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Effects of Different Nitrogen Sources and Sodium Bicarbonate on Growth and Nutrient Uptake in Two Garlic Genotypes: A Hydroponic Study

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ABSTRACT

Soil alkalinity is a major constraint to agricultural production worldwide. Nitrogen sources significantly influence the pH of the nutrient solution; nitrate tends to raise the pH and make the solution more alkaline, whereas ammonium lowers the Ph. This study was conducted as a factorial experiment based on a completely randomized design with three factors: sodium bicarbonate at three concentrations (0, 10, and 20 mM), nitrogen source at three levels (ammonium sulfate, ammonium nitrate and calcium nitrate at a concentration of 5 mM), and two garlic genotypes (white and purple), with three replications in a hydroponic greenhouse at the Faculty of Agriculture, Vali-e-Asr University of Rafsanjan. Alkalinity stress and nitrogen sources had statically significant on morphological parameters and nutrient uptake of garlic genotypes. Also, the results showed that increasing sodium bicarbonate concentration led to a decrease in shoot fresh and dry weight, leaf length and width, bulb diameter, and number of cloves in both garlic genotypes. Manganese and sodium concentrations increased with higher sodium bicarbonate levels, particularly under calcium nitrate and ammonium sulfate treatments. Nitrate sources, especially calcium nitrate and ammonium nitrate, promoted vegetative growth parameters. Ammonium nitrate also increased iron and copper concentrations. Additionally, ammonium sulfate and calcium nitrate improved nutrient uptake. Based on the findings, the white garlic genotype demonstrated superior performance in most measured traits compared to the purple genotype. The interaction between nitrogen form and nutrient solution pH was shown to influence nutrient uptake efficiency, stress tolerance, and overall plant performance. These findings highlight the critical role of targeted nitrogen management in alleviating the negative impacts of alkaline stress on garlic cultivation. By addressing nutrient imbalances and promoting better growth performance, appropriate nitrogen source strategies can enhance the resilience and productivity of garlic genotypes under hydroponic conditions, thereby supporting more efficient and sustainable agricultural practices.

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1. Introduction

Purple garlic, also known as hardneck garlic, is a variety of garlic (*Allium sativum*) that has a purple or red-striped outer layer and is characterized by a hard, fibrous neck (Ammarellou, 2017). Shahdad purple garlic is of great importance due to its unique properties, especially in traditional medicine and for medicinal purposes. This garlic has a reddish-purple color and has anti-inflammatory, antibacterial properties and contains large amounts of vitamin C and antioxidants. Therefore, it is useful for strengthening the immune system, treating infections and strengthening the kidneys. Shahdad purple garlic has a spicy and fragrant taste that is also suitable for use in cooking. While research studies have investigated different garlic genotypes in Iran, specifically focusing on their traits, yield, and resistance to pests like thrips, there's not a specific focus on purple garlic as a distinct genotype within Iranian garlic studies.

Soil alkalinity and salinization have increasingly emerged as significant environmental concerns, posing serious threats to agricultural productivity (Qiu *et al*., 2022). Evidence indicates that the detrimental effects of alkaline stress often surpass those caused by salinity alone (Liu *et al*., 2010; Kumar *et al*., 2024). The main contributors to soil alkalinity (sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) tend to be more damaging to plants than neutral salts such as sodium chloride (NaCl) or sodium sulfate (Na_2SO_4), largely due to their higher pH and their disruptive effect on plant metabolism (Hassan *et al*., 2022). Alkaline stress imposes complex challenges, particularly to root physiology (Yang *et al*., 2024). The impact is twofold: in addition to osmotic and ionic toxicity, elevated pH can reduce the solubility and mobility of essential nutrients, leading to their precipitation or a decline in ionic activity (Fang *et al*., 2021). Such conditions can impair root function, disrupt cellular integrity (Msimbira and Smith, 2020), and hinder uptake of crucial anions like nitrate, phosphate, and chloride, further affecting the plant's ability to maintain ionic balance, potassium-to-sodium selectivity, hormonal stability, and cellular pH homeostasis (Yang *et al*., 2008). As a result, plants in alkaline soils must contend with physiological drought, ion toxicity, and disrupted nutrient acquisition mechanisms (Xu *et al*., 2023). The increase in bicarbonate and carbonate concentrations in the rhizosphere (key contributors to soil alkalinity) can severely limit nitrogen uptake and plant growth (Valdez-Aguilar and Reed, 2008). High pH alone has been shown to inhibit shoot and root development in certain sensitive species (Lu *et al*., 2025). In calcareous soils, it is often the elevated concentration of bicarbonate, rather than the high pH, that primarily restricts root elongation and shoot development (Bolan *et al*., 2023). Bicarbonate specifically reduces shoot growth by limiting leaf expansion, shoot biomass, and stem elongation, largely due to bicarbonate-induced chlorosis, which in turn reduces photosynthetic efficiency (Valdez-Aguilar, 2005). Excessive bicarbonate typically leads to nutrient deficiency symptoms, most notably iron chlorosis. This condition is linked to reduced chlorophyll biosynthesis, often due to impaired iron transport or low iron solubility in alkaline substrates (Vélez-Bermúdez and Schmidt, 2023). Over accumulation of bicarbonate can also reduce zinc content, inhibit photosystem II (PSII) function, and diminish photosynthetic performance indicators such as net photosynthesis rate (PN), water use efficiency (WUE), and chlorophyll fluorescence parameters (Fo, Fv/Fm, ETR) (Zhao and Wu, 2017). Moreover, bicarbonate toxicity can suppress the activity of carbonic anhydrase (CA), a key enzyme involved in carbon metabolism, through disruption of zinc availability (Escudero-Almanza *et al*., 2012). The pH of the nutrient solution has a pronounced effect on nutrient solubility, particularly iron and phosphorus. While moderately acidic conditions (pH ~5.8) optimize nutrient availability, higher pH values (>6.5) can lead to nutrient deficiencies

(Anderson *et al*., 2017). Under alkaline conditions, nutrient uptake is further constrained due to ionic imbalance, with significant limitations on chloride and nitrate uptake and reduced potassium-sodium selectivity, thereby disturbing cellular ionic homeostasis (Malekzadeh *et al*., 2024). In addition, elevated pH can promote precipitation of key ions like calcium, magnesium, and iron, restricting their absorption (Yang *et al*., 2007). Alkalinity-induced nutrient disorders stem not only from sodium ion toxicity but also from elevated bicarbonate concentrations, which impede nutrient mobility and transport (Hasanuzzaman and Nahar, 2024). Optimal micronutrient solubility generally occurs at pH values between 5.8 and 6.4 (Fernández and Hoeft, 2009). In calcareous soils, excessive calcium carbonate and high pH are associated with iron deficiencies (Bolan *et al*., 2023). Furthermore, alkaline stress may enhance calcium and sodium uptake while restricting potassium accumulation (Zhanwu *et al*., 2014). The interaction between pH and nitrogen fertilization significantly influences nutrient dynamics in plant tissues, as nitrogen uptake is modulated by its chemical form (ammonium vs. nitrate) and pH conditions (Zsoldos and Haunold, 1982; Dal Molin *et al*., 2020).

The form and concentration of nitrogen fertilizer profoundly affect nutrient absorption and shoot/root development by altering the rhizosphere pH (Sathiyavani *et al*., 2017; Zhang *et al*., 2023). Ammonium uptake tends to acidify the rhizosphere, enhancing micronutrient availability, whereas nitrate absorption elevates rhizosphere pH due to hydroxide ion release, potentially impairing nutrient availability (Marschner, 2011; Ruan *et al*., 2007; Custos *et al*., 2020). In saline-alkaline soils, the presence of bicarbonate compounds the adverse effects of salinity, resulting in complex and compounded stress conditions (Sharma *et al*., 2024). In garlic-producing regions, nutrient deficiencies and water stress often co-occur, severely limiting nitrogen, phosphorus, and sulfur mineralization and root uptake. Studies have demonstrated that garlic yield and quality can be significantly enhanced with nitrogen fertilization (Kevlani *et al*., 2023). Thus, a balanced nutrient management strategy is essential to improve soil fertility and mitigate nutrient limitation in garlic cultivation (Diriba-Shiferaw *et al*., 2015). Nitrogen is a critical macronutrient required for plant development and yield (Fathi, 2022; Raina and Mazahar, 2022). It plays a fundamental role in amino acid and protein synthesis, nucleic acid structure, chlorophyll formation, and numerous metabolic processes. Nitrogen limitation can reduce photosynthesis, leaf area index, dry matter accumulation, and overall productivity, while also delaying vegetative and reproductive development (Javed *et al*., 2022). Fertilization remains a rapid and effective strategy for enhancing crop performance, with nitrogen being the most yield-limiting nutrient in many agricultural systems worldwide (Diriba-Shiferaw *et al*., 2015). While nitrogen fertilizers such as urea, ammonium nitrate, and ammonium sulfate, are widely used, their environmental footprint particularly in terms of greenhouse gas emissions and biodiversity loss—cannot be overlooked (Tyagi *et al*., 2022; Walling and Vaneeckhaute, 2022). Plants primarily absorb nitrogen as ammonium (NH_4^+) or nitrate (NO_3^-), with nitrate generally preferred due to its lower toxicity and higher mobility. Organic nitrogen sources must be mineralized into these inorganic forms before plant uptake. Nitrate is rapidly assimilated and transported to shoots, while ammonium uptake tends to acidify the rhizosphere and enhance micronutrient solubility (Roosta and Schjoerring, 2007). The contrasting physiological effects of nitrate and ammonium are largely due to their differing impacts on ion balance, rhizosphere pH, respiration, and energy metabolism (Escobar *et al*., 2006; Li *et al*., 2024). High nitrate uptake at low pH is attributed to proton-mediated transport, whereas ammonium absorption can suppress root growth under acidic conditions (Naeem *et al*., 2023). Interestingly, ammonium uptake enhances phosphorus absorption, while nitrate favors cation uptake and restricts anion absorption. Excess ammonium may suppress nitrogen assimilation in roots and shoots and lead to reduced biomass production, especially in legumes like green beans (Roosta and Schjoerring, 2007). According to the reports provided, the objective of this study was to evaluate and identify effective nitrogen source management strategies for mitigating the adverse effects of alkaline stress on garlic plants grown under hydroponic conditions.

