Original Research

Research on Crop Ecophysiology
Vol.20/1, Issue 1 (2025), Pages: 1 - 14

Evaluation of Pre and Postharvest Treatments on Growth Parameters and Photosynthetic Pigments in Iceberg Lettuce

MOHAMMAD REZA FEREYDOUNIAN[1,3] AND MEHRDAD JAFARPOUR[2,3] *

1- *Department of Horticultural Sciences, Isf. C., Islamic Azad University, Isfahan, Iran*
2-*Associate professor, Department of Horticultural Sciences, Edibe and Medicinal Mushrooms Research Center, Isf. C., Islamic Azad University, Isfahan, Iran (Corresponding Author)*
3-*Institute of Agriculture, Water, Food, and Nutraceuticals, Isf.C., Islamic Azad University, Isfahan, Iran*

*Corresponding author's E-mail: mehrdad.jafarpour@iau.ac.ir

Received: 15 October 2024 Accepted: 25 January 2025

ABSTRACT

This study examines the effects of various pre-harvest treatments on the postharvest quality of iceberg lettuce (*Lactuca sativa* var. capitata), a highly perishable vegetable with considerable commercial importance. The primary aim was to identify the most effective concentrations of these treatments—comprising oxalic acid, biochar, and wood vinegar in enhancing growth parameters and chlorophyll content. A factorial experimental design was employed in a randomized complete block layout with three replicates. Treatments included a control with distilled water, three levels of oxalic acid (1.5, 2.5, and 3.5 mM), three rates of biochar (150, 250, and 350 g per plant), and three concentrations of wood vinegar (2, 3, and 4 mL L^{-1}). Postharvest, the plants were processed in two ways: either left uncut or cut, and then stored at 4°C for 0, 2, 4, 6, and 8 days. The results indicated that pre-harvest treatments, especially biochar at 150 g per plant and oxalic acid, significantly improved key quality traits such as leaf number, plant volume, and chlorophyll content during storage. Biochar at this optimal concentration consistently demonstrated superior performance in maintaining leaf quality, overall plant vigor, and pigment content. Additionally, uncut samples generally preserved these qualities better than cut ones, highlighting the detrimental effects of processing stress. In conclusion, biochar at 150 g per plant emerged as the most effective treatment for promoting growth and extending postharvest longevity of iceberg lettuce. These findings suggest that optimizing pre-harvest practices with biochar can significantly enhance lettuce shelf life and quality, offering a sustainable strategy for growers.

Keywords: Biochar application, Chlorophyll content, Postharvest quality, Storage longevity.

INTRODUCTION

Iceberg lettuce (*Lactuca sativa* var. capitata) is a widely consumed vegetable belonging to the Asteraceae family, appreciated for its crisp texture and nutritional benefits. However, it is highly perishable, and postharvest quality is a significant concern for growers, wholesalers, and retailers. The deterioration of iceberg lettuce after harvest can lead to considerable economic losses due to wilting, discoloration, and reduced sensory and nutritional qualities (Abdullah & Zakaria, 2024). Factors such as cultivar, storage conditions, and postharvest treatments play crucial roles in maintaining the quality of lettuce (Lee *et al.*, 2017). As a result, enhancing the growth, productivity, and storage potential of iceberg lettuce is a critical goal for both producers and consumers (Lee *et al.*, 2022).

One of the primary challenges faced during the storage of iceberg lettuce is its susceptibility to enzymatic browning, which occurs when the tissue is damaged, leading to the formation of brown pigments that negatively affect visual appeal and marketability (Abdullah & Zakaria, 2024). The rejection of visually unappealing products by consumers significantly impacts the commercialization chain, particularly in export markets (Martínez-Ispizua *et al.*, 2022). To mitigate these postharvest losses, innovative pre-harvest treatments have been explored, including the application of natural elicitors such as oxalic acid, biochar, and wood vinegar.

Oxalic acid is a naturally occurring organic acid known for its ability to inhibit ethylene biosynthesis, thereby delaying the ripening process and extending the storage life of fruits and vegetables (Eroğul *et al.*, 2023). Its application in horticultural crops has been shown to alleviate the adverse effects of abiotic stresses, enhance disease resistance, and reduce postharvest chilling injury and browning (Zhang *et al.*, 2023). Importantly, oxalic acid is recognized for its safety, with no negative impacts on human health or the environment, making it a viable option for improving the postharvest quality of iceberg lettuce (Liao *et al.*, 2023).

Biochar, a carbon-rich material produced from the pyrolysis of biomass, has gained attention for its potential to enhance soil health and crop productivity (Coumar *et al.*, 2016). By improving the physical, chemical, and biological properties of the soil, biochar can positively affect plant growth and resilience (Hasan *et al.*, 2018). Its application as a pre-harvest treatment for iceberg lettuce could help improve overall plant health, thereby contributing to better postharvest quality and extended shelf life.

Wood vinegar, a by-product of biochar production, contains various organic acids and phenolic compounds that can enhance plant growth and stress resistance (Rehman *et al.*, 2015). The presence of compounds such as acetic acid and propionic acid in wood vinegar can stimulate photosynthesis, while phenolic compounds exhibit antioxidant properties that protect plants from oxidative stress (Yang *et al.*, 2016). Utilizing wood vinegar as a pre-harvest treatment may further improve the quality and longevity of iceberg lettuce during storage.

