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Effects of Marjoram, Amino and Humic Acid on Nitrogen Content, Quantitative and Qualitative Characteristics and Shelf Life of Bell Pepper cv. Inspiration

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ABSTRACT
This study investigated the effects of marjoram (*Origanum majorana*) extract, amino acids, and humic acid on several agronomic and chemical characteristics, as well as the post-harvest shelf life of greenhouse bell peppers (cv. 'Inspiration'). The experimental design comprised ten treatments: a control, three concentrations of humic acid (0.2, 0.4, and 0.6 g/L), amino acids (0.5, 1, and 1.5 g/L), and marjoram extract (3%, 6%, and 9%). These treatments were applied via foliar application for 12 consecutive weeks, starting five days after seedling transplanting once the plants were fully established. The experiment was conducted in a randomized complete block design (RCBD) with ten treatments, each replicated three times, with each replicate consisting of three bell pepper plants. Following analysis of variance (ANOVA), mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% and 1% probability levels. Furthermore, a separate factorial experiment was conducted to evaluate fruit weight loss, considering two factors: (A) treatments and (B) storage duration. Post-treatment measurements included fruit nitrate and nitrite levels, calcium and potassium content, fruit diameter, fruit number per plant, and the percentage of weight loss at 14 and 21days post-harvest. The results indicated that the 0.6 g/L humic acid treatment resulted in the lowest nitrite levels, while the 1.5 g/L amino acid treatment yielded the lowest fruit nitrate content. Additionally, the marjoram extract demonstrated the most favorable results regarding fruit calcium and potassium levels, fruit diameter, and yield (number of fruits per plant). Furthermore, this study revealed a significant positive impact on reducing post-harvest weight loss. These findings provide valuable practical insights for agricultural producers, stakeholders, and consumers in sustainable crop management.
Keywords: *Ca, K, Fruit Nitrate, Marjoram extract, Nitrite*

INTRODUCTION

Bell peppers (*Capsicum annuum* L.) are a globally important vegetable, cultivated extensively in both field, greenhouse environments and soilless growing systems. Part of the Solanaceae family, they are broadly categorized as either hot (pungent) or sweet (non-pungent) peppers [29, 57]. Their widespread cultivation stems from their numerous health benefits and growing consumer demand. Bell peppers are particularly prevalent in Asia, Central America, and Europe [56]. Bell pepper production has increased significantly in recent years due to rising consumer demand. This increased demand also drives a growing interest in healthier production methods, including reduced reliance on chemical fertilizers [15].

Marjoram (*Origanum majorana* L.), a member of the Lamiaceae family, is an aromatic perennial herb commonly known as sweet marjoram. Cultivated globally since ancient times, it's valued both as a medicinal plant and a flavorful seasoning. Its nutritional richness is well-documented [23, 53]. Sweet marjoram, an aromatic perennial herb belonging to the Lamiaceae family [1, 50], is recognized for its significant phenolic content and inherent antibacterial properties [35]. Natural plants contain a diverse array of effective and complex chemical components [30]. Supporting this, various studies have documented the antifungal, antibacterial, and antioxidant capabilities of marjoram species [50]. The essential oil of this plant is primarily composed of carvacrol, thymol, p-cymene, gamma-terpinene, α-pinene, and myrsene

[51]. Medicinal plants contain valuable natural resources whose scientific identification can play a very important role in the health of society [40]. The chemical profile of marjoram includes mainly essential oils alongside other bioactive compounds such as polyphenols, flavonoids, sterols, triterpenes, alkaloids, coumarins, tannins, and saponins [Della-Pepa]. Although fertilizers serve as crucial nutrient sources in agricultural systems [7, 19], the intensified use of chemical fertilizers over recent decades has given rise to substantial environmental issues [16, 19]. The adverse effects of extensive application of inorganic fertilizers on herbal products have been well addressed. Chemical fertilizers by contaminating the soil and water are a great risk for human being's health [24]. Consequently, advancements in agricultural cultivation technologies aim to enhance crop yields and quality while concurrently reducing environmental impacts [25].

