during the 2024 autumn season

	Treatments	Nitrate concentration in heads (mg g^{-1} dry weight)	Carbohydrates in heads (%)	Protein (%) in leaves	Carotene in leaves (mg 100 g^{-1} fresh weight)
A X C	C_0 A_0	0.14	12.99	0.036	4.62
	C_1 A_0	0.18	16.30	0.036	6.49
	C_0 A_1	0.18	14.45	0.038	4.82
	C_1 A_1	0.16	16.66	0.035	6.48
	C_0 A_2	0.13	18.83	0.037	5.90
	C_1 A_2	0.15	16.35	0.042	5.68
	LSD 0.05	0.03	2.673	0.006	1.19
C X B	C_0 B_0	0.14	14.64	0.032	4.97
	C_1 $_0$B	0.17	16.38	0.039	5.78
	C_0 B_1	0.16	15.80	0.039	5.81
	C_1 B_1	0.15	14.35	0.037	5.72
	C_0 B_2	0.15	15.84	0.040	4.57
	C_1 B_2	0.16	18.58	0.037	7.14
	LSD 0.05	N.S	2.673	0.006	1.19
B X A	B_0 A_0	0.15	14.35	0.031	5.41
	B_1 A_0	0.17	13.79	0.040	5.53
	B_2 A_0	0.16	15.79	0.037	5.72
	B_0 A_1	0.19	14.74	0.036	5.50
	B_1 A_1	0.15	15.35	0.032	5.74
	B_2 A_1	0.16	16.58	0.040	5.70
	B_0 A_2	0.13	17.43	0.039	5.22
	B_1 A_2	0.14	16.08	0.042	6.02
	B_2 A_2	0.15	19.26	0.039	6.14
	LSD 0.05	0.05	3.581	0.006	N.S

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Table 4C. Effect of the individual treatments of foliar spraying with kaolin, azolla extract, and willow extract on the qualitative traits of lettuce leaves during the 2024 autumn season

Treatments		Nitrate concentration in heads (mg g^{-1} dry weight)	Carbohydrates in heads (%)	Protein (%) in leaves	Carotene in leaves (mg 100 g^{-1} fresh weight)
C	C_0	0.15	15.42	0.037	5.11
	C_1	0.16	16.44	0.038	6.21
	LSD 0.05	N.S	N.S	N.S	0.54
A	A_0	0.16	14.64	0.036	5.55
	A_1	0.17	15.56	0.036	5.65
	A_2	0.14	17.59	0.040	5.79
	LSD 0.05	0.02	1.802	N.S	N.S
B	B_0	0.15	15.51	0.035	5.38
	B_1	0.15	15.07	0.038	5.77
	B_2	0.16	17.21	0.039	5.85
	LSD 0.05	N.S	1.802	N.S	N.S

Results from Tables 3 and 4 showed that the nutritional quality of lettuce leaves significantly improved, as indicated by the greater concentrations of essential nutrients (nitrogen, phosphorus, calcium), carbohydrates, proteins, and carotenoids. Several synergistic effects contribute to this improvement. The azolla and willow extracts, rich in organic nutrients and bioactive compounds, improved soil fertility by increasing nutrient availability while enhancing soil physical properties, thereby promoting root development and reducing salt accumulation (Mandal et al., 2022; Mphande et al., 2020; Yadav et al., 2021). Kaolin application played a complementary role by forming a protective layer that reduced transpiration rates and improved water use efficiency (Brito et al., 2019). The combination of kaolin with azolla extract proved particularly effective, significantly increasing leaf area and chlorophyll concentration which enhanced photosynthetic efficiency leading to an increase in the dry weight of the plant. Furthermore, the organic compounds in azolla extract (Table 1) promoted root development and stimulated beneficial soil microorganisms involved in nitrogen cycling (Al-Sayed et al., 2022; Maswada et al., 2021). These extracts also contained growth-promoting amino acids and organic compounds that improved chlorophyll synthesis and carbohydrate metabolism (Mansour et al., 2020; Marzouk et al., 2022). Kaolin also plays an additional role in regulating plant hormones, as it increases the levels of IAA, GA, and CK, while reducing ABA, which contributes to improving vegetative traits and enhancing nutrient uptake (Al-Dulaimi et al., 2021; Jibril et al., 2022). Willow extract is rich in IBA hormone and bioactive compounds, which promote root growth, enhance nutrient absorption, and improve lettuce quality (Chen et al., 2021; Curtasu & Nørskov, 2024). These findings are supported by Calvo et al. (2014), Li et al. (2021), and Mahdavikia et al. (2019), who demonstrated that increased levels of plant pigments such as chlorophyll improve light absorption efficiency, which improves the nutritional status of the plant. Organic fertilizers also improve the physical and chemical properties of soil and increase enzyme activity associated with soil fertility results consistent with those reported by Al-Sultani & Al-Janabi (2022) and Hussain et al. (2020) when using Azolla extract in nutrient solutions, and with Mohamed et al. (2021), who confirmed the role of Azolla in improving soil and water properties. Willow extract's effectiveness stems partly from its salicylic acid concentration (Table 2), which boosts chlorophyll and carotenoid concentrations in leaves while enhancing antioxidant activity and preventing protein oxidation (Mossa et al., 2018; Sghiar et al., 2023). The supplied