This study aims to investigate the effects of pre-harvest treatments with oxalic acid, biochar, and wood vinegar on the postharvest quality and longevity of iceberg lettuce. By evaluating leaf size, leaf count, plant volume, and chlorophyll content during various storage periods, we seek to determine the effectiveness of these treatments in maintaining the freshness and nutritional integrity of both whole and cut iceberg lettuce. Ultimately, this research will contribute to developing strategies that enhance the postharvest quality of this valuable vegetable, benefiting growers and consumers alike.

MATERIALS AND METHODS

Experimental design

This study was conducted in a private greenhouse in Velashan County, Isfahan Province, at an altitude of 1,604.82 meters above sea level. The experimental design was a factorial design following a complete randomized block design with three replications. The treatments included various pre-harvest applications and application levels. These involved a control with distilled water, oxalic acid sourced from Merck Germany at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5) and wood vinegar applied at three concentrations 2, 3, and 4 mL. L^{-1} (S2, S3, and S4). Additionally, biochar was applied at three rates 150, 250, and 350 g per plant (B150, B250, and B350) .

The iceberg lettuce plants were grown in soil with 30 cm spacing between plants and rows, resulting in about nine plants per square meter. They were irrigated via a drip system every seven days. The pre-harvest treatments were applied when the plants were 40 days old, in three rounds spaced a week apart, with the first application at 40 days, and the last, one week before harvest .

After harvesting, plants were processed in two ways: either left uncut or cut, and then stored in polyethylene bags at 4°C for five different durations: 0, 2, 4, 6, and 8 days. During each storage period, measurements of leaf size, plant volume, and chlorophyll content were conducted to evaluate how the treatments influenced postharvest quality.

Growth traits

The total number of leaves on each lettuce plant was determined by counting all the visible leaves. The length of each leaf was measured using a ruler, with measurements recorded in millimeters. For this process, the length of each leaf was measured from the base of the leaf stem to the tip. The volume of each iceberg lettuce plant was estimated by measuring its physical dimensions specifically, length, width, and height.

Photosynthetic pigments

For determining leaf chlorophyll, approximately 5 g of fresh leaf tissue was homogenized with 80% acetone, then filtered and diluted to a final volume of 10 mL. The absorbance of this extract was measured at wavelengths of 663 nm and 647 nm using a spectrophotometer (U-2100, JASCO, Japan), following the method described by Lichtenthaler (1987). The concentration of chlorophyll a was calculated using the following formulae:

Chlorophyll a = (19.3 × A663 - 0.86 × A647) Volume / 100 Mass

Chlorophyll b = (19.3 × A647 - 3.6 × A663) Volume /100 Mass

Total chlorophyll = Chlorophyll a + Chlorophyll b

Statistical analysis

The research was carried out utilizing a factorial experiment design following a complete randomized block design with three replications. Variance analysis of the data with SPSS software and also to compare the mean of the desired traits, the LSD test was used at a five percent probability level**.**

RESULTS AND DISCUSSION

The results of the variance analysis showed that the simple effect of storage durations had a significant impact at 1% level on leaf length, plant volume, chlorophyll a, chlorophyll b, and total chlorophyll, at the same time it did not significantly affect the number of leaves. The simple effect of pre-harvest treatments significantly influenced chlorophyll b at 5% level and had significant effects at 1% level on the number of leaves, leaf length, plant volume, chlorophyll a, and total chlorophyll. The simple effect of processing significantly affected leaf length and plant volume at 1% level, but did not have a significant impact on the number of leaves and photosynthetic pigments.

The interaction effect between storage durations and pre-harvest treatments did not significantly affect growth traits and photosynthetic pigments in lettuce. The interaction effect between storage durations and processing had a significant impact at 1% level on plant volume, while it did not significantly affect the number of leaves, leaf length, and photosynthetic pigments. The interaction effect between pre-harvest treatments and processing significantly influenced leaf length at 1% level, but did not have a significant impact on other traits. The three-way interaction of storage durations, pre-harvest treatments, and processing did not significantly affect any of the growth traits or photosynthetic pigments (Table 1).

Table 1. Analysis of variance ofstorage durations, treatment and processing on some growth parameters and photosynthesis pigments of the iceberg lettuce

Sources of changes	df	Mean squares					
		Number of leaf	Length of leaf	Volume of plant	Chlorophyll a	Chlorophyll b	Total chlorophyll
Block	2	4.57 n.s	0.07 n.s	1.39 n.s	0.00245 n.s	0.00003 n.s	0.00278 n.s
Storage durations	5	2.12 n.s	944.89 **	2.83 **	0.43631 **	0.11673 **	1.00336 **
Pre-harvest treatments	9	71.54 **	2709.27 **	7.56 **	0.00694 **	0.00286 *	0.01783**
Processing	1	189.00 n.s	639040 **	2.79 **	0.00174 n.s	0.00009 n.s	0.00104 n.s
Storage durations × Pre-harvest treatments	45	0.45 n.s	16.24 n.s	1.52 n.s	0.00022 n.s	0.00075 n.s	0.00063 n.s
Storage durations × Processing	5	0.39 n.s	19.31 n.s	5.38 **	0.00016 n.s	0.00096 n.s	0.00085 n.s
Pre-harvest treatments ×Processing	9	8.44 n.s	374.36 **	1.45 n.s	0.00007 n.s	0.00045 n.s	0.00029 n.s
Storage durations × Pre-harvest treatments ×Processing	45	0.65 n.s	7.95 n.s	1.21 n.s	0.00011 n.s	0.00040 n.s	0.00036 n.s
Error	238	2.25	8.27	0.89	0.00100	0.00127	0.00307
CV (%)		9.11	6.25	10.78	19.07	16.23	20.19

ns: not significant, * significant at $P < 0.05$ and ** significant at $P < 0.01$ probability level, df: degree of freedom.

