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The Impact of Certain Growth Treatments on Some Physiological Traits and Vitamin C Content in the Nirvin Pepper Cultivar

PEZHMAN HADIYAN[1], MEHRDAD JAFARPOUR[2*]

1-MSc., Department of Horticultural Science, Isfahan(khorasgan) Branch, Islamic Azad University, Isfahan, Iran

2- Associate Professor, Edible and Medicinal Mushroom Research Center and Department of Horticultural Science, Isfahan(khorasgan) Branch, Islamic Azad University, Isfahan, Iran

[*] Corresponding author E-mail: Mehrdad.jafarpour@gmail.com

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ABSTRACT

Capsicum annuum L. from the Solanaceae family is one of the most important and widely consumed vegetables in the world due to its valuable nutritional content. This study investigates the effects of growth-promoting treatments on certain growth characteristics and vitamin C content in the Nirvin pepper cultivar. The treatments included wood vinegar (1.25- 2.5– 3.33 ml per liter), biochar (100–200-300 g per plant), gibberellic acid (25–50-75 ml per liter), Crecer organic fertilizer (2.5–5-7.5 ml per liter), humic acid (150– 200- 250 mg per liter), fulvic acid (5– 6.5- 8 g per liter), and a mixture of fulvic acid + humic acid (5- 6.5- 8 g and 150– 200- 250 mg per liter, respectively). These were applied in 84 plots (each with two plants), using a randomized complete block design with three replications, alongside a control treatment (distilled water spray). The results showed that growth regulators significantly influenced the number of flowers, fruit diameter, pedicel length, and vitamin C content in bell peppers. The best results in terms of overall physiological traits and vitamin C content were achieved with applying fulvic acid and gibberellic acid. Although other treatments also showed a significant positive effect, leading to an increase in most traits compared to the control, they indicated a clear and substantial impact on growth characteristics and vitamin C content in bell peppers. Therefore, the use of growth regulators is recommended to enhance the quantity and vitamin C content of bell peppers**.**

Keywords:Wood vinegar, Biochar, Gibberellic acid, Humic acid, Fulvic acid

INTRODUCTION

One of the major challenges facing the export of agricultural products from Iran to other countries is the low quality of these products, particularly in terms of post-harvest physiology, shelf life, and, more importantly, the poor nutritional value of the products due to the excessive use of chemicals during production. Consequently, the presence of illegal and chemical substances in agricultural products imposes significant health and social costs on consumers each year (Ghorbani *et al*., 2009). Organic farming, which is a key component of sustainable agriculture, has gained widespread acceptance in recent decades, primarily due to global concerns about health, food security, and environmental sustainability. In this context, international support has played a crucial role in promoting this culture (Biao *et al*., 2003).

Capsicum annuum L., from the Solanaceae family (a commonly consumed vegetable in tropical and subtropical regions), is a rich source of essential vitamins and minerals. It is also high in antioxidants and vital compounds such as vitamin C, carotenoids, phenolic compounds, and potassium, which together contribute to the nutritional value of peppers (Daneshvar, 2008). Pepper production ranks third among vegetables in developing countries, after potatoes and tomatoes. The global production of peppers, growing at an annual rate of 5%, is approximately 28.4 million tons from 3.3 million hectares of land, both for dried and fresh consumption (FAO, 2007). Based on the above, it is essential to find ways to produce this crop that are not only safe but also increase its quantity and quality.

Pyrolysis is a process that has been widely used for centuries as an alternative method for managing a wide range of organic waste materials (Shinogi & Kanri, 2003). During this process, organic materials burn slowly and steadily in the absence of oxygen or in low-oxygen conditions (Abdel-fattah *et al*., 2015), reducing the weight, volume, and unpleasant odors of biomass, thus making production easier (Shinogi & Kanri, 2003). Specifically, pyrolysis acid and biochar, produced from the pyrolysis process, have received considerable attention due to their value and usefulness (Oh *et al*., 2012).

