



RESEARCH ARTICLE

# Foliar application of humic acid on growth and biomass improvement of bok choy and red leaf lettuce

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## Abstract

Humic acid (HA) is an organic product that is applied as a foliar spray to stimulate the growth and health of various horticultural and agricultural crops. However, its impact on the growth, yield, and health of exotic vegetable crops such as bok choy (*Brassica rapa* L.) and red leaf lettuce (*Lactuca sativa* L.) needs to be further explored. The current study aimed to assess the impact of foliar application of HA on the growth and biomass production of bok choy and red leaf lettuce. Notably, the foliar spray of 2% HA resulted in increased plant height, leaf length and width, leaf area, and overall plant biomass production in both bok choy and red leaf lettuce compared to the control group. However, the foliar spray of HA did not significantly affect the shoot and root length, nor the number of leaves of bok choy compared to a control. Moreover, the application of HA significantly improved the shoot length and the number of leaves of red leaf lettuce compared to the control group. Hence, HA emerges as a promising natural resource for enhancing agricultural production sustainably. It could serve as a superior growth enhancer for both bok choy and red leaf lettuce.

## Keywords

humic acid; foliar application; bok choy; red leaf lettuce; growth; biomass

## Introduction

Due to the constant use of inorganic fertilizers and chemicals in crop cultivation, agriculture can lead to various detrimental effects on soil quality and ecosystem function, including environmental pollution, reduction of soil microbial population, lowering of soil organic matter, reduction of solubility and mobility of soil micro and macronutrients, and more (1). Alternatively, the use of organic fertilizers in crop production is the best way to reduce the utilization of synthetic fertilizers and agrochemicals while improving the physicochemical and biological properties of the soil (2). Humic acid (HA) is an important organic fertilizer widely applied in agriculture systems to enhance soil fertility and quality, nutrient uptake efficiency, as well as the growth, health, and yield of various important field crops (3). It is the main component of humic substances (HS) along with humin and fulvic acid, which are naturally synthesized from the decomposition of organic matter such as plants and animals (4). Notably, HA consists of 37.2 to 75.8% carbon, 7.9 to 56.6% oxygen, 1.6 to 11.7% hydrogen, 0.5 to 10.5% nitrogen, 0.1 to 8.3% sulphur, and other substances

that stimulate plant growth and health, in addition to improving soil quality (5).

Primarily, HA application improved the physiochemical and biological properties of soil by regulating the formation of soil water-stable aggregates, aeration, and pH, increasing water holding and cation exchange capacity of the soil, as well as enhancing the richness and activity of soil microorganisms (6, 7). Besides improving soil quality, it promotes plant growth and health by enhancing the uptake of nutrients, seed germination, formation and development of roots, as well as promoting root and shoot growth (8, 9). Improving soil fertility and stimulating plant growth by application of HA consequently increases the yield and quality of crops (10, 11). Additionally, HA application was found to improve the plant's tolerance to stress conditions such as drought, low temperature, and salinity (9, 12–14), as well as resistance to diseases and crop pests (8, 15, 16). Numerous reports suggest that HA application has positive effects on the growth, health, and yield of various crops, including peanut (11), banana (16), wheat (17), maize (18), sugarcane seedlings (19), cucumber (20), and tomato (21). In rare cases, HA application improved the soil phosphorus availability of lettuce under cultivation (22).

Bok choy (*Brassica rapa* L.) also known as spoon cabbage or Chinese chard, belongs to the family Brassicaceae and thrives best in temperate and subtropical regions with an optimum temperature of 18–20°C (23). It is a biennial green-leaf vegetable crop classified under the Chinese cabbage variety, with a global production of 70.84 metric tons in 2018 (24). Bokchoy is a high-value vegetable, containing fiber, protein, vitamins (A, B, B2, B6, and C), as well as calcium, folic acid, magnesium, copper, and iron (25). Similarly, lettuce (*Lactuca sativa* L.) is an important leafy vegetable belonging to the family Asteraceae and grouped into seven different types, including red-leaf lettuce. According to data published by the Food and Agriculture Organization (FAO), lettuce worldwide production exceeded 20 million tons per year in 2022 (26). It is also an important source of minerals, vitamins, and phytonutrients such as carotenoids, flavonoids, and phenolic acids, including cyanidin, quercetin, caftaric acid, chlorogenic acid, isochlorogenic acid, and chicoric acid (27). These two leafy vegetables are important dietary sources of essential nutrients and antioxidants, contributing to human health (25–27). Interestingly, a foliar application of 2% HA was found to improve the growth and yield of mungbean and olive (28, 29). Hence, a 2% HA spray could be an optimum concentration for enhancing the growth and yield of leafy vegetables. Building on this connection, a 2% HA foliar spray was used in this investigation. However, the effect of HA on the growth and yield of exotic vegetable crops such as bok choy and red leaf lettuce is not well explored and needs further study. With this rationale, the current study was conducted to assess the effect of foliar application of HA on the growth and biomass improvement of bok choy and red leaf lettuce.