The interaction effect of storage durations and pre-harvest treatments on some growth parameters and photosynthesis pigments of the iceberg lettuce

The interaction between storage durations and pre-harvest treatments showed that on the second day of storage, the highest number of lettuce leaves was observed in the treatments B150, B350, OA1.5, and OA2.5 compared to the control treatment. On the eighth day of storage, the treatments with biochar at all three concentrations (B150, B250, B350) and the treatments with oxalic acid at two concentrations (OA1.5, OA2.5) exhibited the highest number of lettuce leaves compared to the control, while the treatment with wood vinegar did not show a significant difference in leaf count compared to the control on day 8. On day 0 of storage, there was no significant difference in leaf length between the pre-harvest treatments and the control; however, on day 8, the leaf length in treatments B150, B350, and OA1.5 had more leaves than the control. In the wood vinegar treatment at all three concentrations (S2, S3, S4), there was no significant difference in leaf length compared to the control at any storage duration. The plant volume on day 8 of lettuce storage in the refrigerator was significantly greater in the treatments B150, B250, B350, and OA1.5 compared to the control, while the wood vinegar treatment at all three concentrations (S2, S3, S4) and the treatments OA2.5 and OA3.5 did not show a significant difference in plant volume compared to the control on day 8. Overall, on days 0 and 6 of lettuce storage in the refrigerator, the highest levels of chlorophyll a were observed in the biochar treatments at all three concentrations (B150, B250, B350) as well as in the oxalic acid treatments at all three concentrations (OA1.5, OA2.5, OA3.5) compared to the control. On day 8 of storage, the treatments OA3.5 and S2, S3 had higher chlorophyll a levels compared to the control, in contrast the other pre-harvest treatments did not show significant differences in chlorophyll a on day 8. The concentrations of chlorophyll b and total chlorophyll on days 0 and 6 were significantly higher in the treatments B150, B250, and B350 compared to the control. However, on day 8 of storage, the highest concentrations of chlorophyll b and total chlorophyll were observed in the treatment OA3.5 compared to the control and other pre-harvest treatments (Table 2).

Table 2. The interaction effect of experimental treatment (storage durations and pre-harvest treatments) on some growth parameters and photosynthesis pigments of the iceberg lettuce

Storage durations	Pre-harvest treatments	Number of leaf (per plant)	Length of leaf (mm)	Volume of plant (cm^3)	Chlorophyll a (mg. g^{-1} Fw)	Chlorophyll b (mg. g^{-1} Fw)	Total chlorophyll (mg. g^{-1} Fw)
	Control	32 a	244.00 abc	0.0016 ab	0.31 fgh	0.11 gh	0.43 hi
	B150	32 a	262.50 a	0.0021 a	0.67 a	0.39 a	1.06 a
	B250	31 ab	247.50 ab	0.0017 ab	0.62 a	0.26 c	0.88 c
	B350	29 bcd	241.50 ab	0.0016 ab	0.59 ab	0.30 b	0.89 c
0	OA1.5	32 a	249.50 ab	0.0018 ab	0.47 bcd	0.22 d	0.70 e
	OA2.5	31 ab	235.50 bcd	0.0015 bc	0.44 bcd	0.22 d	0.66 ef
	OA3.5	29 bcd	228.50 bcd	0.0013 cd	0.42 cde	0.19 e	0.61 fg
	S2	32 a	247.00 ab	0.0017 ab	0.38 def	0.15 ef	0.54 gh
	S3	31 ab	232.00 bcd	0.0014 bc	0.35 efg	0.15 ef	0.51 gh
	S4	29 bcd	226.00 cde	0.0013 de	0.33 efg	0.13 fg	0.47 hi
	Control	27 de	219.50 def	0.0012 ef	0.46 bcd	0.18 e	0.65 ef
	B150	31 ab	230.00 bcd	0.0014 cd	0.30 fgh	0.09 ghi	0.40 ij
	B250	29 bcd	223.00 bcd	0.0012 de	0.26 ghi	0.11 gh	0.38 jk
	B350	32 a	242.00 ab	0.0016 ab	0.23 ghi	0.09 ghi	0.32 jk
2	OA1.5	31 ab	227.00 cde	0.0013 cd	0.21 hi	0.100 ghi	0.31 jk
	OA2.5	29 bcd	221.00 def	0.0012 de	0.18 hi	0.07 ij	0.25 jk
	OA3.5	26 ef	228.00 bcd	0.0013 cd	0.54 bc	0.21 d	0.75 e
	S2	27 de	231.00 bcd	0.0014 cd	0.64 a	0.32 b	0.97 b
	S3	25 fg	216.50 efg	0.0011 ef	0.49 bcd	0.23 d	0.72 e
	S4	26 ef	217.00 def	0.0011 ef	0.43 cde	0.18 e	0.61 ef
	Control	25 fg	198.00 efg	0.0009 hi	0.20 hi	0.04 k	0.24 k
	B150	25 fg	205.00 efg	0.0010 gh	0.39 def	0.18 e	0.58 fg
	B250	26 ef	213.50 def	0.0011 ef	0.34 efg	0.14 fg	0.48 hi
	B350	27 de	217.50 bcd	0.0012 ef	0.36 def	0.16 ef	0.53 gh
4	OA1.5	25 fg	202.50 efg	0.0009 gh	0.33 efg	0.12 fg	0.45 hi
	OA2.5	26 ef	211.50 efg	0.0011 ef	0.27 ghi	0.12 fg	0.40 ik
	OA3.5	27 de	214.50 def	0.0011 ef	0.30 fgh	0.11 gh	0.41 ij
	S2	25 fg	199.50 efg	0.0009 hi	0.23 ghi	0.11 gh	0.34 jk
	S3	26 ef	209.50 efg	0.0010 ef	0.20 hi	0.06 ij	0.27 jk
	S4	27 de	212.00 def	0.0011 ef	0.21 ghi	0.09 hi	0.31 jk
	Control	28.5 bcd	216.50 def	0.0011 ef	0.29 fgh	0.12 fg	0.41 hi
	B150	28.5 bcd	232.50 bcd	0.0014 bc	0.58 ab	0.27 c	0.85 d
	B250	30 bc	231.00 bcd	0.0014 bc	0.67 a	0.26 c	0.94 b
	B350	27.5 cde	217.50 cde	0.0011 ef	0.56 ab	0.28 c	0.84 d
6	OA1.5	28.5 bcd	221.50 cde	0.0012 de	0.44 bcd	0.16 ef	0.60 fg