2. Material and Methods

2.1. Experimental setup

This experiment was conducted during the summer and fall of 2024 in the greenhouse of the Faculty of Agriculture at Vali-e-Asr University of Rafsanjan, Iran. The study was arranged as a factorial experiment in a completely randomized design (CRD) with three replications and five plants per replicate. Three factors were considered: bicarbonate stress at three levels (Distilled Water, 10, and 20 mM), nitrogen source at three levels (ammonium sulfate, ammonium nitrate, and calcium nitrate, each at a concentration of 5 mM), and genotype at two levels (white and purple garlic). The experiment aimed to investigate the interaction between different nitrogen sources and sodium bicarbonate stress on two garlic genotypes under hydroponic conditions.

Garlic cloves of the 'Hamedan' cultivar were obtained from the National Institute of Genetic and Biological Resources of Iran, and local landrace cloves from Shahdad were collected. To eliminate surface contamination, the cloves were disinfected with 5% commercial bleach (sodium hypochlorite) and rinsed three times with distilled water. The cloves were then transplanted into a hydroponic system with a growing medium consisting of 70% cocopeat and 30% perlite, established in the greenhouse of the Faculty of Agriculture, Vali-e-Asr University. Five cloves from each genotype were planted in 5-liter pots (Figure 1). Environmental condition in greenhouse was a temperature of 25/15 (day/night), a photoperiod of 16/8 h (day/night) under 250 $\mu molm^{-2}s^{-1}$, and a relative humidity of 50 ± 5%. After one month of plant establishment, treatments were applied for two months. These included three levels of sodium bicarbonate (0, 10, and 20 mM) and three nitrogen sources (ammonium nitrate, ammonium sulfate, and calcium nitrate, each at 5 mM), as detailed in Table 1. During and after the treatment period, various morphological, physiological, and nutritional parameters were measured. A modified Hoagland nutrient solution was used tailored to the specific nitrogen source used in each treatment. Stock solutions were made, and for each week, modified Hogand nutrient solutions were made based on different nitrogen sources and provided to the garlic genotypes.

2.2. Measurement of Parameters

2.2.1. Vegetative Traits

The evaluated vegetative parameters included: fresh weight of shoots and roots, dry weight of shoots and roots, number of bulbs, number of cloves per bulb, bulb diameter, and clove diameter. Bulb diameters were recorded at the widest point using a digital caliper. For fresh weight, plants were carefully removed from the substrate, separated into bulb, leaf, and root parts, and individually weighed using a digital scale. Dry weight was determined by drying the samples at 70 °C for 48 hours in an oven and then reweighing them.

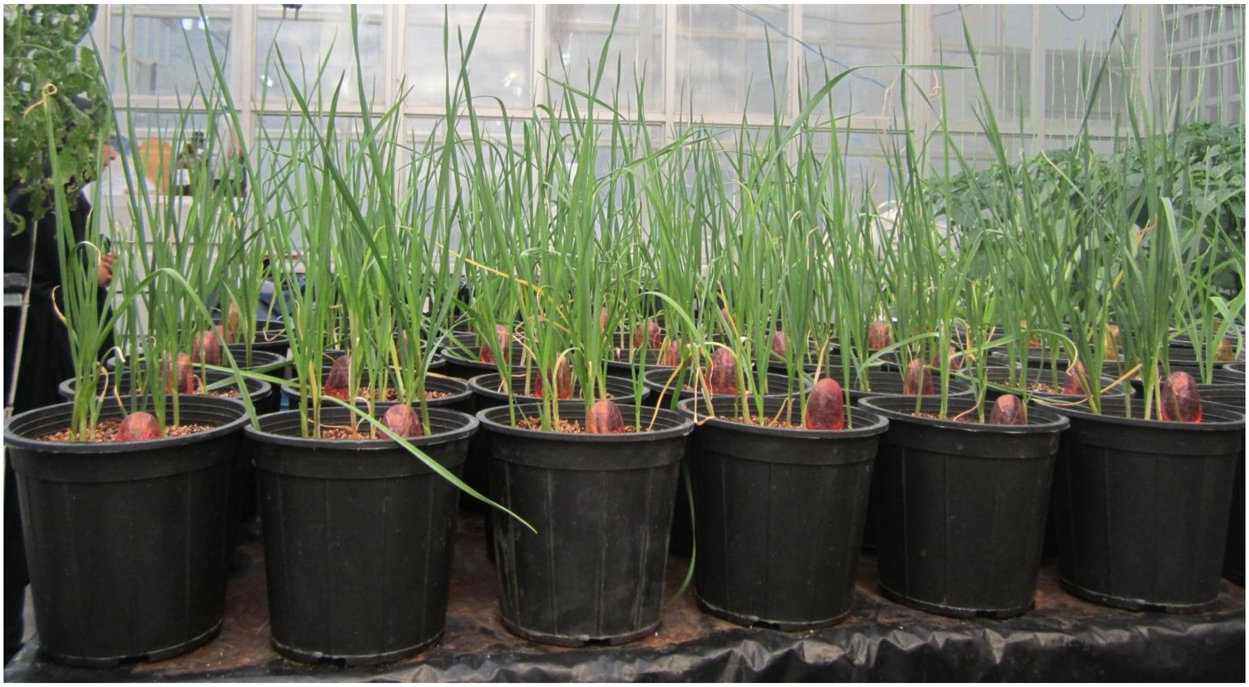


Figure 1. Garlic genotypes (Purple and White) treated with bicarbonate stress at three levels (Distilled Water, 10, and 20 mM), nitrogen source at three levels (ammonium sulfate, ammonium nitrate, and calcium nitrate, each at a concentration of 5 mM).

2.2.2. Measurement of Nutrient Elements

For nutrient analysis, fully expanded mature leaves were randomly sampled at the time of harvest. The analyzed nutrients included potassium (K), sodium (Na), chloride (Cl), calcium (Ca), and magnesium (Mg). To prepare extracts, 0.5 g of oven-dried and ground shoot and root tissue was weighed and subjected to ashing in a muffle furnace—initially at 250 °C for 30 minutes, followed by 550 °C for 3 hours. After ashing, 5 mL of 2N hydrochloric acid was added to each sample, and the final volume was adjusted to 50 mL using distilled water. This extract was directly used for quantifying Na, K, Mg, and Ca. Potassium and sodium concentrations were measured using a flame photometer (JENWAY PEP7, Germany). Calcium was determined via titration: 5 mL of extract was poured into a 50 mL Erlenmeyer flask, diluted with distilled water to 25 mL, and then mixed with 5 drops of 4N sodium hydroxide and ~0.5 g of a mixed indicator (0.5 g ammonium purpurate + 100 g potassium sulfate). After thorough mixing, titration was performed with EDTA (prepared from 1.86 g EDTA-Na and 0.5 g $MgCl_2H_2O$ in 1 L of water), and the endpoint was noted by a color change from red-orange to violet. Calcium content was then calculated using the following equation (Klute, 1986):

$$Ca^{2+}\ (\mathrm{mg/L}) = \left(\frac{\text{EDTA volume} \times \text{EDTA normality}}{\text{Extract volume}}\right) \times 1000$$

Magnesium was determined by complexometric titration. A 5 mL aliquot of extract was diluted to 25 mL, followed by the addition of 10 drops of ammonium chloride buffer and 4 drops of Eriochrome Black T indicator.

The solution was titrated with EDTA until the color shifted from wine red to blue/green. The calcium content (previously determined) was subtracted from this value, and magnesium was calculated using the same formula (Ryan *et al*., 2001):

$$Mg^{2+}\ (\text{mg/L}) = \left(\frac{\text{EEDTA volume} \times \text{EDTA normality}}{\text{Extract volume}}\right) \times 1000$$

Nitrogen content was measured using a Kjeldahl apparatus (Peco, Iran), while sulfur content was analyzed spectrophotometrically following the method of Estefan *et al*. (2013).

2.3. Experimental Design and Data Analysis

The experiment was conducted using a factorial arrangement of three factors within a completely randomized design (CRD), incorporating three replicates per treatment. Data analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). A two-way analysis of variance (ANOVA) was employed to evaluate the main and interaction effects of the experimental factors. To compare treatment means, Duncan's multiple range test (DMRT) was applied as a post hoc procedure, with statistical significance set at $p \leq 0.05$. Results are presented as means ± standard error (SE).