Amino acids represent an organic nitrogen form that serves as a fundamental constituent of all living cells, thereby contributing to enhanced plant growth and performance [10, 37]. Extensive research has validated that the application of amino acids via foliar spraying at various growth stages positively impacts both plant yield and crop quality [17]. On the other hand, foliar spraying is oneof the most effective ways to provide the traceelements needed by plants [41]. Contemporary studies have increasingly focused on optimizing foliar application protocols for amino acids. Indeed, amino acids form the backbone of protein structures and consequently play a crucial role in facilitating plant development [21]. As a result, foliar supply of amino acids can serve as a significant substrate for protein biosynthesis [43]. Over recent decades, the pivotal role of amino acids in promoting plant growth and aiding defense mechanisms against stress has become more evident, consequently sparking heightened interest within both fundamental and applied plant science disciplines [49]. Research indicates that foliar application of amino acids enhances the energy efficiency of plants, elevates the rate of photosynthesis, and consequently aids in increasing yield [17]. Numerous researchers have emphasized the significance of utilizing amino acids to improve the growth and productivity of various plant crops [32]. Various studies have documented increased plant yield in greenhouse tomatoes, Chinese cabbage, and leaf radish following the application of amino acids [15]. Additionally, a study demonstrated a significant rise in soybean yield subsequent to foliar spraying with amino acids [25]. In another investigation focusing on lettuce, it was observed that foliar application of amino acids at a concentration of 500 mg/L resulted in enhanced leaf area, an increased number of leaves, and greater leaf chlorophyll content [32].

Humic acid, a form of organic matter derived from plant and animal residues, enhances soil and crop quality through microbial decomposition, particularly when applied in conjunction with mineral fertilizers [28]. Indeed, this compound is widely recognized as an agricultural biostimulant that confers multifaceted benefits to plant development [32]. Humic acid, classified as a natural organic matter, significantly influences plant growth and quality [32, 42]. Humic substances are black or brown organic compounds characterized by high molecular weight and complex structures, originating from the decomposition of plant or animal residues [27]. By employing various mechanisms within plants, humic acid enhances nutrient availability, promotes plant growth and yield, and mitigates the detrimental effects of stress [55]. This organic fertilizer can directly exert positive effects on key plant growth components, including cell permeability, respiration, photosynthesis, and cell elongation [9]. Furthermore, humic substances not only induce significant changes in nutrient uptake and primary plant metabolism but may also strongly influence secondary metabolism [6]. Depending on their solubility at different pH levels, humic substances encompass humic acid, humin, and fulvic acid [27]. Research indicates that humic acid enhances the uptake of both micro and macro elements in plants, thereby promoting plant growth by increasing the bioavailability of nutrients [52].

Vegetables are widely recognized as essential for human food security and nutrition, serving as the most accessible source of the vitamins and minerals required for health. Currently, there is insufficient understanding of both the economic and nutritional value of vegetables, underscoring the urgency to provide healthy diets for all [46]. Nitrogen (N) stands as one of the most crucial plant nutrients, often being the most limiting factor, and it plays a pivotal role in determining vegetable quality [48]. Nitrogen is an integral component of both protein and chloroplast structure. However, excessive nitrogen application can negatively impact nutritional quality and characteristics, notably leading to the accumulation of nitrates in edible tissues [Martínez-Moreno]. Nitrogen (N) is a key element governing the functions and dynamics of diverse terrestrial ecosystems [22]. Nitrite (NO2) and nitrate (NO3) constitute part of the natural nitrogen cycle. These water-soluble compounds consist of nitrogen and oxygen atoms. Nitrite can react with other substances, such as amines or amino acids found in food, leading to the formation of nitrosamines, some of which are classified as carcinogenic when consumed in excess [11]. Several factors contribute to the increased accumulation of nitrates within protected cultivation systems, including limited light availability, variations in light quality, high water supply, elevated temperatures, pH levels, aeration management (including high CO_2 levels), and the quality of the nutrient solution [33].

Nitrate accumulation is widely regarded as detrimental to both the environment and human health. Fruits and vegetables typically contain higher levels of nitrate compared to nitrite. Notably, increased levels of humidity (> 60%), annual rainfall (> 1500 mm), and average temperature (> 10 °C), along with fertilizer application, result in a significant increase in nitrate/nitrite accumulation within vegetables and fruits ($p<0.05$). Fertilizer application stands as a primary controllable and influential factor contributing to pollutant residues, necessitating careful management [20]. Research indicates that the risk of nitrate and nitrite contamination poses a very serious threat [58]. In recent years, human activities, particularly the extensive use of nitrogen fertilizers and the alteration of the natural nitrogen cycle, have threatened various environmental aspects. Excessive nitrogen fertilizer use contributes to the decline in plant species richness and biodiversity. Furthermore, active nitrogen in diverse forms damages the ozone layer and intensifies the greenhouse effect. Consequently, the long-term environmental repercussions are severe and must not be overlooked [22].

As previously mentioned, the excessive use of chemical fertilizers poses a major challenge in modern agriculture. Therefore, this study aimed to investigate methods for reducing reliance on chemical fertilizers and promoting sustainable development within the agricultural sector. To the best of our knowledge, limited research has specifically examined the effects of marjoram medicinal plant extract, humic acid, and amino acids on greenhouse bell pepper parameters and post-harvest shelf life. The results of this study hold significant potential to be beneficial in this context. Consequently, this research can contribute effectively to the development and enhancement of agricultural product quality, the production of healthy food items, and the enhancement of food security. Therefore, given the above, this study aimed to investigate the effects of *Origanum majorana* extract, amino acids, and humic acid on nitrate/nitrite content, some quantitative and qualitative traits, and the post-harvest shelf life of greenhouse bell pepper.