	OA2.5	30 bc	233.00 bcd	0.0014 bc	0.47 bcd	0.18 e	0.66 ef
	OA3.5	27.5 cde	206.00 efg	0.0010 fg	0.41 cde	0.19 e	0.60 fg
	S2	28.5 bcd	218.00 def	0.0012 ef	0.35 efg	0.14 fg	0.49 gh
	S3	30 bc	230.00 cde	0.0014 cd	0.37 def	0.16 ef	0.54 fg
	S4	27.5 cde	203.00 efg	0.0009 gh	0.33 efg	0.11 gh	0.44 hi
8	Control	23 g	180.50 g	0.0006 i	0.18 hi	0.05 jk	0.24 k
	B150	30 bc	227.50 bcd	0.0013 cd	0.30 fgh	0.12 fg	0.43 hi
	B250	27.5 cde	200.50 efg	0.0009 gh	0.24 ghi	0.12 fg	0.36 jk
	B350	28.5 bcd	213.00 def	0.0011 ef	0.21 hi	0.09 ghi	0.30 jk
	OA1.5	30 bc	225.00 cde	0.0013 de	0.22 ghi	0.10 ghi	0.32 jk
	OA2.5	27.5 cde	198.50 fg	0.0009 hi	0.17 i	0.06 ij	0.23 k
	OA3.5	23 g	197.50 fg	0.0008 hi	0.48 bcd	0.23 d	0.71 e
	S2	23 g	188.00 g	0.0007 i	0.39 def	0.16 ef	0.56 fg
	S3	23 g	185.50 g	0.0007 i	0.31 efg	0.12 gh	0.44 hi
	S4	23 g	183.50 g	0.0007 i	0.23 ghi	0.11 gh	0.35 ik

Within a column in each treatment means followed by the same letter are not significantly different at $P<0.05$ according to least significant different test. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). Storage durations included five different periods 0, 2, 4, 6, and 8 days.

The interaction effect of storage durations and processing treatments on some growth parameters and photosynthesis pigments of the iceberg lettuce

The interaction between treatment storage durations and processing showed that, overall, the number of leaves in the cut treatments decreased compared to the uncut treatments at all storage durations in the refrigerator. Additionally, there was no significant difference in the number of leaves among the cut treatments across different storage durations. At all storage durations, the leaf length and plant volume in the uncut treatments were greater than in the cut treatments, with the lowest leaf length and plant volume observed in the cut treatments on days 6 and 8 compared to day 0 (Table 3).

The observed reduction in plant volume in the cut treatments over the storage period can be largely attributed to the physiological and structural changes induced by cutting. The cutting process causes tissue damage, which not only directly compromises the integrity of the plant tissue but also increases its susceptibility to deterioration (Suo *et al.,* 2021). This damage results in higher respiration rates and moisture loss through transpiration, both of which accelerate weight loss and quality decline during storage (Suo *et al.*, 2021). Additionally, the disruption of plant tissues creates entry points for microorganisms, which can colonize the damaged areas and further hasten deterioration. This microbial invasion, combined with increased respiration, leads to faster tissue breakdown, corroborating the lower plant volume and poorer quality observed in the cut samples (Suo *et al.,* 2021). Our findings reinforce the importance of minimal processing to maintain plant vigor and maximize storage longevity.

In general, the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll did not show significant differences between the cut and uncut treatments at various storage durations. However, from day 0 to day 8, the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll exhibited a downward trend (Table 3).