Table 1. Hoagland nutrient solution (Hoagland, 1950) was used based on different nitrogen sources

Calcium Nitrate (Hoagland Complete)	Stock Concentration	Volume consumed (mL/L)	Ammonium Nitrate (Hoagland Modified)	Stock Concentration	Volume consumed (mL/L)	Ammonium sulfate (Hoagland modified)	Stock Concentration	Volume consumed (mL/L)
$Ca(NO_3)_2*4H_2O$	1M	5	NH_4NO_3	1M	5	$(NH_4)_2SO_4$	1M	7.5
KNO_3	1M	5	KNO_3	1M	5	K_2SO_4	1M	2.5
KH_2PO_4	1M	1	$CaCl_2$	1M	2	CaCl2	1M	2
-	-	-	KH_2PO_4	1M	1	KH_2PO_4	1M	1
$MgSO_4*7H_2O$	1M	2	$MgSO_4*7H_2O$	1M	2	$MgSO_4*7H_2O$	1M	2
$ZnSO_4*7H_2O$	0.25 g/L	1	$ZnSO_4*7H_2O$	0.25 g/L	1	$ZnSO_4*7H_2O$	0.25 g/L	1
$CuSO_4*5H_2O$	0.08 g/L	1	$CuSO_4*5H_2O$	0.08 g/L	1	$CuSO_4*5H_2O$	0.08 g/L	1
Na_2MoO_4	0.02 g/L	1	Na_2MoO_4	0.02 g/L	1	Na_2MoO_4	0.02 g/L	1
$MnSO_4*H_2O$	1.83 g/L	1	$MnSO_4*H_2O$	1.83 g/L	1	$MnSO_4*H_2O$	1.83 g/L	1
H_3BO_3	0.6 g/L	1	H_3BO_3	0.6 g/L	1	H_3BO_3	0.6 g/L	1
Fe-EDDHA	10 g/L	1	Fe-EDDHA	10 g/L	1	Fe-EDDHA	10 g/L	1

3. Results

3.1. Fresh Weight of Shoots

The analysis of variance indicated that genotype had a significant effect at the 5% level, while bicarbonate concentration, nitrogen sources, and their interactions were significant at the 1% level (Table 2). The combination of ammonium nitrate without bicarbonate (control) produced the highest shoot fresh weight (10.26 g) in the white genotype. The lowest shoot fresh weight (1.56 g) was recorded in the same genotype when treated with ammonium sulfate without bicarbonate. In the white genotype, shoot fresh weight under calcium nitrate remained unaffected by bicarbonate levels, while under ammonium nitrate; increasing bicarbonate concentration resulted in a decline. With ammonium sulfate, a slight increase was observed at 10 mM bicarbonate, followed by a reduction at 20 mM. In the purple genotype, shoot fresh weight decreased in all treatments with bicarbonate except for ammonium nitrate at 10 mM, which caused an increase (Table 3).

3.2. Dry Weight of Shoots

The analysis of variance (ANOVA) revealed that genotype, bicarbonate concentration, nitrogen source and most of their interactions (with the exception of the genotype × bicarbonate interaction) had a significant effect on shoot dry weight at the 1% level (Table 2). The highest dry weight recorded was 1.65 g, achieved through the combination of ammonium nitrate and 10 mM bicarbonate in the purple genotype. Conversely, the lowest dry weight of 0.43 g was observed in the white genotype treated with ammonium sulfate in the absence of bicarbonate (Table 3). In the white genotype, plants supplied with nitrate did not exhibit a clear response to bicarbonate levels. However, those receiving ammonium showed an increase in dry weight at 10 mM bicarbonate, followed by a decline at 20 mM. In the purple genotype, the only treatment that resulted in an increase in shoot dry weight was the combination of ammonium nitrate and 10 mM bicarbonate; all other treatments did not demonstrate a significant effect of bicarbonate on dry weight (Table 3).

Table 2. Analysis of variance of vegetative parameters of two garlic genotypes under the influence of nitrogen and sodium bicarbonate sources in hydroponic cultivation

S.O.V	df	Shoot fresh mass	Shoot dry mass	Root fresh mass	Root dry mass	Bulb length	Bulb diameter	Number of bulbs
Genotype (G)	1	2.33*	0.7**	71.9**	0.56**	8.6ns	140.4**	3.62**
Nitrogen Source (N)	2	8.4**	0.6**	2.18**	0.02ns	1.3ns	39.1**	1.72**
Bicarbonate (B)	2	49.8**	0.3**	4.14**	0.03*	14.3ns	18.1ns	2.8**
G×N	2	17.5**	0.9**	4.28**	0.12**	10.8ns	12.8ns	4.01**
G×B	4	2.12**	0.3**	1.17**	0.07**	18.8ns	3.7ns	5.19**
N×B	2	10.22**	0.06ns	0.20**	0.07**	4.7ns	77.9**	5.62**
G×N×B	4	25.5**	0.36**	2.08**	0.12**	12.9ns	17.2ns	0.26ns
Experimental error	35	0.48	0.068	0.01	0.008	6.23	8.06	0.25
CV	-	11.48	12.63	5.15	16.11	13.4	16.1	18.6

ns, *, and ** indicate non-significant, significant at the 5% and 1% probability levels of Duncan's test, respectively.

Table 3. Interaction of nitrogen sources and different concentrations of sodium bicarbonate (alkalinity stress) on fresh and dry weight of shoots and roots of two garlic genotypes in hydroponic cultivation

Nitrogen Source (5mM)	Sodium Bicarbonate (mM)	Garlic Genotype	Shoot fresh mass (g)	Shoot dry mass (g)	Root fresh mass (g)	Root dry mass (g)
Calcium Nitrate	Distilled water (Control)	White	3.7f	1.63a	4.7b	0.5b
	10		3.6f	1.35 abc	4.3c	0.49b
	20		3.9f	1.58a	3.4e	0.55ab
Ammonium Nitrate	Distilled water (Control)		10.2a	1.22a-e	3.7d	0.39bc
	10		8.28bc	1.39ab	2.7f	0.26cd
	20		5.8de	.82def	2.1g	0.47 cd B
Ammonium sulfate	Distilled water (Control)		1.5g	0.43f	3.26e	0.07e
	10		9.4ab	1.48a	5.9a	0.37a
	20		4.2f	0.7f	3.9d	0.11de
Calcium Nitrate	Distilled water (Control)	Purple	6.07d	0.92b-f	2.02g	0.16de
	10		6.63d	0.63f	2.07g	0.15de
	20		4.40f	0.74f	0.8i	0.11de
Ammonium Nitrate	Distilled water (Control)		4.11f	0.78ef	1.42g	0.15de
	10		7.96c	1.65a	2.06h	0.22de
	20		4.47f	1.27a-d	1.49h	0.14de
Ammonium sulfate	Distilled water (Control)		8.13c	0.89c-f	1.47h	0.46b
	10		5.92de	0.83def	1.34h	0.19de
	20		4.73ef	0.7f	3.97d	0.05e

Means that have the same letter in each attribute are not significant based on Duncan's test at a probability level of 5%.

3.3. Root Fresh Weight

The analysis of variance showed that genotype, sodium bicarbonate concentration, nitrogen source, and their interactions significantly affected root fresh weight at the 1% probability level (Table 2). According to the mean comparison results, the highest root fresh weight (5.91 g) was obtained in the white garlic genotype when treated with ammonium sulfate combined with 10 mM sodium bicarbonate. The lowest value (0.80 g) was recorded in the purple genotype under the treatment of calcium nitrate with 20 mM bicarbonate (Table 3). In the white genotype, root fresh weight declined with increasing bicarbonate concentration in all treatments except for ammonium sulfate, where an increase was observed. The highest root fresh weight in this genotype occurred under 10 mM bicarbonate combined with ammonium sulfate. In the purple genotype, an increase in bicarbonate level led to an increase in root fresh weight only when ammonium sulfate was applied. In contrast, under calcium nitrate and ammonium nitrate, the 10 mM bicarbonate treatment resulted in higher root fresh weight compared to both the control and the 20 mM level (Table 3).

3.4. Root Dry Weight

Statistical analysis revealed that sodium bicarbonate concentration significantly affected root dry weight at the 5% level, while genotype and the interaction of genotype, nitrogen source, and bicarbonate concentration were significant at the 1% level. However, the nitrogen source alone did not have a significant effect on the root dry weight of garlic genotypes (Table 2). Based on the mean comparison of nitrogen source and bicarbonate level interactions, in the white genotype, bicarbonate had no noticeable effect on root dry weight when calcium nitrate or ammonium nitrate was used. However, the highest root dry weight was observed with ammonium sulfate at 10 mM bicarbonate. In the purple genotype, root dry weight decreased with rising bicarbonate levels only in the presence of ammonium sulfate. No significant changes were seen when plants were supplied with calcium nitrate or ammonium nitrate under varying bicarbonate concentrations (Table 3).

3.5. Bulb Length

The analysis of variance for bulb length showed no significant effects from genotype, nitrogen sources, sodium bicarbonate concentrations, or their interactions (Table 2).