MATERIALS AND METHODS

This study investigated the effects of marjoram extract, amino acids, and humic acid on nitrate/nitrite content, selected quantitative and qualitative characteristics, and post-harvest shelf life of greenhouse-grown bell pepper (cv. 'Inspiration'). The experiment was laid out in a randomized complete block design (RCBD) with 10 treatments, each replicated three times. Each replicate consisted of three colored bell pepper plants. Data were subjected to analysis of variance (ANOVA), and mean comparisons were performed using the Least Significant Difference (LSD) test at the 5% and 1% probability levels. Additionally, weight loss was evaluated in a separate 2-factor factorial experiment (A: treatments; B: storage duration at 14 and 21 days). Before sowing, soil physicochemical properties were analyzed. Seeds were sown in October 2024 in seedling trays filled with a mixture of peat moss (50%), coco peat (30%), and perlite (20%). Seedlings were transplanted into the main greenhouse beds 35 ± 5 days later, in November. Foliar sprays were applied weekly for 12 consecutive weeks under a drip irrigation system. At the peak of fruit production, 10 fruits were randomly harvested from each replicate of every treatment and transferred to the laboratory for analysis.

The research was conducted in a commercial greenhouse in Roudan County, Hormozgan Province, southern Iran (geographical coordinates: X = 518,613; Y = 3,025,192). Greenhouse conditions were maintained at 22–25 °C during the day and 14–17 °C at night, with a relative humidity of 60–80%. The photoperiod varied from 13 to 14.5 h depending on the experimental week.

In this study, the amino acid fertilizer Aminabon (Free Amino Acids 50), produced by CIBON AGRONUTRIENTES SL, Spain, was selected. This product contains 50% free amino acids. The humic acid fertilizer utilized was NATURAL GREEN HUMIC Acid, manufactured by Agroland in Greece. This fertilizer contained 10% potash and a combined 90% humic acid and fulvic acid. The bell pepper cultivar tested was 'Inspiration', a hybrid cultivar used for producing red bell peppers featuring blocky, four-lobed fruits. It is known for its strong plants, high yields, and fruits of suitable thickness for the market. The bell pepper cultivar 'Inspiration' is owned by RIJK ZWAAN, Netherlands.

Table 1 Analysis of the amount of some mineral element in the used extract.

Element	Value	Element	Value
Fe^{2+}	22 mg/kg	P_2O_5	0.68 %
Cu^{2+}	1 mg/kg	K_2O	0.12 %
Zn^{2+}	35 mg/kg	Ca^{2+}	0.11 %
B^-	0.038 %	Mg^{2+}	0.06 %
pH	5.08	N(Total)	0.04 %
EC	4.11 ds/m	Mn^{2+}	4.6 mg/kg

The treatments in this study were as follows:

- Control: Pure water
- Humic acid: 0.2 g/L, 0.4 g/L, and 0.6 g/L
- Amino acid: 0.5 g/L, 1 g/L, and 1.5 g/L
- Marjoram extract: 3%, 6%, and 9%.

In this experiment, to prepare the marjoram extract, dried marjoram plant material was first mixed in a ratio of 1:15 (one part dry plant material to 14 parts water). The mixture was then left at room temperature (22 to 24 degrees Celsius) for 72 hours to allow the plant material to infuse into the water. Subsequently, the obtained solution was passed through a filter. The filtered solution was then diluted with water to achieve the desired concentrations of 3%, 6%, and 9% and used as the test material. During the bed preparation stage before planting, necessary precautions were taken. Furthermore, pest and disease control was conducted uniformly across all treatments, with the required pesticides applied only if necessary.

In this experiment, the diameter of the fruits was measured using a caliper immediately after harvest for each treatment and the data were recorded. Additionally, the number of fruits per plant was counted and recorded for each treatment.

Measurement of Calcium and Potassium in the Fruit

To measure the desired elements, after harvesting the fruits from each treatment, they were dried in an oven. Subsequently, the dried samples were ground, and 2 grams of each sample was ashed at 550 °C. After ashing, 10 ml of 2 N hydrochloric acid was added to the samples, which were then placed in a water bath at 100°C for 10 minutes. The samples were then filtered using Whatman No. 2 filter paper and the filtrate was brought to a final volume of 100 ml. The amount of potassium was measured using a flame photometer.

Fruit calcium was measured via titration with potassium permanganate. For this procedure, 20 ml of the ash solution was transferred to a beaker, and approximately 100 ml of distilled water and two drops of methyl red reagent were added. Using a pH meter and ammonium hydroxide solution (1+1), the pH of the solution was adjusted to approximately 5.6-7, at which point the solution turned yellow. The pH was then lowered to approximately 2.5-3 using hydrochloric acid (1+3) until the solution turned pink. Finally, the solution was titrated with 0.1 N potassium permanganate solution until a stable pink color persisted for at least 30 seconds [3].