## Materials and Methods

### Planting material, humic acid, and growth media

Red leaf (RZ-Cherokee) and bok choy (Tokito-Choko) seedlings were grown in 98-cell plug portray containing a mixture of coco peat and vermicompost mixture (3:1). Specifically, seeds were sown individually in each cell of the portray containing the growth media. After seed germination, seedlings were watered once every two days by sprinkling and supplied with a 100 ppm water-soluble NPK (19:19:19) foliar spray at five-days intervals up to 20 days of sowing (DOS), as used for the study. The humic acid (HA) used for the study was purchased from Sigma-Aldrich (CAS number: 1415-93-6). A 2% HA solution was prepared and used for foliar spray. The soil used for growing the plants was collected from a farmer's field located at Kookalthorai, Kothagiri, Nilgiris. The physiochemical characteristics of collected soil used for the study were examined, and the results are provided in Table 1.

### Experimental design, treatments, and plant growth

A pot culture experiment was conducted using a Randomized Complete Block Design (RCBD) with two treatments and six replications in a Polyhouse at Kookalthorai, Kothagiri, Nilgiris. The experimental treatments included a control group without HA foliar spray (CT) and a group with a 2% HA foliar spray (HA). 20 days old seedlings were transplanted into 2.5 kg plastic pot containing 2.0 kg of soil mixture prepared by mixing red soil and sand at a ratio of 3:1. In a total of 24 pots, 12 pots were allocated for each red leaf and bok choy. 10 days after transplanting, a 2% HA foliar spray was applied. All the pots were watered once every two-day with 100 mL of Hoagland's nutrient solution and 200 mL water. Plant growth-related observations for red leaf lettuce and bok choy were recorded 5 days after the HA spray.

### Growth attributes

Plants were gently uprooted with minimum disturbance to the roots and washed with running tap water to remove soil particles adhering to the root surface. First, growth-related phytomorphological parameters such as shoot length (cm), root length (cm), plant height (cm), leaf length (cm), leaf width (cm), leaf number, and leaf area (cm<sup>2</sup>) were recorded. Subsequently, the plant samples were dried in a hot air oven at 78°C for 3 days. Plant biomass production was measured and expressed as mg g<sup>-1</sup> DW (30–32).

### Statistical analysis

Statistical analysis was conducted using the software SPSS (version 16.0). The effects of foliar application of HA on the growth of red leaf lettuce and bok choy compared to the control were analysed. Significant differences between the treatments were assessed using an independent sample student's t-test at  $p > 0.05$  and  $p > 0.01$  levels of significance. All data were summarized using descriptive statistics and presented in bar diagrams with standard error bar, expressing the mean value and standard error (mean  $\pm$  SE). Pearson's correlation analysis ( $p < 0.05$ ) was performed to examine the relationship between the

variables influenced by the foliar application of HA in red leaf and bok choy.

## Results and Discussion

Application of HA, whether as soil amendment, seed treatment, or foliar spray, has been found to improve growth, health, yield, and quality of various agricultural and horticultural crops. This improvement is attributed to enhanced seed germination, root growth, nutrient uptake, water use efficiency, and photosynthetic efficiency. However, the effect of foliar spray of HA on the growth and yield of bok choy and red leaf lettuce remains a gap in the research and needs further exploration (10, 11, 19, 21, 29, 33–35). Hence, the present study demonstrates that foliar application of 2% HA enhances the growth and biomass production of leafy vegetables such as bok choy and red leaf lettuce.