3.6. Bulb Diameter

Analysis of variance for bulb diameter revealed that genotype, nitrogen source, and the interaction between genotype and bicarbonate concentration had significant effects at the 1% probability level (Table 2). The white garlic genotype exhibited a greater bulb diameter compared to the purple genotype (Figure 2A). Among the nitrogen sources, plants supplied with calcium nitrate developed larger bulb diameters, while no significant difference was observed between ammonium nitrate and ammonium sulfate treatments (Figure 2B). The interaction between nitrogen source and sodium bicarbonate concentration (alkalinity stress) showed that increasing bicarbonate levels led to a decrease in bulb diameter. The highest bulb diameters were observed in the absence of bicarbonate (0 mM) for all three nitrogen sources, whereas the lowest diameters were recorded at the highest bicarbonate concentration (20 mM) across all nitrogen sources (Figure 2C).

3.7. Number of Cloves per Bulb

The analysis of variance for the number of cloves per bulb indicated that genotype, nitrogen source, and sodium bicarbonate concentration had significant effects at the 1% level. However, the three-way interaction among genotype, nitrogen source, and bicarbonate concentration was not significant (Table 2). In all nitrogen treatments, the purple genotype produced more cloves per bulb than the white genotype, although the differences among nitrogen sources were not statistically significant in either genotype (Figure 3A). Increasing bicarbonate concentrations led to a reduction in clove number in both garlic genotypes. The highest number of cloves was observed under control conditions (0 mM bicarbonate) and at 10 mM bicarbonate across all nitrogen sources (Figure 3B). The lowest clove number was recorded in the white genotype under 20 mM sodium bicarbonate (Figure 3C).

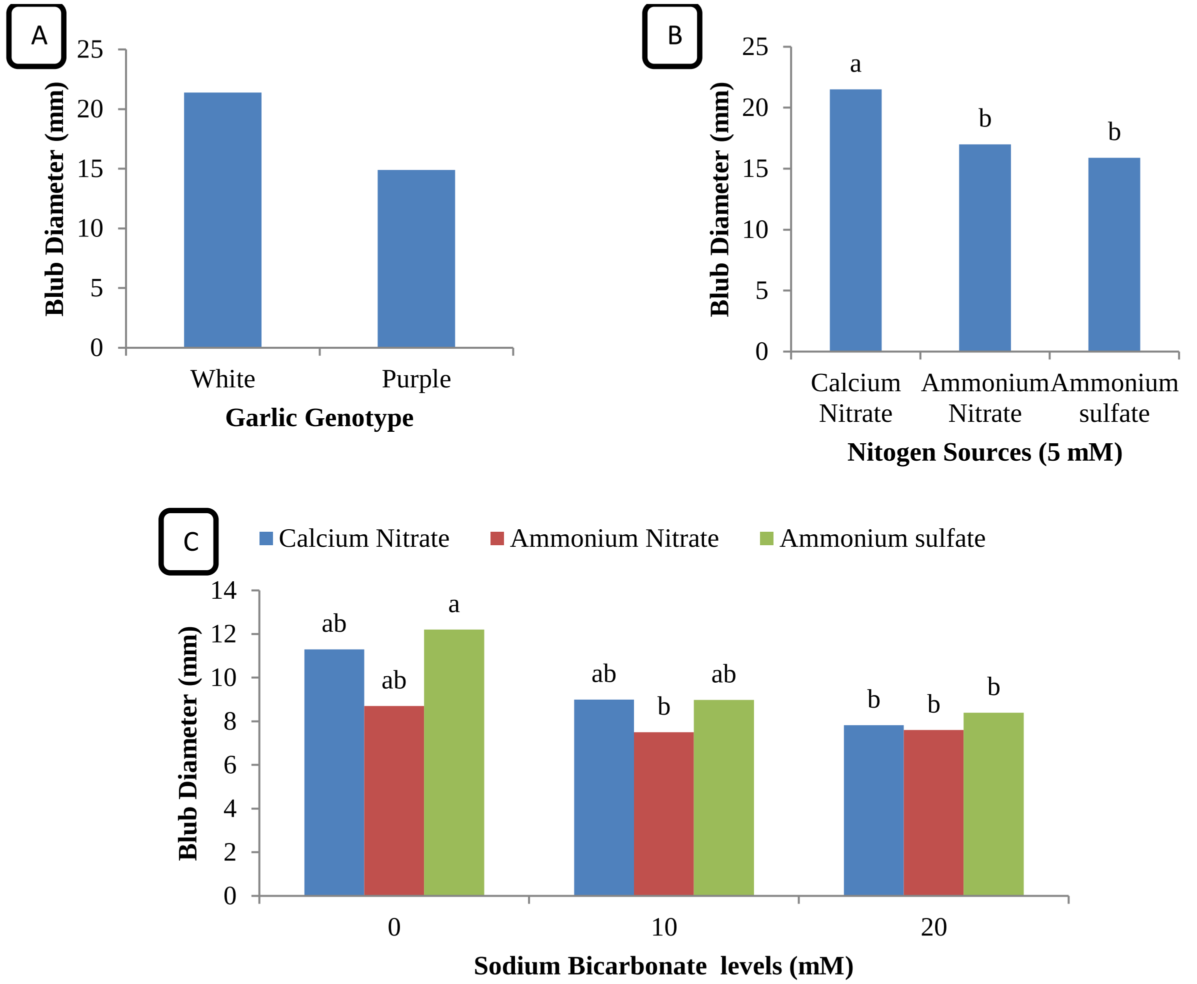
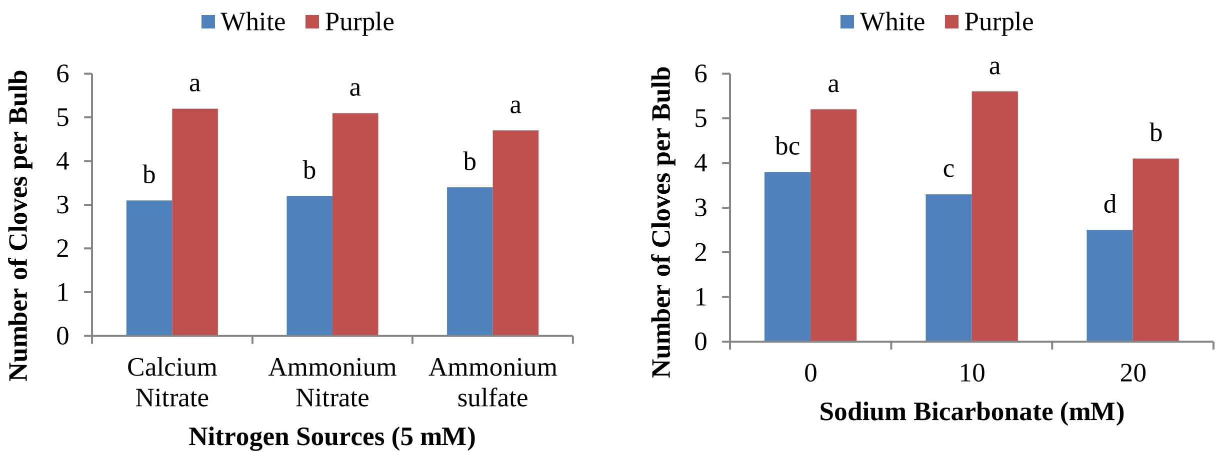


Figure 2A-C. The effect of garlic genotype (A), Nitrogen Sources (B) and interaction of Nitrogen Sources and Sodium Bicarbonate levels (C) on bulb diameter in hydroponic systems



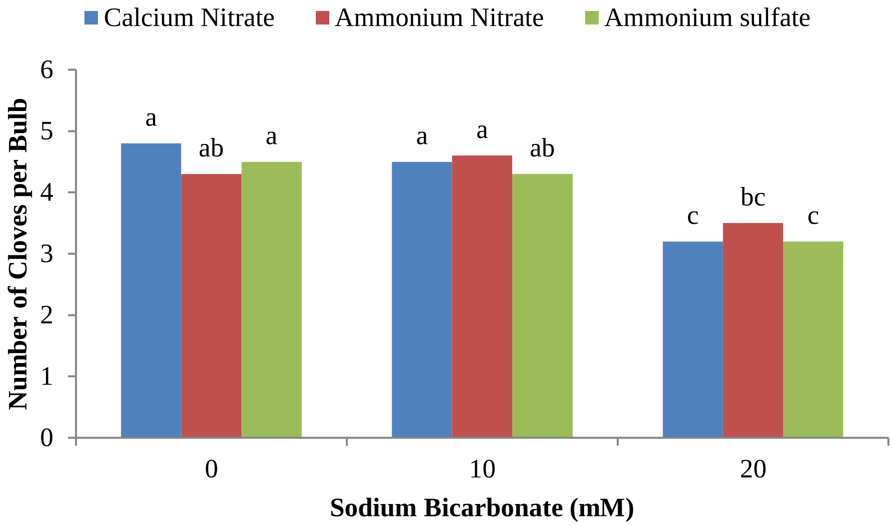


Figure 3A-C. The effect of Nitrogen Sources (A), Sodium Bicarbonate levels (B) and interaction of Nitrogen Sources and Sodium Bicarbonate levels (C) on number of Cloves per Bulb in hydroponic systems

3.8. Nitrogen Content

Analysis of variance showed that genotype, nitrogen source, bicarbonate concentration, and their interactions had a significant effect ($p \leq 0.01$) on nitrogen content in the aerial parts of garlic (Table 4). In both genotypes, nitrogen content decreased with increasing bicarbonate concentration across all nitrogen sources. However, in plants treated with ammonium sulfate, nitrogen content increased in both genotypes as bicarbonate levels rose, and a similar trend was observed in the purple genotype under ammonium nitrate treatment. The highest nitrogen content in the aerial parts (2.62% dry weight) was recorded in the purple genotype at 10 mM bicarbonate with calcium nitrate, while the lowest value (0.26% dry weight) was observed in the same genotype treated with ammonium nitrate under control conditions (Table 5).