Measurement of Nitrate and Nitrite in the Fruit

The levels of nitrate and nitrite were measured using the method for determining nitrite and nitrate in fruits, vegetables, and their products via molecular spectroscopy, as specified in the standard of the Institute of Standards and Industrial Research of Iran (ISIRI No. 4106) [38]. This method is one of the most common standard methods for determining nitrate and nitrite concentrations in food, including vegetables and fruits. It is based on chemical reactions involving color formation, using sulfonic acid and N-(1-naphthalyl) ethanolamine (NED-H) or tetrazolium derivatives (such as the Griess reagent) [38].

Measurement of Weight Loss Percentage

To determine the effect of treatments on weight loss percentage, 20 fruits from each treatment were stored under ambient conditions (temperature 24°C, relative humidity 50%). On the 14th and 21st days of storage, weight loss was calculated and expressed as a percentage.

Statistical Analyses

After confirming the normality of the data using the Kolmogorov-Smirnov and Shapiro-Wilk tests with SPSS 20.0 software, and performing an analysis of variance (ANOVA) using SAS 9.01 software in a randomized complete block design, the comparison of means was conducted using the LSD test at probability levels of 5 and 1 percent. Furthermore, a separate experiment was conducted as a factorial design with two factors to evaluate weight loss: (A) treatments and (B) storage duration (at 14 and 21 days). (Tables 2 & 3).

RESULTS

As shown in the analysis of variance table (Table 2), treatments had a significant effect on all measured traits at the 1% probability level.

Table 2 Analysis of variance of 10 treatments on the study trait.

Sources	DF	MS					
		Fruit Nitrate	Fruit Nitrite	Fruit Ca	Fruit K	Fruit Diameter	Number of fruits
Repetition	2	84.0 ns	5.105 *	0.005 ns	0.057 ns	0.996 *	5.7 ns
Treatment	9	493.4 **	2.249 **	0.146 **	0.779 **	1.038 **	15.3 **
Error	18	82.3	0.946	0.004	0.044	0.192	3.5
C.V%		21.5	22.6	2	7.5	5.4	14.8

Effects of Treatments on Nitrate and Nitrite Contents of Greenhouse Bell Pepper

Regarding the fruit nitrate content, significant differences were observed among the different treatments. Comparison of the treatments showed that the highest fruit nitrate content with average value (45 mg/kg) was obtained by the application of humic acid at 0.2 g/L. The lowest average fruit nitrate content with an average of 30.7 mg/kg was observed under the treatment containing 1.5 g/L of amino acid, (Figure 1).

The results of this study regarding the fruit nitrite content also indicated that significant differences were observed between the treatments. In this regard, the highest fruit nitrite content with value of 5.87 mg/kg was obtained using 9% marjoram treatment. Conversely, the lowest fruit nitrite content with average value of 2.87 mg/kg was observed in the 0.6 g/L humic acid treatment (Fig. 2).

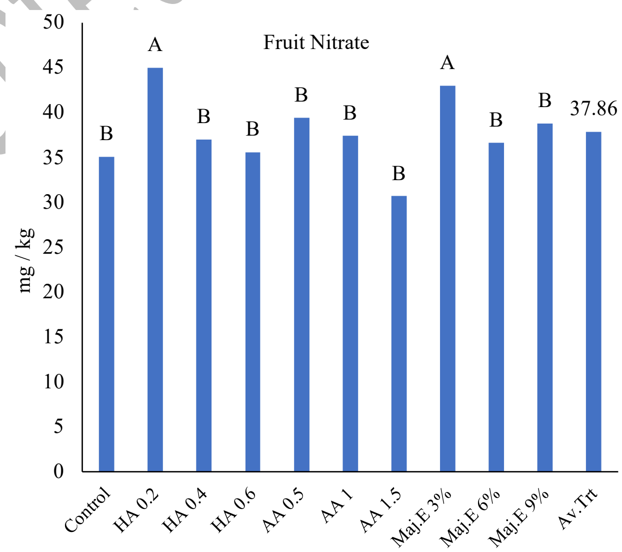

Fig. 1 Effect of humic acid, amino acid and marjoram extract on nitrate levels of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