### Phytomorphological traits of bok choy and red leaf lettuce

Morphological traits of plants are important factors that determine the efficiency of plants in utilizing available light energy and gas exchange capacity. Additionally, they contribute to the spatial configuration of plants, which, in turn, influences yield and quality improvement (31). In particular, the yield of leafy vegetables is directly related to morphological traits such as plant height, shoot and root length, number of leaves, and biomass accumulation (16, 31, 33). HA is widely used in agriculture and brings about morphological changes in various crops by supplying essential nutrients and growth factors that enhances growth, health, and yield (3, 20). In the present study, compared to the control, foliar application of 2% HA significantly improved the plant height of bok choy by 8.77%; however, shoot and root length were not statistically affected (Table 1 and; Fig. 1). Similarly, the foliar spray of HA significantly enhanced shoot length (18.84%) and plant height (13.60%) of the red leaf lettuce compared to the control, while root length was not affected (Fig. 1). This enhancement in plant height of leafy vegetables by HA application is known to promote shoot and root growth by supplying nutrients and regulating the ability of plants to uptake nutrients, as well as augmenting the synthesis of plant growth-stimulating endogenous hormones such as auxin and cytokinins and metabolic enzymes (20, 36, 37). Similarly, HA-treated banana plantlets recorded higher pseudo-stem height, girth, and weight, as well as root length and weight (16). The application of HA extracted from both pig manure and food waste-based vermicompost significantly improved the growth of tomato and cucumber by enhancing plant height due to hormone-like activity (38). Additionally, maize plants treated with HA recorded higher induction of lateral roots, as well as root diameter and length, resulting in increased uptake of available soil nutrients (37). These improvements in plant height and root length of bok choy and red leaf lettuce by foliar application of HA have a positive impact on biomass production, which could lead to yield enhancement (16, 36).

Usually, the leaf area index (LAI) is considered an imperative parameter to determine the photosynthetic efficiency of plants, which is an important yield-contributing characteristic (39). In the present study, leaf length (16.43%), leaf width (23.61%), and leaf area (26.30%) were significantly improved in bok choy by the foliar application of 2% HA compared to the control, while the number of leaves (6.52%) was not influenced by HA application (Table 1 and 2; Fig. 2). Similarly, foliar application of 2% HA significantly improved leaf length (21.78%), leaf width (56.86%), leaf area (65.59%), and the number of leaves (21.88%) in red leaf compared to the control (Fig. 2). This increased leaf area and related parameters such as leaf width, leaf area, and the number of leaves are attributed to the production of enlarged primordium cells and progressive increase in the size of the cells during initiation at the meristem and subsequent growth. This process is stimulated by the synthesis of endogenous plant growth hormones and enzymes, as well as the supply of nutrients through the application of HA substances as a foliar spray (38, 40). HA-treated banana plantlets also recorded a higher number of leaves (16). Furthermore, application of HA through seed treatment increased the number of leaves per corn plant (18). Additionally, olive cuttings treated with HA along with GA<sub>3</sub> significantly improved leaf area and leaf fresh and dry weight, consequently increasing chlorophyll content and potentially affecting the photosynthesis rate (28).