Table 4. Analysis of variance of nutrient elements of two garlic genotypes under the influence of nitrogen and sodium bicarbonate sources in hydroponic cultivation

S.O.V	Df	Nitrogen	Potassium	Phosphorus	Calcium	Magnesium	Sulfur	Sodium
Genotype (G)	1	0.56**	23.88**	0.01**	0.015**	0.088**	0.27*	51.7**
Nitrogen Source (N)	2	2.52**	1.38**	0.03**	0.005*	0.04**	0.14ns	19.58**
Bicarbonate (B)	2	0.74**	0.16**	0.01**	0.004**	0.07**	0.08ns	12.38**
G×N	2	2.19**	0.80**	0.02**	0.036**	0.254ns	0.033*	8.63**
G×B	4	1.21**	0.44**	0.001ns	0.074**	0.001**	0.08ns	14.97**
N×B	2	2.86**	1.11**	0.003ns	0.064**	0.056**	0.07ns	2.46**
G×N×B	4	0.57**	2.11**	0.003ns	0.037**	0.079**	0.27**	5.92**
Experimental error	35	0.002	0.04	0.0015	0.0007	0.0008	0.06	0.035
CV	-	4.13	2.57	19.77	5.60	5.83	24.6	2.31

ns, *, and ** indicate non-significant, significant at the 5% and 1% probability levels of Duncan's test, respectively.

Table 5. Interaction of nitrogen sources and different concentrations of sodium bicarbonate (alkalinity stress) on macronutrient elements of two garlic genotypes in hydroponic cultivation

Nitrogen Source (5mM)	Bicarbonate Stress (mM)	Garlic Genotype	Nitrogen (%DM)	Potassium(%DM)	Calcium (%DM)	Magnesium (%DM)	Sulfur (%DM)	Sodium (%DM)
Calcium Nitrate	Distilled water (Control)	White	1.88c	0.93a	0.55bcd	0.18k	1.18a-c	0.78g
	10		1.48f	0.84de	0.48e	0.25j	1.19a-c	0.74h
	20		0.68j	0.87bcd	0.40fg	0.35hi	1.30a	1.01c
1Ammonium Nitrate	Distilled water (Control)		1.79d	0.85cd	0.55bcd	0.75a	0.79b-e	0.44k
	10		0.46k	0.93e	0.59bc	0.26j	1.16a-d	0.64i
	20		0.94h	0.83de	0.39g	0.52f	1a-d	0.96d
Ammonium sulfate	Distilled water (Control)		0.84i	0.9ab	0.43efg	0.64v	1.29ab	0.55j
	10		0.66j	0.89b	0.71a	0.62cd	0.83a-e	0.56j
	20		1.67e	0.88bc	0.53d	0.43g	1.24abc	0.77hg
Calcium Nitrate	Distilled water (Control)	Purple	1.22g	0.69h	0.52d	0.66bc	0.47a-c	0.78g
	10		2.62a	0.81ef	0.38g	0.57de	1.13a-d	1.12a
	20		0.68g	0.59i	0.71a	0.56ef	0.7de	0.92e
Ammonium Nitrate	Distilled water (Control)		0.26l	0.69h	0.54cd	0.70b	1.12a-d	1.07b
	10		0.90hi	0.71h	0.60b	0.51f	0.79cde	1.04c
	20		0.62j	0.83de	0.43efg	0.43g	1.01a-d	0.98d
Ammonium sulfate	Distilled water (Control)		1.91e	0.79fg	0.45ef	0.3gh	0.89a-e	0.84f
	10		2.43b	0.76g	0.26h	0.33i	1.29ab	0.65i
	20		1.60e	0.83de	0.42fg	0.56ef	1.30a	0.80g

Means that have the same letter in each attribute are not significant based on Duncan's test at a probability level of 5%.

3.9. Potassium Content

Analysis of variance revealed that genotype, bicarbonate concentration, nitrogen source, and their interactions significantly affected potassium concentration ($p \leq 0.01$) in the aerial parts (Table 4). In both white and purple garlic genotypes, potassium content decreased with increasing bicarbonate levels when treated with calcium nitrate. In the white genotype, potassium levels also decreased under increasing bicarbonate stress when supplied with ammonium sulfate or ammonium nitrate. However, in the purple genotype, potassium content increased under these same nitrogen sources as bicarbonate levels rose (Table 5).

3.10. Phosphorus Content

Analysis of variance indicated significant effects ($p \leq 0.01$) of genotype, nitrogen source, bicarbonate concentration, and the genotype × nitrogen source interaction on phosphorus content in the aerial parts (Table 4). Increasing bicarbonate concentration led to a general decline in phosphorus content in both genotypes, with the lowest levels observed at 20 mM bicarbonate (Figure 4A). The highest phosphorus concentration was found in the white genotype treated with ammonium sulfate, while the lowest was recorded under calcium nitrate treatment in the same genotype (Figure 4B).

3.11. Calcium Content

Calcium content in the aerial parts was significantly affected ($p \leq 0.01$) by genotype, nitrogen source, bicarbonate concentration, and their interactions—except for the genotype × nitrogen source interaction (Table 4). Generally, calcium content decreased with increasing bicarbonate concentrations. However, an increase was observed in the purple genotype under calcium nitrate and in the white genotype under ammonium sulfate. The highest calcium content (0.71 g per dry weight) was found in the purple genotype treated with calcium nitrate at 20 mM bicarbonate and in the white genotype treated with ammonium nitrate at 10 mM bicarbonate. Conversely, the lowest calcium content (0.26 g per dry weight) was observed in the purple genotype treated with ammonium sulfate at 10 mM bicarbonate (Table 5).

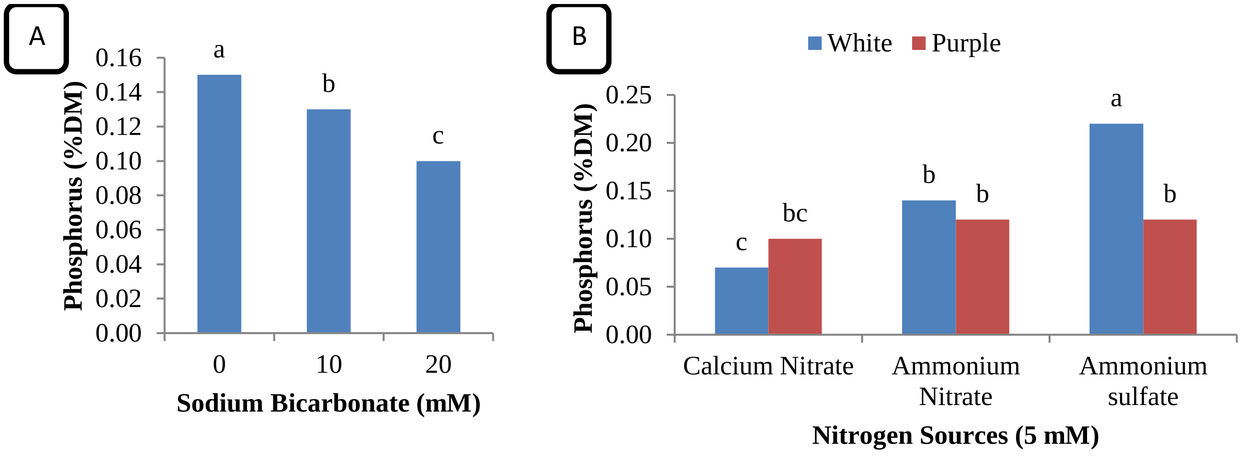


Figure 4A and B. The effect of Sodium Bicarbonate levels (A) and interaction of Nitrogen Sources and garlic genotypes (B) on phosphorus content in hydroponic systems.

3.12. Magnesium Content

The analysis showed that genotype, nitrogen source, bicarbonate concentration, and their interactions significantly affected magnesium content in the aerial parts of garlic ($p \leq 0.01$) (Table 4). In general, magnesium levels declined with increasing bicarbonate stress in both genotypes. However, an increase was recorded in the white genotype under calcium nitrate and in the purple genotype under ammonium sulfate. The highest magnesium content (0.75% dry weight) was observed in the white genotype treated with ammonium nitrate under control conditions (no bicarbonate), while the lowest (0.18% dry weight) occurred in the same genotype under calcium nitrate and no bicarbonate (Table 5).

3.13. Sulfur

Analysis of variance for sulfur concentration in garlic aerial parts revealed that the main effect of genotype and the interaction between genotype and nitrogen source were significant at the 5% probability level, while the three-way interaction of genotype, nitrogen source, and sodium bicarbonate concentrations was significant at the 1% level. However, the main effects of nitrogen source and bicarbonate concentration and their interaction were not statistically significant (Table 4). An increase in sulfur content was observed in the purple genotype under sodium bicarbonate stress when ammonium sulfate was applied, and in the white genotype when calcium nitrate was used. The highest sulfur concentration (1.30% of dry weight) was recorded in the white genotype with calcium nitrate and in the purple genotype with ammonium sulfate under 20 mM sodium bicarbonate. The lowest sulfur content (0.47% of dry weight) was observed in the purple genotype with calcium nitrate in the absence of bicarbonate (Table 5).