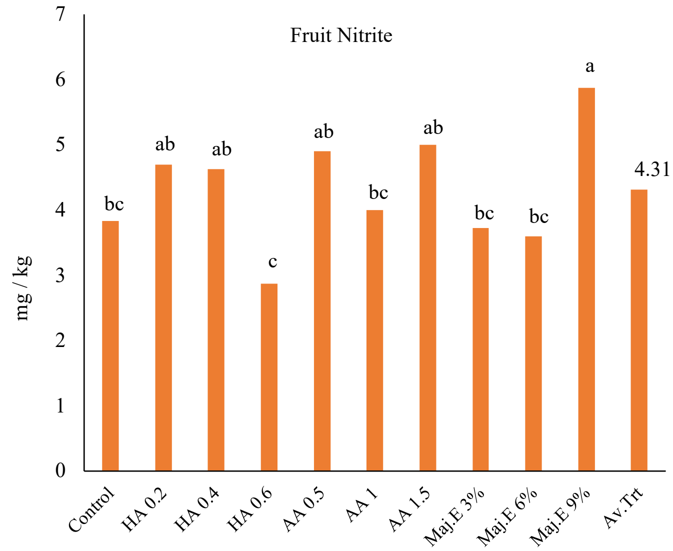

Fig. 2 Effect of humic acid, amino acid and marjoram extract on nitrite levels of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

Effects of Treatments on Calcium and Potassium Contents of Greenhouse Bell Pepper

The results of this study also showed significant effect of treatments on fruit calcium content. results revealed that the highest average fruit calcium content (3.67 mg/g) was obtained with the use of 9% marjoram extract. The lowest fruit calcium content with an average of 3.07 mg/g was observed under the control treatment, (Figure 3).

Regarding the fruit potassium content, the results also indicated significant differences between the treatments. In this regard, the highest fruit potassium content with value 3.68 mg/g was obtained in the 9% marjoram treatment. The lowest fruit potassium content with value 2.04 mg/g was observed in the 0.5 g/L amino acid treatment (Figure 4).

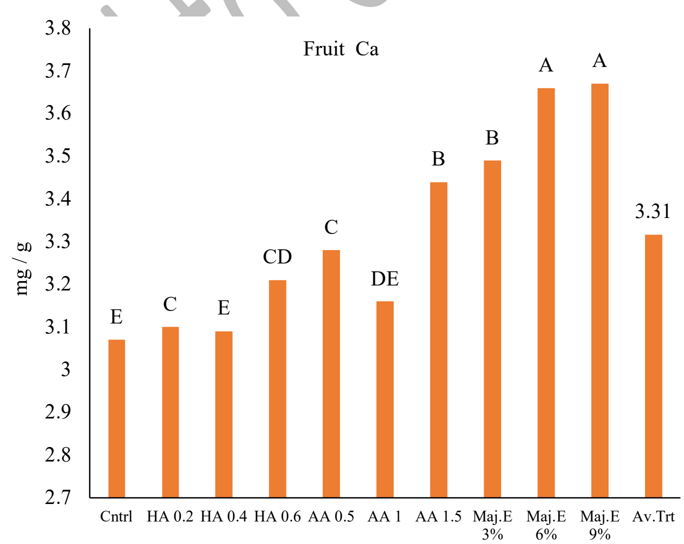

Fig. 3 Effect of humic acid, amino acid and marjoram extract on the fruit calcium content of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

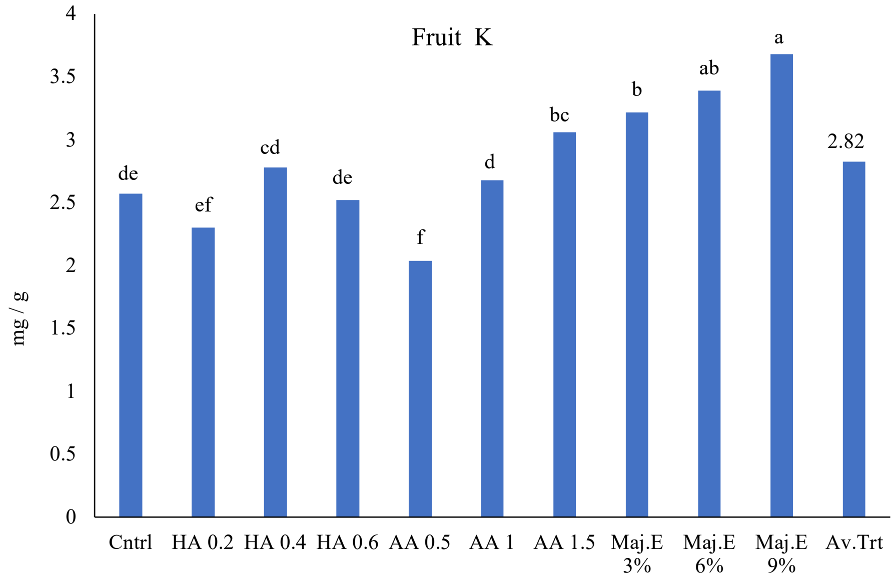

Fig. 4 Effect of humic acid, amino acid and marjoram extract on fruit K content of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

Effects of Treatments on fruit Diameter and Fruits Number per Plant

The results also showed significant differences between the different treatments regarding the fruit diameter trait. Comparison of the results revealed that the highest average fruit diameter (8.9 cm) was obtained with the application of 3% marjoram extract. The lowest average fruit diameter was also observed under the control treatment, with an average of 7 cm. These values were significantly different from each other (Figure 5).