Table 3. The interaction effect of experimental treatment (processing and storage durations treatments) on some growth parameters and photosynthesis pigments of the iceberg lettuce

Processing	Storage durations	Number of leaf (per plant)	Length of leaf (mm)	Volume of plant (cm^3)	Chlorophyll a (mg. g^{-1} Fw)	Chlorophyll b (mg. g^{-1} Fw)	Total chlorophyll (mg. g^{-1} Fw)
No cutting	0	32.2 a	309.10 a	0.0019 a	0.59 a	0.26 a	0.86 a
	2	32.2 a	301.60 b	0.0018 b	0.43 b	0.19 b	0.63 b
	4	32.2 a	297.80 c	0.0017 c	0.35 c	0.13 c	0.49 c
	6	32.2 a	295.00 d	0.0017cd	0.28 d	0.11 c	0.39 d
	8	32.2 a	292.30 e	0.0016 d	0.21 e	0.08 d	0.29 e
Cutting	0	23.6 b	152.00 f	0.0009 e	0.58 a	0.29 a	0.87 a
	2	23.25 b	133.75 g	0.0006 f	0.42 b	0.17 b	0.60 b
	4	22.71 b	132.14 gh	0.0006 fg	0.34 c	0.14 c	0.48 c
	6	23.25 b	129.75 hi	0.0005 gh	0.26 d	0.12 c	0.39 d
	8	23.25 b	127.25 i	0.0005 h	0.18 e	0.07 d	0.26 e

Within a column in each treatment means followed by the same letter are not significantly different at $P<0.05$ according to least significant different test. Storage durations were set at five different storage durations points: 0, 2, 4, 6, and 8 days. The postharvest processing treatments included no cutting and cutting

The interaction effect of pre-harvest treatments and processing on some growth parameters and photosynthesis pigments of the iceberg lettuce

The interaction effect between pre-harvest treatments and processing showed that the number of lettuce leaves in the uncut condition was higher in the biochar treatment at all three concentrations (B150, B250, B350) compared to the control group. Conversely, in the cut condition, the highest number of leaves was observed with oxalic acid treatments at all three concentrations (OA1.5, OA2.5, OA3.5) compared to the control (Figure 1).

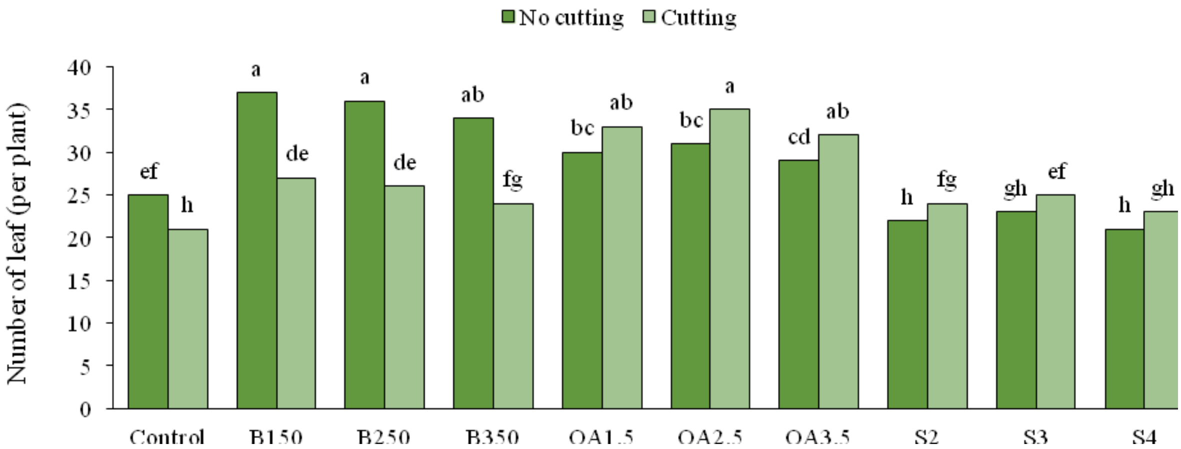


Figure 1. The interaction effect of pre-harvest treatments and processing on number of leaf of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

Overall, in both uncut and cut conditions, the shortest leaf length was observed in the wood vinegar treatment across all three concentrations (S2, S3, S4). The most extended leaf length was recorded in the uncut condition, particularly in the B150, B250, and B350 treatments compared to the control (Figure. 2).

The beneficial effects of biochar on soil health are well-documented. Its porous structure and high adsorption capacity create a conducive environment for soil microorganisms, facilitating nutrient retention and reducing nutrient leaching by limiting the dissolution and migration of water-soluble ions. This slow and sustained nutrient release enhances soil fertility, promotes microbial activity, and supports healthy root and plant growth. When roots develop well, they can efficiently supply water and mineral nutrients to the leaves, thereby encouraging overall plant vigor (Ren *et al.*, 2021).

Regarding wood vinegar, it contains a complex mixture of bioactive substances known to promote plant growth. Under optimal concentrations, these compounds can act synergistically to enhance plant development and increase dry matter accumulation (Zhu *et al.*, 2022). However, the effects of wood vinegar are dose-dependent, and excessive application may lead to inhibitory effects due to potential phytotoxicity or nutrient imbalances. In contrast to the apparent contradiction with Zhu *et al.* (2022), their findings highlight the growth-promoting properties of wood vinegar at suitable concentrations, which align with the beneficial compounds it contains. The differences in our results could be attributed to several factors, such as variations in plant species, application methods, concentrations, or environmental conditions. In our study, perhaps the concentrations or application frequency of wood vinegar exceeded optimal levels, resulting in less favorable outcomes, such as reduced leaf length.