3.14. Sodium

The results of analysis of variance indicated that genotype, sodium bicarbonate concentration, nitrogen sources, and their interactions had significant effects on sodium concentration in garlic aerial parts at the 1% probability level (Table 4). In the white garlic genotype, sodium concentration increased with rising bicarbonate levels across all nitrogen sources. In contrast, in the purple genotype, this increase was only observed with calcium nitrate, while sodium levels decreased under ammonium nitrate and ammonium sulfate treatments as bicarbonate concentration increased (Table 5).

3.15. Iron

The analysis of variance showed that genotype, bicarbonate concentrations, nitrogen sources, and their interactions significantly affected iron content in garlic shoots at the 1% probability level (Table 6). In both genotypes and across all nitrogen sources, iron concentration declined with increasing bicarbonate levels. The highest iron content (424.43 mg kg^{-1} dry weight) was recorded in the purple genotype with ammonium nitrate and no bicarbonate, while the lowest value (15.96 mg kg^{-1} dry weight) was observed in the white genotype under 20 mM bicarbonate and ammonium sulfate (Table 7).

Table 6. Analysis of variance of nutrient elements of two garlic genotypes under the influence of nitrogen and sodium bicarbonate sources in hydroponic cultivation

S.O.V	df	Iron	Cupper	Manganese	Zinc
Genotype (G)	1	1185**	128.4**	29939**	332.5**
Nitrogen Source (N)	2	2236**	1947**	5122**	893.7**
Bicarbonate (B)	2	480507**	428**	13048**	17.9**
G×N	2	3613**	68.0**	1550**	23.7**
G×B	4	3986**	586.5**	21**	42.2**
N×B	2	1224**	797.3**	21**	91.8*
G×N×B	4	1665**	478.01**	1895**	29.06**
Experimental error	35	60.2	1.89	16.8	1.72
CV	-	4.69	6.85	5.58	4

ns, *, and ** indicate non-significant, significant at the 5% and 1% probability levels of Duncan's test, respectively.

3.16. Manganese

Analysis of variance showed significant effects of genotype, bicarbonate concentration, nitrogen source, and their interactions on manganese content in garlic aerial parts ($p \leq 0.01$) (Table 6). In the white genotype, manganese concentration increased with increasing bicarbonate concentration in all nitrogen treatments, whereas in the purple genotype, it decreased across all nitrogen sources. The highest manganese concentration was found in the white genotype with calcium nitrate at 10 mM bicarbonate, and the lowest in the purple genotype with calcium nitrate at 20 mM bicarbonate (Table 7).

Table 7. Interaction of nitrogen sources and different concentrations of sodium bicarbonate (alkalinity stress) on micronutrient elements of two garlic genotypes in hydroponic cultivation

Nitrogen Source (5mM)	Bicarbonate Stress (mM)	Garlic Genotype	Iron (mg Kg^{-1} DM)	Copper (mg Kg^{-1} DM)	Manganese (mg Kg^{-1} DM)	Zinc (mg Kg^{-1} DM)
Calcium Nitrate	Distilled water (Control)	White	345.1c	67.4f	23.9gh	20.1d
	10		116.06e	208.9a	22.5h	11.9fg
	20		80.67g	119.2c	25.5g	10.2g
1Ammonium Nitrate	Distilled water (Control)		358.03b	29.6h	33.2cdf	11.8g
	10		97.03f	75.2e	29.2f	15.8e
	20		59.4h	39.1h	29.3f	26.5c
Ammonium sulfate	Distilled water (Control)		335.3c	91.06d	41.3b	58.1a
	10		123.7e	116.4c	32.7e	25c
	20		15.9j	126.4b	35.4c	14.9e
Calcium Nitrate	Distilled water (Control)	Purple	293.8d	19.8j	26.2g	11.3g
	10		90.4gf	49.5g	30.4f	24.8c
	20		34.3i	11.06k	22.9h	4.7i
Ammonium Nitrate	Distilled water (Control)		424.4a	54g	35.2d	9.7gh
	10		94.05gf	81.3e	44.6a	7.6h
	20		36.6i	47.7g	33.06e	14.2ef
Ammonium sulfate	Distilled water (Control)		349.1b	48.2g	41.8b	24.1c
	10		97.5f	90.1d	39.7b	54.2b
	20		30.57i	47.9g	44.1a	16.0e

Means that have the same letter in each attribute are not significant based on Duncan's test at a probability level of 5%.

3.17. Zinc

Analysis of variance revealed that genotype, bicarbonate concentration, nitrogen source, and their three-way interaction significantly affected zinc content in garlic at the 1% probability level. The interaction between bicarbonate levels and nitrogen sources was also significant at the 5% level (Table 6). Zinc concentration decreased with increasing bicarbonate concentration across all nitrogen sources and both genotypes. However, an increase in zinc content was observed in the purple genotype with ammonium sulfate and in the white genotype with calcium nitrate under higher bicarbonate levels. The highest zinc concentration (44.63 mg kg^{-1} dry weight) was observed in the purple genotype with ammonium nitrate at 10 mM bicarbonate, while the lowest (22.56 mg kg^{-1} dry weight) occurred in the white genotype with calcium nitrate at the same bicarbonate level (Table 7).

3.18. Copper

According to the analysis of variance, genotype, bicarbonate concentration, nitrogen source, and their interactions significantly affected copper concentration in garlic shoots at the 1% level (Table 6). In both genotypes, copper content decreased under calcium nitrate and ammonium sulfate treatments as bicarbonate concentration increased. Conversely, copper content increased with rising bicarbonate concentrations when ammonium nitrate was used. The highest copper concentration (58.16 mg kg^{-1} dry weight) was found in the white genotype with ammonium sulfate in the absence of bicarbonate. The lowest value (4.79 mg kg^{-1} dry weight) was recorded in the purple genotype treated with calcium nitrate at 20 mM bicarbonate (Table 7).