Furthermore, the results regarding the average number of fruits per plant indicated significant differences between the treatments. In this regard, the highest number of fruits per plant (15 fruits/plant) was observed in the amino acid treatment at 0.5 g/L. The lowest number of fruits per plant (7.3 fruits/plant) was observed in the control treatment. Significant differences were observed between these treatments (Figure 6).

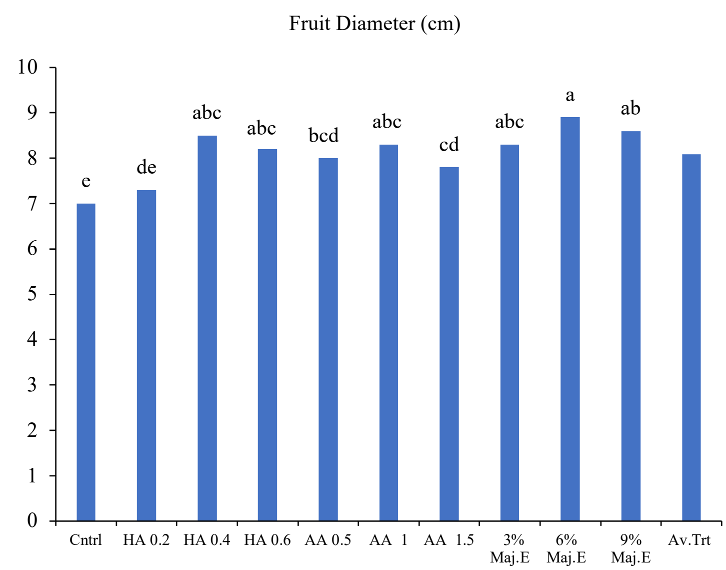

Fig. 5 Effect of humic acid, amino acid and marjoram extract on the fruit diameter of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

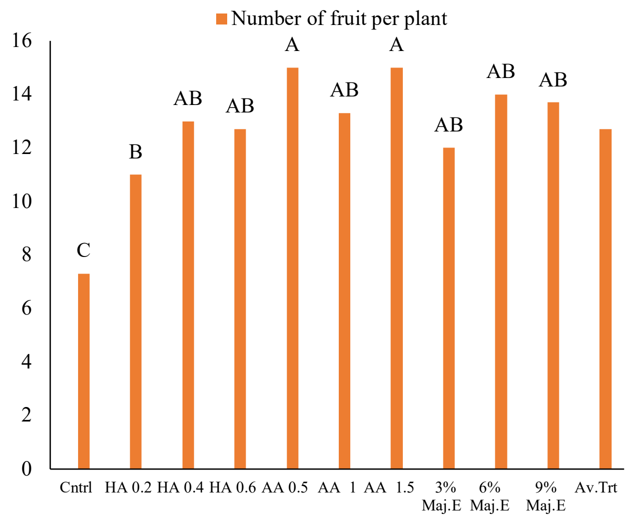

Fig. 6 Effect of humic acid, amino acid and marjoram extract on the fruit number per plant of greenhouse bell pepper.
HA=Humic Acid, AA=Amino Acids and Maj.E=Marjoram Extract.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

Effects of Treatments on Weight loss of Greenhouse Bell Pepper

Weight loss percentage was evaluated using a two-factor factorial design, with Factor A comprising 10 treatments and Factor B consisting of two storage durations (14 and 21 days). The results indicated significant differences between storage durations as well as among treatments. However, since the interaction between storage duration and treatments was not significant, it can be concluded that the treatments exhibited a consistent effect on fruit weight loss across both storage periods. As previously mentioned, the results indicated significant main effects for both treatments and storage duration on fruit weight loss under the specified storage conditions (24°C and 50% relative humidity) (Table 3). In this regard, the lowest fruit weight loss (20.0%) observed using the 9% marjoram and 0.4 g/l humic acid treatments on the 14th day of storage. The highest weight loss (23.3%) was observed in the control treatment on the 14th day of storage. There was significant difference between them. On the 21st day after storage, the lowest fruit weight loss (30.7%) was obtained using 3% marjoram treatment. The highest fruit weight loss (37.3%) was obtained in the control treatment, on the 21st day of storage. Regarding the average percentage of fruit weight loss on the 14th and 21st days of storage, a significant difference was observed (Figure 7).

Table 3 Analysis of variance of data regarding the effect of treatments on the percentage of fruit weight loss during storage.