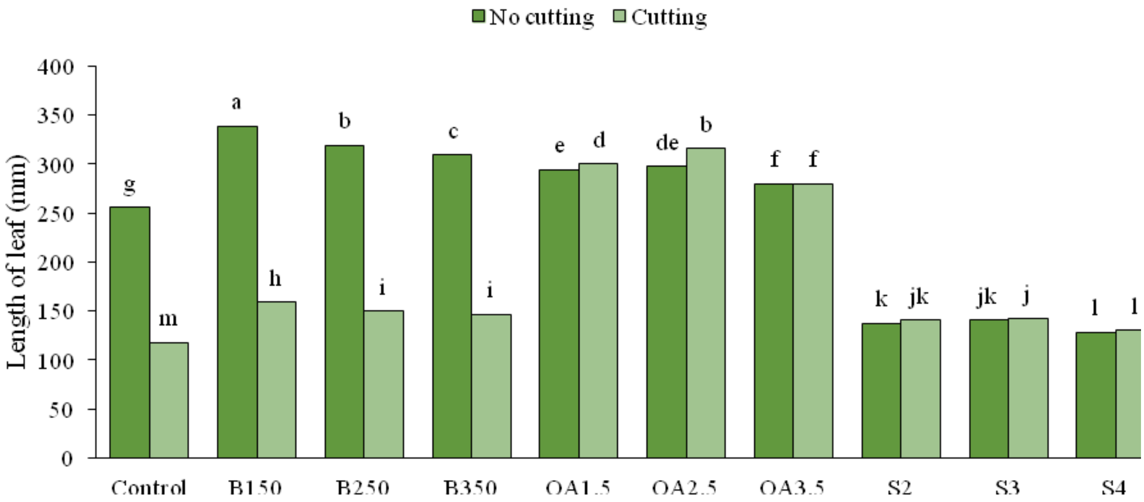


Figure 2. The interaction effect of pre-harvest treatments and processing on leaf of length of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

Regarding plant volume, in the uncut condition, the most significant volume was seen in the B150, B250, and B350 treatments compared to the control. In the cut condition, the highest plant volume was observed in the treatments with OA2.5, OA1.5, and S3, respectively, compared to the control (Figure. 3).

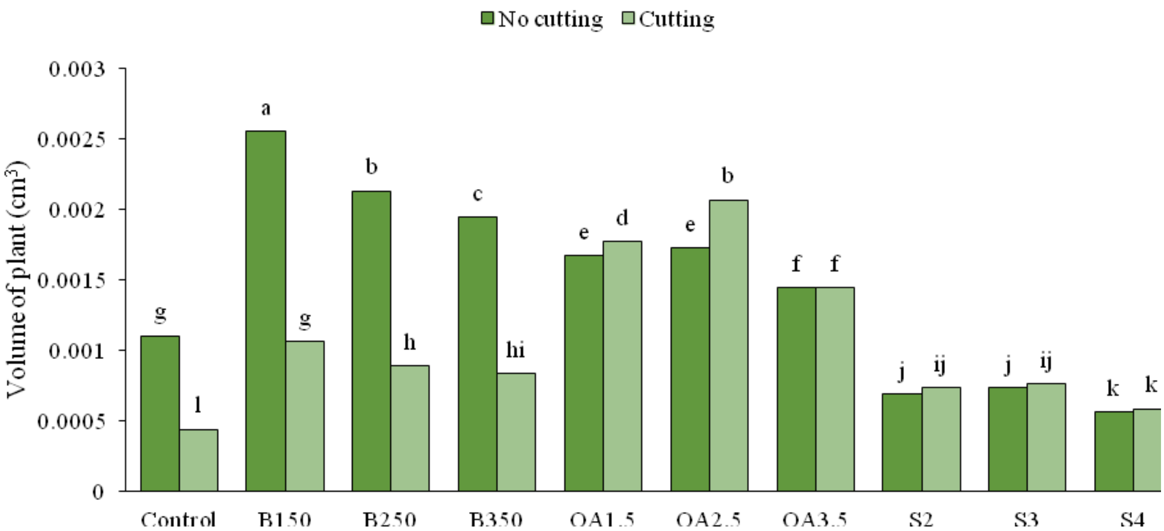


Figure 3. The interaction effect of pre-harvest treatments and processing on volume of plant of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3,

and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

In the cut condition, the treatments with B150, OA2.5, and S3 showed the highest chlorophyll-a content compared to the control, while the lowest chlorophyll a concentration was found in the control group (Figure 4).