### Plant biomass production of bok choy and red leaf lettuce

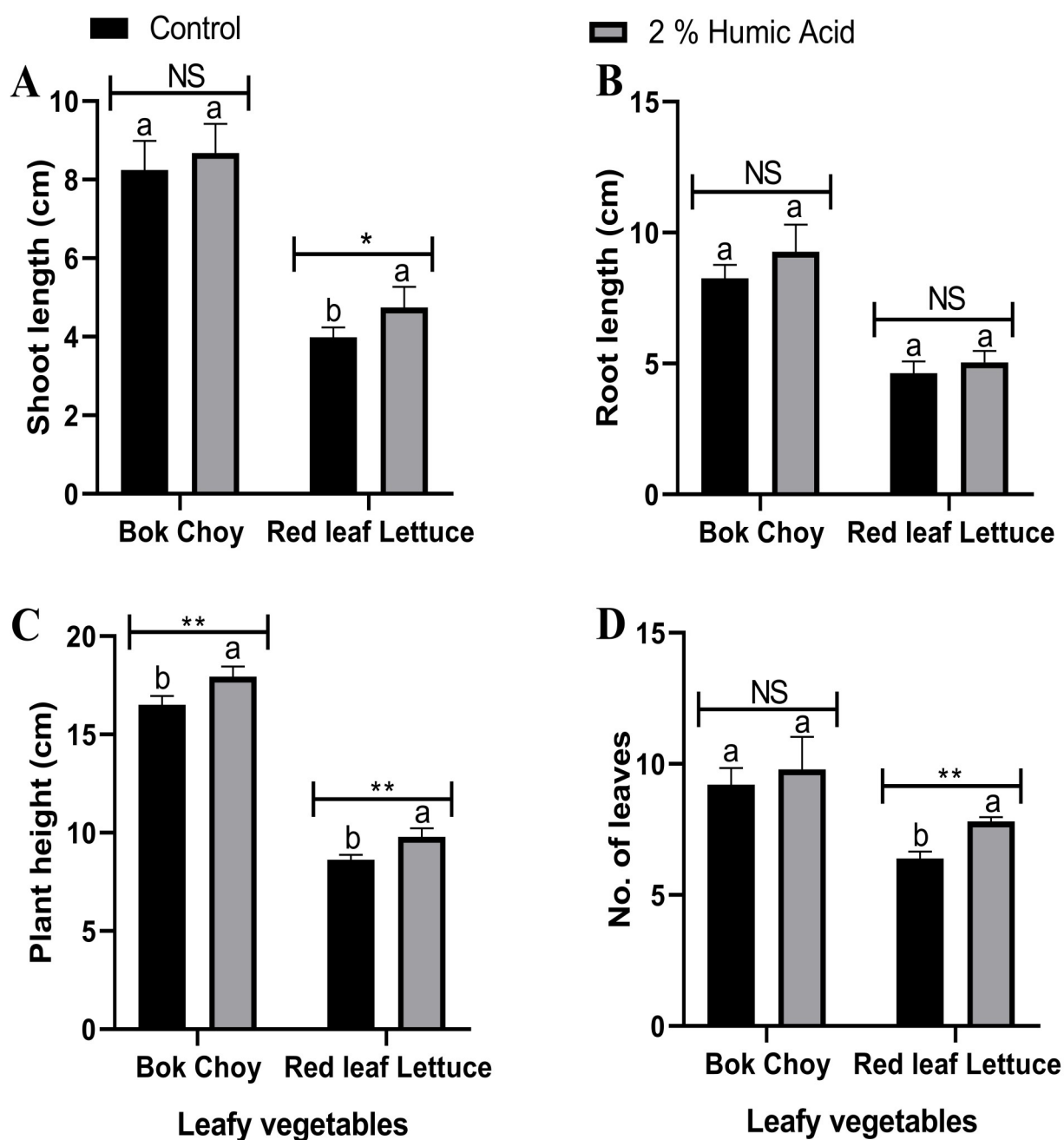
The presence of various nutritional and growth factors in HA could increase plant growth (38). HA consists of 37.2 to 75.8% carbon, 7.9 to 56.6% oxygen, 1.6 to 11.7% hydrogen, 0.5 to 10.5% nitrogen, 0.1 to 8.3% sulfur, and other substances responsible for stimulating plant growth (5). In the present study, compared to the control, foliar application of 2% HA significantly improved plant biomass production (26.88%) of bok choy (Table 1 and 3; Fig. 2). Similarly, foliar spraying of 2% HA significantly improved plant biomass production (27.71%) of the red leaf compared to the control (Table 1 and 2; Fig. 2). The increased plant biomass production by foliar application of HA is attributed to enhanced uptake and supply of nutrients and stimulation of plant growth hormones and enzymes (30, 38). Likewise, banana plantlets treated with HA recorded higher pseudo-stem weight as well as root weight (16). Moreover, soil application of humic acid at the rate of 60 kg ha<sup>-1</sup> was found to improve plant height and dry weight of *Zea mays* L. (32). The shoot and root weight

**Table 1.** Physical and chemical properties of soil used for the study.

Soil properties	
Color	Red soil
Texture	Sandy clay
pH	5.38 (±0.02)
Electrical conductivity (dS m <sup>-1</sup> )	0.35 (±0.01)
Soil organic carbon (mg g <sup>-1</sup> soil)	2.36 (±0.06)
Available nitrogen (mg kg <sup>-1</sup> soil)	176.0 (±1.12)
Available phosphorus (mg kg <sup>-1</sup> soil)	212.5 (±2.73)
Available potassium (mg kg <sup>-1</sup> soil)	275.0 (±2.92)

**Table 2.** Foliar application of HA on growth and biomass production of bok choy. Data in the table are expressed as mean  $\pm$  SE. Mean values of the two treatments were compared by independent sample student t-test at  $p \leq 0.05$ . MD- mean difference between, HA- humic acid applied, t- t-test value, p- probability of significance at two-tailed test.