S.O. V	D.F	S.S	M.S	F. value
(A) Time	1	2112.3	2112.3	578.7 **
(B) Treatment	9	166.3	18.5	5.06 **
(AB) Treatment × Time	9	26.4	2.9	0.80 ns
Error	40	16.0	3.6	
Total	59	2451.0		
C.V%=6.9				

ns, and ** not significant and significant p<0.01, respectively.

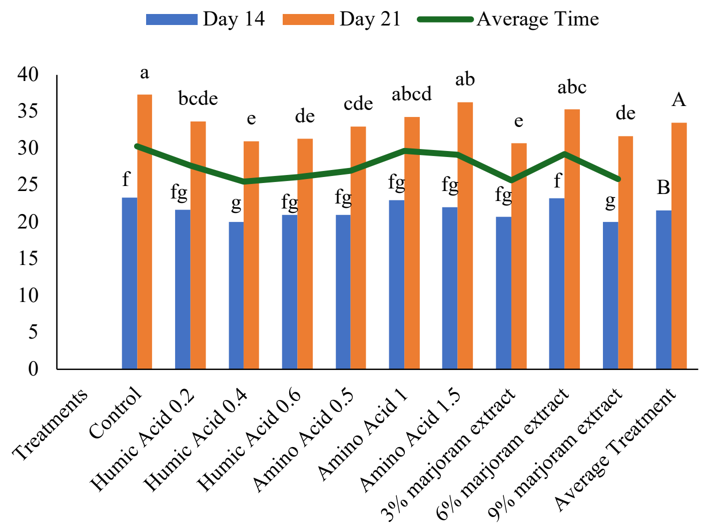

Fig. 7 Interaction of humic acid, amino acid and marjoram extract on the percentage of fruit weight loss on the 14th and 21st days of greenhouse storage of bell pepper.
Means of columns followed by the same letter are not significantly different according to the 5% LSD test.

DISCUSSION

Nitrogen, as a key element in plant growth and development, plays a crucial role in regulating both yield and crop quality [54]. However, excessive nitrogen fertilization not only leads to a waste of resources but also causes a series of environmental issues, including nitrate pollution of groundwater and increased greenhouse gas emissions .The results of this study indicate that the 0.6 g/L humic acid treatment exhibited lower nitrite content compared to the control and other treatments. Furthermore, the 1.5 g/L amino acid treatment resulted in a lower and more favorable nitrate content. In this regard, and considering that the average nitrate content in fruits from humic acid treatments was higher than that from amino acid treatments, it can be inferred that humic acid may influence nitrate uptake through its interaction with plasma membrane H^+-ATPase [42]. Comparison of the average accumulated concentrations of nitrate and nitrite ions across the bell pepper samples revealed that these average concentrations were significantly different from each other. Therefore, considering that nitrogen is a constituent element of amino acids, nucleic acids, purine bases, alkaloids, and chlorophyll [13]. and that vegetable production in greenhouses often involves excessive nitrogen fertilizer (N) application—which represents a pathway for pollutants to enter the food chain [7, 8]. The observed differences necessitate further attention in this regard. Over-application of fertilizers elevates nitrate bioavailability to plants and boosts nitrate leaching losses from the soil profile [18]. Foliar sprays for greenhouse bell peppers incorporating controlled levels of amino acids and humic acids, are suggested as a practice. Considering the critical role of nitrate uptake and distribution in plants, which is influenced by environmental impacts and the quality of agricultural produce [47], it is imperative to prevent the misuse, imbalance, and overuse of nitrogen fertilizers [39]. Research appears limited regarding the uptake and accumulation patterns of nitrate and nitrite in greenhouse bell peppers. This study also advocates for the use of controlled concentrations of amino acids and humic acid in foliar applications for greenhouse bell peppers. Nevertheless, the significance of nitrate dynamics in plants due to environmental issues and product quality means that the common occurrence of nitrate in water supplies, vegetables, fruits, and processed foods, potentially causing negative health outcomes, has escalated into a worldwide public health concern. Consequently, to safeguard and improve food quality, need to rigorously enforce production standards [7]. Therefore, aligning with the study's outcomes, the concurrent application of ammonia-based fertilizers with foliar treatments containing amino acids and humic substances is advised against in colored bell pepper cultivation. The rationale is that nitrification converts ammonia into nitrite, which subsequently tends to accumulate within the fruit tissue. Concerning strategies to mitigate nitrate and nitrite contamination, numerous approaches have been suggested. Yet, elevating the knowledge among farmers and producers regarding the detrimental impacts of these substances stands as the paramount and foundational measure. Moreover, fostering broader public understanding among consumers about the risks associated with nitrate and nitrite could stimulate legislative and practical actions aimed at curbing excessive food contamination and enhancing regulatory oversight by pertinent bodies [2].