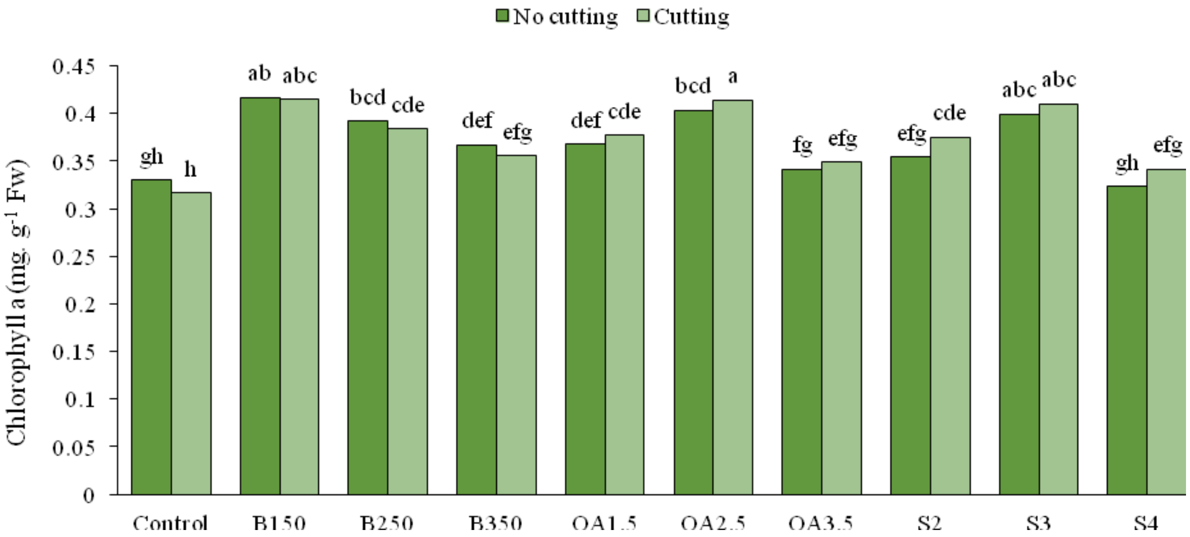


Figure 4. The interaction effect of pre-harvest treatments and processing on chlorophyll a of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

The chlorophyll-a concentration in both cut and uncut conditions was significantly higher in the B150 treatment compared to the control; other treatments did not show significant differences from the control regarding chlorophyll-a content (Figure5).

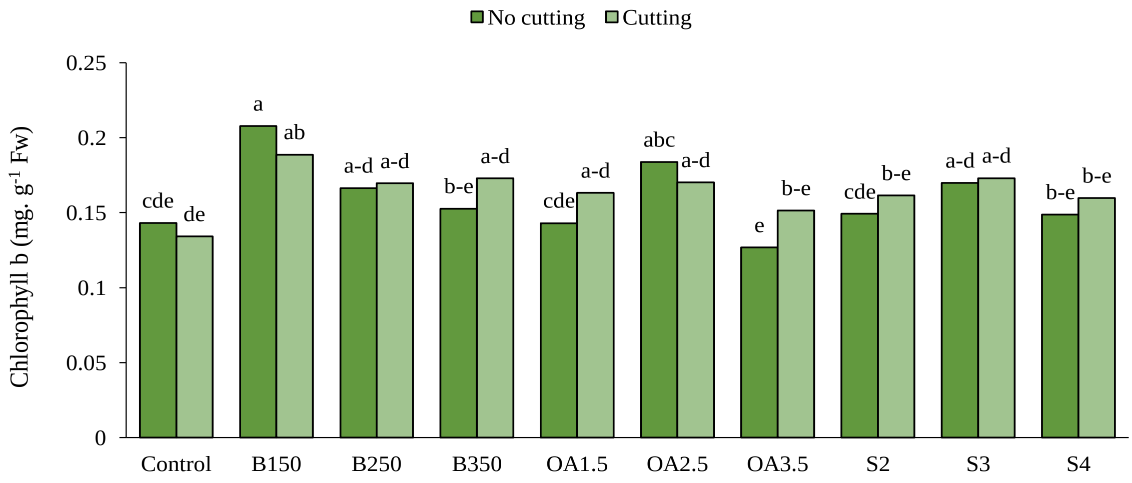
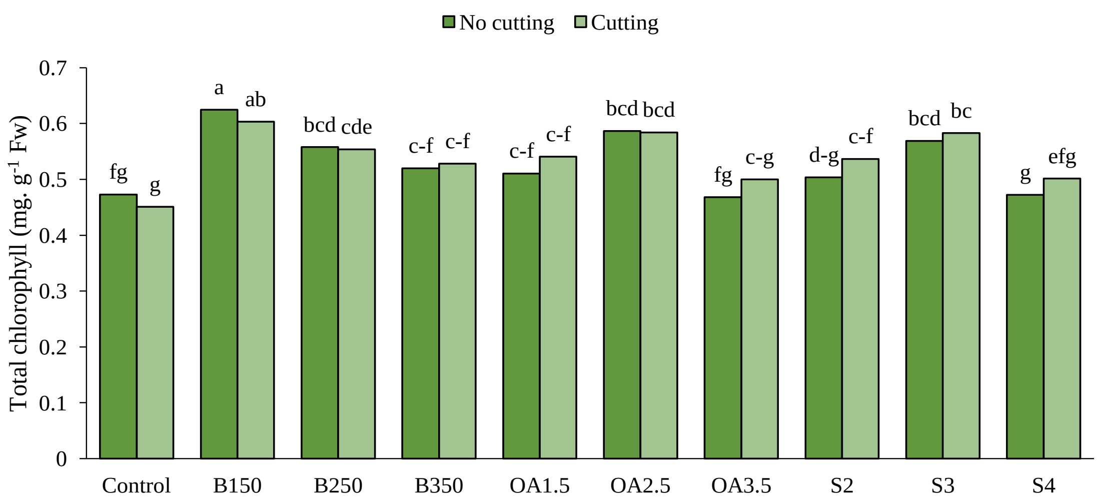


Figure 5. The interaction effect of pre-harvest treatments and processing on chlorophyll b of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

Finally, the highest total chlorophyll concentration in both conditions was observed in the B150 treatment compared to the control (Figure 6).



igure 6. The interaction effect of pre-harvest treatments and processing on total chlorophyll of the iceberg lettuce. Pre-harvest treatments included: distilled water (control), oxalic acid at three concentrations 1.5, 2.5, and 3.5 mM (OA1.5, OA2.5, and OA3.5); biochar at three application rates 150, 250, and 350 g per plant (B150, B250, and B350); and wood vinegar at three concentrations 2, 3, and 4 mL. L^{-1}. (S2, S3, and S4). The postharvest processing treatments included no cutting and cutting.