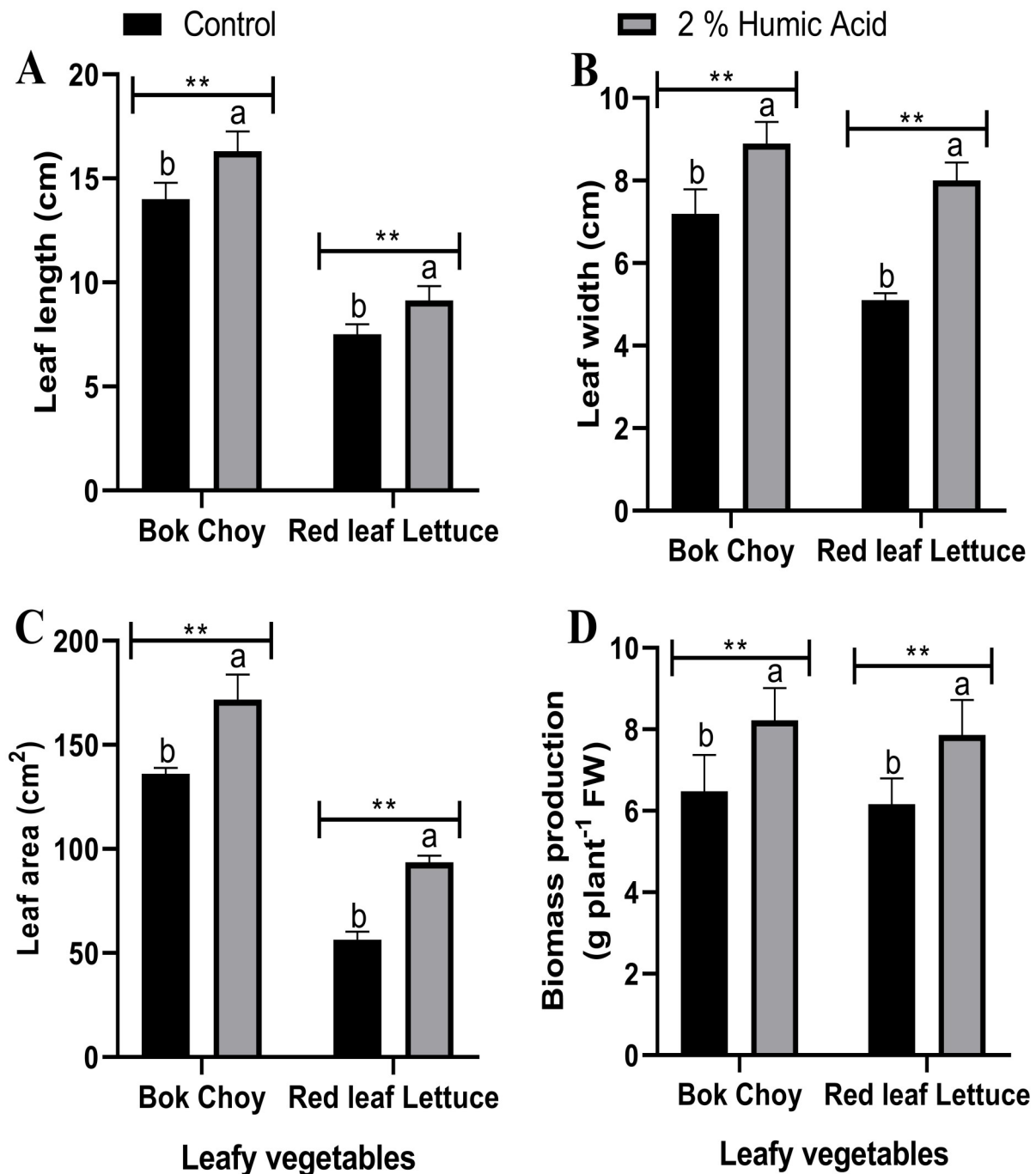
Variables	Control	HA	MDP	t	p
Shoot length (cm)	8.24 ( $\pm 0.30$ ) <sup>a</sup>	8.68 ( $\pm 0.30$ ) <sup>a</sup>	5.32	-1.02	0.331
Root length (cm)	8.26 ( $\pm 0.21$ ) <sup>a</sup>	9.27 ( $\pm 0.43$ ) <sup>a</sup>	12.21	-2.13	0.059
Plant height (cm)	16.50 ( $\pm 0.19$ ) <sup>b</sup>	17.95 ( $\pm 0.21$ ) <sup>a</sup>	8.77	-5.13	<0.001
No. of leaves	9.20 ( $\pm 0.26$ ) <sup>a</sup>	9.80 ( $\pm 0.50$ ) <sup>a</sup>	6.52	-1.06	0.315
Leaf length (cm)	14.00 ( $\pm 0.32$ ) <sup>b</sup>	16.30 ( $\pm 0.39$ ) <sup>a</sup>	16.43	-4.53	<0.001
Leaf width (cm)	7.20 ( $\pm 0.24$ ) <sup>b</sup>	8.90 ( $\pm 0.21$ ) <sup>a</sup>	23.61	-5.31	0.001
Leaf area (cm <sup>2</sup> )	136.03 ( $\pm 1.16$ ) <sup>b</sup>	171.82 ( $\pm 4.84$ ) <sup>a</sup>	26.30	-7.19	<0.001
Plant biomass production (g)	6.48 ( $\pm 0.36$ ) <sup>b</sup>	8.22 ( $\pm 0.32$ ) <sup>a</sup>	26.88	-3.59	0.005