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nutrients, particularly nitrogen, play crucial roles in fundamental biological processes including nucleic acid (DNA/RNA) synthesis, protein production, and auxin biosynthesis, all of which support cell division and meristematic growth (Abdelgawad et al., 2020). Phosphorus plays fundamental roles in plant metabolism as a structural component of nucleic acids and energy compounds (ATP and ADP), essential nutrients collectively stimulate cell expansion and vegetative growth. The observed increases in magnesium, calcium, iron, and copper (Table 1,2) concentrations further contributed to chlorophyll production and growth efficiency (Battacharyya et al., 2015), consistent with findings by Hassan et al. (2021) regarding nitrogen's positive effects on leaf number, area, and biomass accumulation. Studies have indicated that azolla extract improve the nutritional value of lettuce (Zulfiqar et al., 2019). Foliar application of kaolin has a direct effect on improving vegetative traits and reducing water loss, thereby enhancing nutrient uptake and plant growth (Abd El-Gawad et al., 2019). Organic extracts release nitrogen gradually, reducing nitrate accumulation in lettuce, consistent with the findings of Martinez-Ballesta et al. (2020), who demonstrated that lettuce treated with organic extracts contains lower nitrate concentrations. The increases in carotene and protein are directly linked to improved nitrogen uptake and stimulation of biological processes related to it (Glenn et al., 2010), as well as the effect of amino acids in organic extracts that enhance plant nutrition.
Al-Taey et al. (2019) and Martinez-Valero et al. (2020) noted that leafy vegetables can synthesize amino acids, and Kazemi (2014) reported that foliar application of salicylic acid increases protein concentration and plant pigments. Furthermore, the interaction between organic extracts and kaolin was found to increase leaf number, leaf area, and chlorophyll concentration, which in turn boosted protein and carotenoid levels This increases the dry weight of the plant. This is supported by Vlahova et al. (2022), who reported that organically grown lettuce contains higher carotenoid levels compared to conventionally cultivated lettuce.

CONCLUSION

The present experiment shows that spraying leaves with kaolin, azolla extract or willow extract remarkably enhances the quality of lettuce through modulating the nutrient uptake, chlorophyll concentration, and the carbohydrate assimilation with a less accumulation of nitrates. Synergistic effect of treatments (kaolin and organic extract of azolla) enhance the vegetative growth, photosynthetic function and antioxidant pigments (carotenoids). The organics extracts also contributed to soil health and nutrient availability, with prevail in support of sustainable lettuce production with better nutritional quality. These findings highlight the potential of natural biostimulants as eco-friendly alternatives to conventional fertilizers, offering improved crop quality with reduced environmental impact.

JOURNAL DECLARATION

The Second author (**Wafaa A. Hussain**) serves as an editor for Iraqi Journal of Agricultural Sciences but was not involved in the peer review process of this manuscript beyond their role as an author. The authors declare no other conflict of interest.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.
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AUTHOR’S CONTRIBUTION STATEMENT

The author conducted the experiment, performed the statistical analysis, interpreted the data, and wrote and revised the manuscript.

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تحسين جودة الخس من طريق الرش الورقي بالكاؤولين والمستخلصات النباتية

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المستخلص

أجري البحث لتحسين جودة نبات الخس باستخدام الرش بالكاؤولين ومستخلصي الأزولا والصفصاف. نُفذت تجربة عاملية وفق تصميم القطاعات العشوائية الكاملة بثلاثة مكررات، شملت ثلاثة عوامل: الرش بالكاؤولين(0، 4 غم لتر$^{-1}$)، ومستخلص الأزولا (0، 3.75%، 7.5%)، ومستخلص الصفصاف (0، 3% مستخلص أوراق، 3% مستخلص قلف). أظهرت النتائج أن الرش بالكاؤولين أدى إلى زيادة معنوية في نسبة الفسفور والكالسيوم وسمك الورقة ومحتوى الكلوروفيل الكلي والوزن الجاف للأوراق ومحتوى الكاروتين في الأوراق وبمعدلات بلغت 0.668% و2.189% و0.434 ملم ورقة$^{-1}$ و371.7 ملغم 100غم$^{-1}$ وزن رطب و1.620غم نبات$^{-1}$ و6.21 ملغم 100غم$^{-1}$ وزن رطب بالتتابع، في حين أدى الرش بمستخلص الأزولا إلى تحسين نسبة الفسفور والكالسيوم وسمك الورقة والمساحة الورقية ومحتوى الكلوروفيل والوزن الجاف والكربوهيدرات، مع خفض محتوى النترات وبقيم بلغت 0.710% و2.497% و0.446 ملم ورقة$^{-1}$ و384.0 دسم^{2} نبات$^{-1}$ و350.4 ملغم 100غم وزن رطب$^{-1}$ و 1.772غم نبات$^{-1}$ و 17.59% و 0.14 ملغم غم$^{-1}$ وزن جاف بالتتابع قياسا بمعاملة المقارنة. أما الرش بمستخلص الصفصاف فقد ساهم في زيادة الفسفور والكالسيوم والمساحة الورقية والوزن الجاف والكربوهيدرات وبقيم بلغت 0.756% و2.415% و374.69 سم نبات$^{-1}$ و1.687 غم نبات$^{-1}$ و17.21% بالتتابع، كما أظهرت التداخلات الثلاثية بين العوامل تأثيرات معنوية واضحة في معظم المؤشرات المدروسة، مما يؤكد أهمية هذه المعاملات في تحسين الصفات النوعية للخس.

الكلمات المفتاحية: الزراعة الآمنة بيئياً، المحاصيل الورقية، المستخلصات المستدامة، نبات الخس.