In this study, the effects of applying marjoram extract on increasing the calcium and potassium content of fruit, fruit diameter, and the number of fruits in greenhouse bell pepper plants were maximized. The 6% marjoram treatment achieved the highest potassium content and fruit diameter, while the 9% marjoram treatment also showed the highest calcium content and the highest number of fruits per plant, with no significant difference compared to the 1.5 g/L amino acid treatment. These findings align with reports suggesting that marjoram's antioxidant capacity, stemming from its phenolic and methanolic constituents, helps safeguard cellular structures against damage (e.g., lipid peroxidation) and consequently boosts the accumulation of calcium and potassium. These minerals are vital for cell integrity and resilience to stress, a conclusion echoed by Ansar et al. (2020) [5]. Our study further noted that increasing the concentrations of both humic acid and amino acid treatments also elevated the calcium and potassium content within the fruit. Amino acids, fundamental as protein precursors and participants in numerous metabolic pathways [37], are indispensable for plant development. They uniquely influence processes like photosynthesis, act as organic nitrogen sources, and are readily assimilated by plant cells to support protein synthesis and overall growth.

Thus, foliar or root application of liquid amino acid fertilizers can augment the uptake of essential plant nutrients [17], corroborating our experimental observations. Literature also indicates that humic acid positively modulates plant hormone production, nutrient uptake efficiency, protein synthesis, growth dynamics, and overall yield [4]. The presence of functional groups like carboxyl, hydroxyl, ketone, quinoid, and phenolic moieties in humic acid [9], underpins its ability to enhance nutrient absorption and secondary metabolite content e.g., essential oils, [42]. Furthermore, humic acid represents a valuable organic amendment with the capacity to boost agricultural productivity [31], findings entirely consistent with the outcomes of this investigation.

The results of this study indicated that treatments involving marjoram extract minimized fruit weight loss percentages, on days 14 and 21 post-harvest. This observation suggests marjoram's capacity for antioxidant activity and free radical scavenging, consistent with reported properties [14]. The observed antimicrobial (antibacterial, antiseptic, antifungal) properties of sweet marjoram are largely credited to secondary metabolites like thymol and carvacrol. Marjoram's nutritional profile includes notable levels of crude protein (12.80%), crude fat (4.32%), ash (5.62%), crude fiber (19.52%), moisture (5.66%), and carbohydrates [51]. A key bioactive compound, is Carvacrol commonly present in the Lamiaceae plant family is recognized for its diverse pharmacological effects, encompassing anti-inflammatory, antioxidant, antitumor, cytoprotective, and antimicrobial actions [14]. Notable bioactivities attributed to sweet marjoram encompass antibacterial, antioxidant, and antifungal effects [44]. Taxonomically, sweet marjoram (*Origanum majorana*) is classified as an aromatic and medicinal herb within the mint family (Lamiaceae) [1, 53]. Its essential oil composition is rich, featuring terpineol (approx. 29.6%), 2-carene (approx. 20.1%), camphene (approx. 13.4%), and α-pinene (approx. 7.9%), compounds known for their antibacterial properties [12]. Furthermore, marjoram is a significant source of phenolic acids, including gallic, caffeic, dihydroxy (potentially referring to specific compounds like 2,5-dihydroxybenzoic acid), and chlorogenic acids [23, 36 & 45]. The documented presence of these diverse bioactive compounds strongly corroborates the antifungal and antibacterial potential of marjoram extract, findings that are fully consistent with those observed in this research.

CONCLUSION

In this study, the effects of marjoram medicinal plant extract, amino acids, and humic acid on nitrate/nitrite content, and some quantitative and qualitative characteristics, and post-harvest shelf life of greenhouse bell pepper (cv. 'Inspiration') were investigated and evaluated. The results indicated that the 0.6 g/l humic acid treatment resulted in the lowest nitrite levels, while the 1.5 g/l amino acid treatment achieved the lowest fruit nitrate levels. Marjoram extract yielded the most outstanding results in enhancing fruit calcium and potassium content, fruit diameter, and the number of fruits per plant. Additionally, the antifungal and antibacterial effects of marjoram extract, along with its positive impact on reducing post-harvest fruit weight loss percentage, were well substantiated. Considering the cost-effectiveness of Marjoram extract relative to humic and amino acid-based fertilizers, as well as other synthetic alternatives, this study offers significant insights into minimizing production expenses while promoting a reduction in chemical fertilizer consumption. Finally, it is recommended that future researchers conduct similar studies on other plant species to further investigate this subject.

Consent for Publication

The authors give their consent for the publication of their information in the Journal of Medicinal Plants and By-products (JMPB).

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict Of Interest

The authors declare that they have no conflict of interest.

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Authors' contributions

The authors declare that they were involved in all stages of the design and implementation of this study and are fully aware of the research process. The manuscript has also been reviewed by all authors, and all of them have read and approved the final version.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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