**Fig. 1.** Effect of foliar application of HA on (A) shoot length, (B) root length, (C) plant height, and (D) number of leaves of bok choy and red leaf lettuce. Data in the figure are expressed as mean  $\pm$  SE. Mean values of the two treatments were compared by independent sample student t-test at  $p \leq 0.05$  and  $p \leq 0.01$ .

**Table 3.** Foliar application of HA on growth and biomass production of red leaf lettuce. Data in the table are expressed as mean  $\pm$  SE. Mean values of the two treatments were compared by independent sample student t-test at  $p \leq 0.05$ . MD- mean difference between, HA- humic acid applied, t- t-test value, p- probability of significance at two-tailed test.

Variables	Control	HA	MDP	t	p
Shoot length (cm)	3.99 ( $\pm 0.10$ ) <sup>b</sup>	4.74 ( $\pm 0.22$ ) <sup>a</sup>	18.84	-3.148	0.010
Root length (cm)	4.63 ( $\pm 0.18$ ) <sup>a</sup>	5.05 ( $\pm 0.18$ ) <sup>a</sup>	9.08	-1.637	0.133
Plant height (cm)	8.62 ( $\pm 0.10$ ) <sup>b</sup>	9.72 ( $\pm 0.18$ ) <sup>a</sup>	13.60	-5.743	<0.001
No. of leaves	6.40 ( $\pm 0.10$ ) <sup>b</sup>	7.80 ( $\pm 0.01$ ) <sup>a</sup>	21.88	-11.299	<0.001
Leaf length (cm)	7.50 ( $\pm 0.20$ ) <sup>b</sup>	9.13 ( $\pm 0.28$ ) <sup>a</sup>	21.78	-4.675	0.001
Leaf width (cm)	5.10 ( $\pm 0.01$ ) <sup>b</sup>	8.00 ( $\pm 0.18$ ) <sup>a</sup>	56.86	-15.229	<0.001
Leaf area (cm <sup>2</sup> )	56.50 ( $\pm 1.57$ ) <sup>b</sup>	93.55 ( $\pm 1.32$ ) <sup>a</sup>	65.59	-18.122	<0.001
Plant biomass production (g)	6.16 ( $\pm 0.26$ ) <sup>b</sup>	7.87 ( $\pm 0.35$ ) <sup>a</sup>	27.71	-3.910	0.003