4. Discussion

4.1. Vegetative Growth Parameters

The application of nitrogen sources in the presence of sodium bicarbonate significantly enhanced the fresh and dry mass of both shoots and roots up to a concentration of 10 mM bicarbonate, with the greatest improvements observed in the ammonium sulfate treatment. However, when the bicarbonate concentration increased to 20 mM, these parameters declined compared to the control. This decline is likely attributable to the rise in pH caused by elevated bicarbonate and carbonate levels, which induces alkaline stress and negatively affects nitrogen uptake and plant development (Valdez-Aguilar and Reed, 2008; Molina and Covarrubias, 2019). Different nitrogen sources play a crucial role in regulating vegetative growth in plants. Nitrogen form and concentration influence bulb development in bulbous crops. In the current study, the largest bulb diameter and the highest fresh and dry root mass were observed in plants supplied with ammonium sulfate. El-Bakry *et al*. (2024) also reported increased plant height and chlorophyll index in onions following sulfur supplementation. Their findings suggested that sulfur-containing fertilizers promoted shoot development, bulb diameter, and dry matter accumulation. Ozkan *et al*. (2018) reported increases in bulb diameter, and yield with rising sulfur levels, particularly from potassium sulfate. According to Abou Seeda *et al* (2020), sulfur contributes to physiological functions that improve plant quality by enhancing dry matter content. Hamilton *et al*. (1997) evaluated sulfur effects on six clones of onion cultivar TG 1015Y and noted reduced bulb weight under sulfur-deficient conditions. Garlic requires substantial nitrogen inputs, especially during early growth phases, as nitrogen availability strongly influences clove size. Sufficient nitrogen during vegetative stages promotes vigorous growth and optimal leaf development, whereas excessive nitrogen at later stages may reduce both yield and storage quality (Govindasamy *et al*., 2023). Insufficient nitrogen, on the other hand, accelerates maturity and reduces yield (Xiong *et al*., 2018). Retrenches found that nitrogen fertilization enhanced garlic yield and yield components (Abera and Adinew, 2023; Kevlani *et al*., 2023). Our findings align with those of Hore *et al*. (2014), who observed significant improvements in growth traits—such as plant height, leaf number, clove count, and clove weight—due to nitrogen application, which in turn led to improved vegetative growth and increased bulb yield. Nitrogen, a crucial element for plant growth, affects vegetative parameters by influencing various aspects of a plant's development and performance. Nitrogen's role in vegetative growth is primarily linked to its impact on chlorophyll content, photosynthesis, and protein synthesis, which are essential for overall biomass and yield (Flores-Saavedra *et al*., 2024). From a commercial perspective, market preferences play a major role in determining production goals, with demand spanning from small to large bulbs. Tian *et al*. (2017) reported that increase in nitrogen application could enhance plant height, leaf area, leaf count, and biomass accumulation. Gateri (2019) emphasized the importance of nitrate in developmental processes like bulbing in onions, where bulb weight increases with reduced soil nitrogen due to greater leaf expansion. However, excessive nitrogen may favor leaf growth over bulb formation. High garlic yield and quality can be achieved through appropriate nitrogen and sulfur fertilization strategies, including proper rates and application timing (Tesfaye and Bayih, 2024). Nitrogen is essential for ensuring robust vegetative growth in garlic (Kevlani *et al*., 2023). According to Prystupa and Gutierrez-Boem (2022), combining nitrogen and sulfur during early vegetative stages supports vigorous growth prior to the onset of winter. Tilahun *et al*. (2021) found that sulfur enhanced nitrogen and potassium uptake and promoted dry matter production in onions. Similar findings have been reported by Tabatabaei (2009) and Zeinali and Moradi. (2015), confirming that ammonium sulfate increases fresh weight in onion bulbs. Studies by Nasreen *et al*. (2008) also indicated that potassium sulfate boosts onion fresh mass. Our findings are consistent with research by Przygocka-Cyna *et al*. (2020) and Tilahun *et al*. (2021), who collectively confirmed the positive impact of nitrogen and sulfur on onion yield and biomass. Bolandnazar *et al*. (2012) demonstrated that onion biomass increases with potassium sulfate and ammonium nitrate application up to an optimal level. Amanah and Susila (2025) concluded that nitrate, alone or combined with ammonium, enhances dry Shallot t (Allium cepa L. var. aggregatum) weight. Decreases in bulb dry weight following high levels of ammonium or nitrate fertilizers may be explained by the diversion of resources toward vegetative growth during the bulbing phase, which ultimately limits final bulb yield (Attaya *et al*., 2024). According to Matlabi Fard (2015), nitrogen application increased overall yield and nitrogen content in shoots and bulbs while reducing the number of wrapper leaves and the weight of small cloves in garlic. Nitrogen's role in stimulating leaf growth may be attributed to its effects on cell division and leaf elongation, as suggested by Fathi (2022). Tena *et al* (2024) reported that nitrogen fertilization led to the formation of larger onion cloves. In garlic, bulb weight, diameter, and biological yield index significantly increased with nitrogen, phosphorus, and sulfur application, likely due to synergistic effects of these nutrients in providing balanced nutrition (Diriba *et al*., 2015; Kevlani *et al*., 2023). Nitrogen enhances vegetative growth and promotes photosynthetic activity in storage organs, contributing to increased bulb size and weight (Noor *et al*., 2023). In this study, sodium bicarbonate treatment reduced dry bulb weight, neck and bulb diameters, leaf dimensions, plant height, and clove number, depending on genotype and bicarbonate concentration. Roosta *et al*. (2011) studied the interaction of nitrogen form and bicarbonate in green beans, showing that ammonium reduced vegetative growth more than nitrate, and bicarbonate exacerbated ammonium toxicity, likely due to increased nutrient solution pH. In this study, High ammonium-to-nitrate ratios in the nutrient solution decreased total and root dry weights. Generally, alkalinity impairs plant growth by reducing nutrient solubility due to elevated pH levels, a result of bicarbonate accumulation (Kumar, *et al*., 2024). Growth inhibition under high bicarbonate has also been linked to reduced photosynthesis, iron deficiency, or iron precipitation, which damages chlorophyll synthesis (Shahsavandi *et al*., 2020). Valdez-Aguilar (2008) similarly attributed reduced bean growth under alkaline stress to pH-induced ionic imbalance in the rhizosphere.

4.2. Macronutrients and Their Dynamics under Bicarbonate-Induced Alkalinity

To achieve optimal yield and desirable quality in garlic cultivation, a well-balanced supply of both macro- and micronutrients is essential (Shukla *et al*., 2016). Compared to many other crops, garlic exhibits a greater demand for nutrients (Islam *et al*., 2024). Alkalinity stress, triggered by elevated sodium bicarbonate concentrations, disrupts nutrient uptake and causes physiological imbalances that hinder plant development (Sharma *et al*., 2024). These disruptions may stem from the antagonistic effects of high pH on nutrient availability and transport within the plant, or from competition between sodium ions and other essential elements during uptake (Li and Yang, 2023). In this study, increasing sodium bicarbonate concentrations led to a general decline in shoot nitrogen levels across all nitrogen sources and both garlic genotypes. However, an exception was observed in the white genotype under ammonium sulfate, where nitrogen levels rose despite elevated bicarbonate. The nitrogen source significantly influenced the nutrient solution's pH: nitrate raised the pH, making the solution more alkaline, while ammonium lowered it, creating more acidic conditions (Hachiya and Sakakibara, 2017). Nitrogen is indispensable throughout a plant’s life cycle, as it forms the backbone of DNA, RNA, proteins, enzymes, chlorophyll, ATP, and key phytohormones like auxins and cytokinins (Hawkesford *et al*., 2012). It plays a critical role in amino acid synthesis, cell division, protoplasm formation, and essential metabolic processes such as photosynthesis and enzymatic reactions (Zayed *et al*., 2013). A deficiency in nitrogen leads to diminished growth and productivity (Maqsood *et al*., 2014). Decreased total nitrogen content under bicarbonate treatment has been reported in Brassicaceae species (Xia and Wu, 2022), and pea (Barhoumi wt al., 2007). Nitrogen uptake in bulbous plants has been shown to vary based on cultivar, climatic conditions, plant density, and fertilization levels (Farhan *et al*., 2024). Moreover, sulfur can enhance nitrogen absorption by lowering root zone pH (Rizk *et al*., 2012). Phosphorus is another highly required macronutrient, integral to nucleotides, phospholipids, phosphoproteins, and dinucleotides. It also plays a key role in energy transfer, photosynthesis, enzyme regulation, and carbohydrate translocation (Khan *et al*., 2023). Plants deficient in phosphorus typically exhibit stunted growth. Phosphorus availability is strongly pH-dependent, with optimal uptake occurring between pH 5.5 and 7.5 (Chan *et al*., 2021). In this study, leaf phosphorus concentrations declined with higher bicarbonate levels, aligning with findings by Ding *et al* (2021). The reduction in macronutrient levels under high bicarbonate may be due to inhibited root growth (Leibar-Porcel *et al*., 2020). Alkaline environments surrounding roots can precipitate essential ions like phosphorus, reducing their bioavailability (Qetrani *et al*., 2024). Even under mildly alkaline conditions, phosphorus availability in aquaponic systems can be compromised (Da Silva Cerozi and Fitzsimmons, 2016). Potassium is vital for maintaining ionic balance, carbohydrate transport, enzyme activation, and osmotic regulation. It is particularly important for stomatal function, and its deficiency can lead to stomatal closure and reduced photosynthesis (Jones, 2007). As a cofactor in numerous biochemical reactions, potassium's availability diminishes under high pH and sodium stress caused by sodium bicarbonate. The optimal pH range for potassium uptake is 6.0 to 7.5. This study found that potassium levels in the shoots of both garlic genotypes decreased under elevated bicarbonate when calcium nitrate was used as the nitrogen source. Similar declines were observed with ammonium sulfate and ammonium nitrate in the white genotype. Sagervanshi *et al*. (2021) also reported a reduction in potassium content in Vicia faba L. leaves and roots with rising bicarbonate levels. Although differences between 10 and 20 mM bicarbonate treatments were not statistically significant, the decrease is likely due to competition between sodium and potassium ions, reducing potassium uptake (Colla *et al*., 2010). Alkalinity stress often leads to higher sodium accumulation and reduced potassium levels due to pH-mediated inhibition of K^+ uptake and Na^+/K^+ competition (Latef & Tran, 2016). Munns and Tester (2008) noted potassium depletion under alkaline stress in wheat due to this antagonistic interaction. Shoot calcium content decreased with rising sodium bicarbonate levels, although an increase was recorded in the purple genotype under calcium nitrate and in the white genotype under ammonium sulfate. High ammonium supply has been associated with decreased calcium and magnesium levels in plant tissues (Borgognone *et al*., 2013). Bagheri and Roosta (2012) observed significant calcium increases in cabbage leaves under bicarbonate treatment, regardless of cultivar, highlighting the variability of calcium responses. Iron deficiency may also contribute to reduced calcium accumulation (Jeong & Guerinot, 2009). Magnesium is the only metallic element at the center of the chlorophyll molecule, and its deficiency leads to reduced chlorophyll content, thereby impairing photosynthesis (Marschner, 1995). As an essential part of the chlorophyll molecule and a key activator of many enzymes involved in energy metabolism, magnesium plays a vital role in plant growth (Jones, 2007). This study found that magnesium levels declined with increasing bicarbonate, except under calcium nitrate in the white genotype and ammonium sulfate in the purple genotype. Precipitation of magnesium under alkaline stress has also been reported (Shi & Wang, 2005). Sulfur content in garlic shoots increased with sodium bicarbonate concentration in the purple genotype under ammonium sulfate and in the white genotype under calcium nitrate. The highest sulfur levels were observed in shoots treated with 20 mM bicarbonate from those respective nitrogen sources. Nasreen *et al*. (2007) reported linear increases in total nitrogen and nitrate content in onion with increasing nitrogen in hydroponics, while total sulfur and potassium rose initially before declining. El-Bakry *et al*. (2024) found sulfur application enhanced sulfur uptake in onion. Sulfur also promotes the uptake of other nutrients (Diriba *et al*., 2015), and is critical for the synthesis of sulfur-containing amino acids such as cysteine and methionine, which are required for protein production. Beyond improving bulb yield, sulfur can enhances quality traits like flavor and aroma (Marcinkowska and Jeleń, 2022). Sulfur-based secondary compounds not only improve nutritional value and palatability but also contribute to pest and disease resistance (Ullah *et al*., 2008). Other studies have shown that sulfur improves physical and chemical attributes of garlic, including allicin content, pungency, protein, and nutrient levels (Brown & Noah, 2014). Severe sulfur deficiency during bulb formation negatively affects both yield and quality (Przygocka-Cyna *et al*., 2020). Additionally, sulfur is involved in the synthesis of vitamins, glucosides, and enzyme activation (Hill *et al*., 2023). Marschner (1995) noted that plants can absorb up to 30% of their sulfur from the atmosphere, with the remainder needing to be supplied via fertilization. Global studies confirm sulfur’s beneficial impact on crops, and its role in improving micronutrient availability by modifying soil pH (Chorianopoulou and Bouranis, 2022). In the white garlic genotype, shoot sodium levels increased with rising bicarbonate concentrations across all nitrogen sources. This trend was also observed in the purple genotype under calcium nitrate. Elevated sodium uptake can reduce the availability of other ions, particularly potassium (Adams and Shin, 2014). When sodium replaces potassium in various metabolic processes, sodium toxicity may occur (Clausen and Poulsen, 2013). Bagheri and Roosta (2012) found that sodium accumulation in cabbage leaves and roots increased with bicarbonate levels, peaking at 20 mM and lowest in untreated controls. Maintaining high cytoplasmic potassium and low sodium levels is essential to protect enzyme function (Munns & Tester, 2008). Under salt and alkaline stress, most plants exhibit increased sodium uptake and reduced potassium absorption (Shi & Wang, 2005). These findings are consistent with Li *et al*. (2014), who observed higher sodium and lower potassium in wheat seedlings under alkaline stress. In beans, increasing sodium carbonate in the nutrient solution significantly raised sodium accumulation while lowering potassium (Radi *et al*., 2012).