CONCLUSION

Among all treatments tested, biochar at a rate of 150 g per plant proved to be the most effective in improving growth parameters and maintaining chlorophyll content over storage periods. This treatment significantly enhanced leaf number, plant volume, and fluorescence pigments, thereby extending the shelf life of iceberg lettuce. The benefits are likely due to biochar's ability to improve soil health, promote root development, and increase nutrient availability, which collectively enhance plant vigor and postharvest resilience. The promising results of biochar application highlight its potential as a cost-effective, environmentally friendly approach to reduce postharvest losses and improve the economic returns for lettuce producers.

REFERENCES

Abdullah M A, Zakaria S. 2024. Impact of ultraviolet radiation and 1-methylcyclopropane treatments on maintain quality and storability of Iceberg lettuce head during cold storage. Horticulture Research Journal, 2(3): 84-96.

Coumar M V, Parihar R S, Dwivedi A K, Saha J K, Rajendiran S, Dotaniya M L, Kundu S. 2016. Impact of pigeon pea biochar on cadmium mobility in soil and transfer rate to leafy vegetable spinach. Environmental Monitoring and Assessment, 188: 1-10. https://doi.org/10.1007/s10661-015-5028-y.

Eroğul D, Kibar H, Şen F, Gundogdu M. 2023. Role of postharvest oxalic acid treatment on quality properties, phenolic compounds, and organic acid contents of nectarine fruits during cold storage. Horticulturae, 9(9): 1021. https://doi.org/10.3390/horticulturae9091021.

Hasan Ö Z. 2018. A new approach to soil solarization: Addition of biochar to the effect of soil temperature and quality and yield parameters of lettuce (*Lactuca sativa* L. Duna). Scientia Horticulturae, 228: 153-161. https://doi.org/10.1016/j.scienta.2017.10.021.

Lee J S, Chandra D, Son J, 2022. Growth, physicochemical, nutritional, and postharvest qualities of leaf lettuce (*Lactuca sativa* L.) as affected by cultivar and amount of applied nutrient solution. Horticulturae, 8(5): 436. https://doi.org/10.3390/horticulturae8050436.

Lee J S, Chang M S. 2017. Effect of nutrient solution concentration in the second half of growing period on the growth and postharvest quality of leaf lettuce (*Lactuca sativa* L.) in a deep flow technique system. Horticultural Science and Technology, 35: 456–464.

Lichtenthaler H K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology, 1(44): 350-382. https://doi.org/10.1016/0076- 6879(87)48036-1

Liao Y, Li Z, Yang Z, Wang J, Li B, Zu Y. 2023. Response of Cd, Zn translocation and distribution to organic acids heterogeneity in *Brassica juncea* L. Plants, 12(3): 479. https://doi.org/10.3390/plants12030479.

Martínez-Ispizua E, Calatayud Á, Marsal J I, Basile F, Cannata C, Abdelkhalik A, Soler S, Valcárcel J V, Martínez-Cuenca M R. 2022. Postharvest changes in the nutritional properties of commercial and traditional lettuce varieties in relation with overall visual quality. Agronomy, 12(2): 403. https://doi.org/10.3390/agronomy12020403

Rehman R S, Zafar S A, Hur G. 2025. A stage-specific approach: assessing the impact of exogenously applied wood vinegar on growth, yield, and quality of rice (*Oryza sativa* L.). Pakistan Journal of Agricultural Sciences, 62(1).

Ren T, Wang H, Yuan Y, Feng H, Wang B, Kuang G, Wei Y, Gao W, Shi H, Liu G. 2021. Biochar increases tobacco yield by promoting root growth based on a three-year field application. Scientific Reports, 11(1): 21991. https://doi.org/10.1038/s41598-021-01426-9.

Suo R, Wang W, Ma Y, Fu L, Cui Y. 2021. Effect of different root lengths for retaining freshness of hydroponic lettuce. Journal of Agriculture and Food Research, 4: 100151. https://doi.org/10.1016/j.jafr.2021.100151.

Yang J F, Yang C H, Liang M T, Gao Z J, Wu Y W, Chuang L Y. 2016. Chemical composition, antioxidant, and antibacterial activity of wood vinegar from Litchi chinensis. Molecules, 21: 1150. https://doi.org/10.3390/molecules21091150.

Zhang W, Jiang Y, Zhang Z. 2023. The role of different natural organic acids in postharvest fruit quality management and its mechanism. Food Frontiers, 4(3): 1127-1143. http://dx.doi.org/10.1002/fft2.245.

Zhu K, Liu J, Luo T, Zhang K, Khan Z, Zhou Y, Cheng T, Yuan B, Peng X, Hu L. 2022. Wood vinegar impact on the growth and low-temperature tolerance of rapeseed seedlings. Agronomy, 12(10): 2453. https://doi.org/10.3390/agronomy12102453.