**Figure 2.** Effect of foliar application of HA on (A) leaf length, (B) leaf width, (C) leaf area, and (D) biomass production of bok choy and red leaf lettuce. Data in the figure are expressed as mean  $\pm$  SE. Mean values of the two treatments were compared by independent sample student t-test at  $p \leq 0.05$  and  $p \leq 0.01$ .

of tomato and cucumber were reported to be augmented by the application of HA extracted from both pig manure and food waste-based vermicompost, which, in turn, improved the growth of plants (38). Overall, HA was found to stimulate the growth of tested leafy vegetables like bok choy and red leaf by augmenting plant height, shoot and root length, leaf area and biomass production.

### Correlation analysis

Foliar application of 2% HA showed a positive correlation with the growth parameters of bok choy (Tables 4 and 5). In bok choy, plant height was positively correlated with the root length, influenced by HA application. Moreover, plant biomass production, root length, plant height, number of leaves, and leaf width and length of bok choy were positively correlated with leaf area. In red leaf lettuce, plant biomass production was positively correlated with plant height, number of leaves, leaf width, and leaf area. Additionally, shoot length, plant height, number of leaves, leaf length and width, and plant biomass production of red leaf lettuce were positively correlated with leaf area. HA application positively influenced the growth-related variables of bok choy and red leaf lettuce by stimulating the production of indole-3-acetic acid, cytokinins, and other nutritional factors (20, 38). Similarly, plant height and total dry matter production of *Tectona grandis* were positively correlated with the rate of HA application (41). Furthermore, HA applications were found to promote root growth in maize, significantly correlated with HA hydrophobicity association in the rhizosphere region, which leads to the release of auxin-like plant growth promoters and stimulates biochemical activity in plants (42).

### Conclusion

Humic acid (HA) is widely utilized in agricultural production systems to enhance soil quality, nutrient uptake efficiency, as well as growth, health, and yield of various important agricultural and horticultural crops. Notably, HA consists of various nutrients and plant growth-promoting substances that contribute to improved plant growth. The present study concludes that foliar application of 2% HA enhanced phytomorphological traits, leaf area index, and biomass production of leafy vegetables such as bok choy, and red leaf lettuce. Therefore, HA represents a promising organic formulation and a preferred alternative to synthetic chemical fertilizers for sustainable agricultural production systems.

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### Authors' contributions

SA, SR, DS, GR, and KS conceived the idea, planned the work, and drafted the manuscript. SA carried out the experiments and statistical analysis. All authors read and approved the final manuscript.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

**Table 4.** Effect of foliar application humic acid on plant growth and plant biomass production of bok choy.

Variables	Plant biomass production	Shoot length	Root length	Plant height	No. of leaves	Leaf length	Leaf width	Leaf area
Plant biomass production	1	0.265	0.228	0.465	-0.025	0.797**	0.802**	0.620*
Shoot length		1	-0.466	0.344	0.125	0.113	0.108	0.165
Root length			1	0.670*	0.274	0.472	0.516	0.606*
Plant height				1	0.396	0.596*	0.638*	0.782**
No. of leaves					1	-0.258	-0.174	0.630*
Leaf length						1	0.990**	0.582*
Leaf width							1	0.652*
Leaf area								1

\*. Correlation is significant at the 0.05 level (2-tailed); \*\*. Correlation is significant at the 0.01 level (2-tailed)

**Table 5.** Effect of foliar application humic acid on plant growth and plant biomass production of red leaf lettuce.

Variables	Plant biomass production	Shoot length	Root length	Plant height	No. of leaves	Leaf length	Leaf width	Leaf area
Plant biomass production	1	0.386	0.416	0.592*	0.784**	0.529	0.795**	0.761**
Shoot length		1	-0.093	0.733**	0.667*	0.750**	0.582*	0.693*
Root length			1	0.609*	0.528	0.433	0.474	0.509
Plant height				1	0.892**	0.893**	0.787**	0.900**
No. of leaves					1	0.879**	0.926**	0.990**
Leaf length						1	0.721**	0.893**
Leaf width							1	0.950**
Leaf area								1

\*. Correlation is significant at the 0.05 level (2-tailed); \*\*. Correlation is significant at the 0.01 level (2-tailed).

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