4.3. Micronutrients and Their Dynamics under Bicarbonate-Induced Alkalinity

Among all micronutrients, iron (Fe) is required in the largest amounts by plants due to its involvement in two critical physiological processes (Schmidt *et al*., 2020). First, it acts as a cofactor in many oxidation-reduction enzymes (Vigani and Murgia, 2018); second, it is essential for chlorophyll biosynthesis (Kroh and Pilon, 2020). Iron is directly involved in the photosynthetic process, especially influencing Photosystem II more significantly than Photosystem I. A shortage of iron disrupts the D1 protein and thylakoid-associated proteins, impairing photosynthetic efficiency (Higuchi and Saito, 2022). Moreover, iron is vital for the activity and gene expression of Rubisco (ribulose-1,5-bisphosphate carboxylase), a key enzyme in the carbon fixation cycle of C3 plants, which makes up a major portion of chloroplast proteins (Soufi *et al*., 2023). Although iron is generally abundant in soils, its availability becomes limited in calcareous soils due to the presence of bicarbonate (HCO_3^-), which induces iron chlorosis by converting bioavailable Fe^{2+} into the less soluble Fe^{3+} form. For every unit increase in soil pH, the solubility of Fe^{2+} and Fe^{3+} can drop by approximately 100 and 1,000 times, respectively (Nicolic and Romheld, 2002). Bicarbonate accumulation is therefore a major contributor to iron deficiency symptoms, especially in alkaline conditions (Sarkar *et al*., 2022). In this study, increased sodium bicarbonate concentrations led to a notable decline in shoot iron content across all nitrogen sources and both garlic genotypes. The characteristic yellowing (chlorosis) observed is not necessarily due to the absolute absence of iron but rather to its conversion into non-bioavailable forms. This iron deficiency results in decreased chlorophyll content and destruction of chloroplast structures (Li *et al*., 2021). Nitrate and carbonate ions are major contributors to this deficiency (Ning *et al*., 2023). Previous studies also demonstrated that ammonium-based nitrogen enhances iron mobilization from roots to shoots and promotes its reduction into plant-accessible forms in young leaves (Tadayon & Moafpourian, 2010). Likewise, both ammonium nitrate and urea fertilization significantly increased iron content in wheat leaves and grains, with no meaningful difference between the two nitrogen sources (Noor Gholipour & Bagheri, 2006). Plants classified as Strategy I responders typically enhance rhizosphere acidification, ferric reduction, and iron transport when iron availability becomes limited (Jeong and Guerinot, 2009). Leaves suffering from low chlorophyll not only have impaired photosynthesis but also absorb excessive light energy per chlorophyll unit, leading to increased generation of reactive oxygen species (ROS). These ROS cause oxidative stress by damaging DNA, inactivating enzymes, and triggering lipid peroxidation. Plants counteract this via antioxidant defense systems composed of both enzymatic (e.g., catalase, SOD, APX, POX, DHAR) and non-enzymatic (e.g., glutathione, ascorbate, tocopherol) components. Modulating these antioxidant systems may enhance plant tolerance to bicarbonate-induced iron deficiency (Amooaghaie and Roohollahi, 2017). Zinc (Zn) is another essential micronutrient that influences carbohydrate metabolism, cell division, and enzyme function (Suganya *et al*., 2020). It is an integral part of enzymes such as carbonic anhydrase, alcohol dehydrogenase, and glutamate dehydrogenase (Castillo-González *et al*., 2018). Zinc deficiency quickly impairs plant growth and cell division, reduces net photosynthesis, and disrupts electron transport (Zhao and Wu, 2017; Kazemi *et al*., 2024). Kazemi *et al* (2023) found that application of zinc sulfate as a foliar spray can be considered as an efficient strategy to enhance nutrition and production of secondary metabolites in medicinal plants. Sharma *et al*. (2025) identified bicarbonate as a primary cause of zinc deficiency in plant grown on calcareous soils due to its negative effects on both root uptake and translocation of zinc to shoots. In our experiment, increasing sodium bicarbonate reduced shoot zinc content across all nitrogen treatments and both garlic genotypes. However, an increase in shoot zinc was observed in the purple genotype with ammonium sulfate and in the white genotype with calcium nitrate under elevated bicarbonate levels. The role of bicarbonate in zinc and iron chlorosis has been widely recognized (Zhao and Wu, 2017). It hinders both uptake by roots and subsequent translocation to aerial parts (Valdez Aguilar and Reed, 2010), similar to its effect on iron (Roosta, 2011). At higher pH values, typical under bicarbonate stress, soluble zinc species like $Zn(OH)_3^-$ and $Zn(OH)_4^{2-}$ become more prevalent but are poorly absorbed (Forno *et al*., 1975). The antagonistic relationship between zinc and iron under these conditions has also been reported (Basar and Ozgumus, 1999). Manganese (Mn), a critical cofactor in various enzymatic reactions including the TCA cycle, also plays a structural role in the water-splitting protein of Photosystem II (Alejandro *et al*., 2020). It is part of the superoxide dismutase complex, which neutralizes ROS and protects plant cells (Schmidt, S.B. and Husted, 2019). In our findings, bicarbonate treatment increased manganese content in the white garlic genotype but decreased it in the purple one across all nitrogen sources. The use of ammonium fertilizers acidifies the rhizosphere due to proton release during uptake, thus enhancing the solubility and uptake of micronutrients such as Mn, Zn, and Fe in calcareous soils (Naeem *et al*., 2023). Combined application of nitrate and ammonium further improves plant growth and nutrient absorption (Yang *et al*., 2025). Copper (Cu), a cofactor in numerous metabolic processes including photosynthesis and ATP synthesis, also displayed altered distribution under bicarbonate stress (Pandey, 2020). Our results indicated that shoot Cu content decreased with bicarbonate treatment in both genotypes when using calcium nitrate or ammonium sulfate. In contrast, ammonium nitrate application resulted in increased Cu levels in both genotypes. The highest shoot Cu content was observed in the white genotype under ammonium sulfate without bicarbonate. High pH reduces Cu bioavailability due to precipitation of copper with phosphate and carbonate ions (Kumar *et al*., 2021).

Conclusion

This study demonstrates that garlic genotypes respond distinctly to varying nitrogen sources and sodium bicarbonate (alkalinity) stress, significantly influencing growth parameters, nutrient content, and overall plant development. The white and purple genotypes exhibited differential sensitivity to bicarbonate concentrations, with notable effects on shoot and root biomass, nutrient uptake, and bulb characteristics. Ammonium nitrate combined with low bicarbonate levels generally promoted shoot fresh and dry weight, particularly in the white genotype, while ammonium sulfate enhanced root fresh and dry weight at moderate bicarbonate concentrations. Increasing bicarbonate stress predominantly reduced growth metrics such as bulb diameter and clove number across both genotypes, highlighting the inhibitory effects of alkalinity on garlic development. Nutrient analyses revealed complex interactions; for example, nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients like iron, manganese, zinc, and copper were all significantly affected by genotype, nitrogen source, and bicarbonate levels. Generally, bicarbonate stress caused declines in most nutrient concentrations, though some exceptions—such as increased nitrogen content under ammonium sulfate—indicate adaptive nutrient uptake responses. Overall, the findings underscore the importance of selecting appropriate nitrogen sources and managing bicarbonate levels to optimize garlic growth and nutrient status under alkaline conditions. This knowledge can guide improved agronomic practices for garlic cultivation in environments prone to alkalinity stress, ensuring better yield and quality outcomes.

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