Pyrolysis acid (wood vinegar) is a dark brown liquid (reddish-brown) produced by condensing the vapors and smoke from the thermal pyrolysis of wood or any lignocellulosic material (Firouzbehi *et al*., 2020). In other words, wood vinegar is a brown (reddish) liquid with a strong odor, produced by the distillation of gases from the burning of wood in an anaerobic environment (Nurhayati *et al*., 2005). The primary components of wood vinegar are methanol and acetic acid. Other components include acetone, methyl acetate, aldehyde, allyl alcohol, furans, furfural, and formic, propionic, and butyric acids. Wood vinegar also has antioxidant and antifungal properties, is easily produced, and does not have a harmful or destructive impact on the environment (Tiilikkala *et al*., 2010). Another study mentions that the most important components of pyrolytic lignin acid are methanol and acetic acid, along with methyl acetate, acetone, aldehyde, furfural, formic acid, propionic acid, and butyric acid. Additionally, wood vinegar contains 15 elements, including both macro and micro-elements such as calcium, chromium, cadmium, potassium, iron, copper, sodium, manganese, aluminum, zinc, arsenic, phosphorus, molybdenum, boron, and lead. Many of these elements play a vital role in the plant's essential processes, particularly photosynthesis (Zulkarami *et* *al*., 2011). This material, in addition to its use in combating fungi and insects, also plays an important role in enhancing plant growth and productivity. Other applications of this compound include improving soil structure, controlling plant growth, accelerating root development, and enhancing stem, leaf, flower, and fruit development, as well as increasing the yield of orchards. Furthermore, using wood vinegar in livestock farms, diluted at a 1:50 ratio, helps eliminate unpleasant odors from the environment (Brown *et al*., 2003). On the other hand, one of the positive effects of wood vinegar is its impact on plant growth and yield, attributed to the presence of compounds such as methanol and furfural (Nurhayati *et al*., 2005).

Biochar, another compound produced from pyrolysis, is made from charcoal derived from wood waste, agricultural residues, and biomass, which are used as fertilizers. Soil can retain biochar for thousands of years. This material contains various amounts of carbon, hydrogen, nitrogen, oxygen, sulfur, and ash (Smider & Singh, 2014). Pyrolysis of these materials produces energy and biochar, which contains relatively high levels of plant nutrients such as phosphorus, potassium, magnesium, and calcium. Adding biochar to soil, by absorbing soluble organic carbon, changes the chemical composition of the soil (Pitikainen *et al*., 2000). Biochar has several positive effects on agricultural soils, including increasing soil organic matter, improving water retention capacity, enhancing cation exchange capacity, modifying soil pH for better nutrient cycling, reducing nutrient leaching from the soil, and decreasing water and fertilizer requirements (Mia *et al*., 2014; Rondon *et al*., 2007; Nishio, 1996).

Organic fertilizers, due to their organic content, have numerous beneficial effects on the physical, chemical, biological properties, and fertility of the soil. Therefore, they are considered a key factor in soil fertility and productivity. The use of organic fertilizers increases soil organic matter and humus, improves chemical properties such as soil pH, cation exchange capacity, enhances the efficiency of soil microorganisms, and increases nutrient availability, ultimately boosting soil fertility and plant yield (Renato *et al*., 2003).

Gibberellic acid (GA), one of the most important plant growth regulators, plays a key role in various physiological processes in plants. This hormone affects cell elongation, flowering induction, parthenocarpic fruit production (seedless fruits), root elongation, leaf growth, seed germination, and improves both the quantity and quality of fruits. GA also delays fruit ripening and increases the internodal distance (Jones & Carbonell, 1984). Studies have shown that gibberellic acid improves fruit size and yield by promoting more efficient use of nutrients for reproductive growth (flowering and fruiting), enhancing photosynthetic efficiency, reducing respiration, increasing sugar transport and accumulation, and supporting other metabolic processes (Georgi *et al*., 2010; Naeem *et al*., 2001; Ouzounidou *et al*., 2008).

Free radicals (reactive oxygen species) possess one or more unpaired electrons, which allows them to damage and disrupt important biological molecules such as proteins, nucleic acids, carbohydrates, and lipids (Nanosombat & Wimuttigosol, 2011). Antioxidants are compounds that inhibit free radicals. They neutralize oxidizing agents by transferring hydrogen atoms, chelate metals, and catalytically reduce oxidation reactions, thus protecting biological molecules from degradation. Antioxidants used in the food industry are available both naturally and synthetically (Khalili & Ebrahimzadeh, 2014).

Vegetables are among the most important sources of carbohydrates and proteins, rich in vitamins and minerals (Dehghan Tanha *et al*., 2018). Various antioxidant compounds, such as phenolic compounds, vitamin C, and carotenoids, are found in pepper fruits. After parsley, bell peppers have the highest amount of vitamin C among vegetables, with a single medium-sized pepper providing twice the daily requirement of vitamin C. Therefore, in terms of improving the body's relative resistance and nutritional physiology, bell peppers are highly nutritious and possess numerous medicinal and culinary properties. They can be consumed fresh or used as a condiment in pickles, brine, and stuffed dishes (Hallmann & Rembialkowska, 2012). Other reports confirm that peppers contain significant compounds such as carotenoids, vitamins A and C, and small amounts of essential oils, which also act as strong antioxidants and help neutralize free radicals (Shariati *et al*., 2010). Regular consumption of red bell peppers reduces serum triglycerides and glucose levels while providing twice the daily requirement of vitamin C (Babaei Garmkhany *et al*., 2015; Shamsaie Mehrgan, 2019).

Methodology

This study was conducted in the years 2024-2025 in a greenhouse located in the cities of Tiran and Karun, with geographical coordinates of longitude 50.9 and latitude 32.8. The plants under study were 5-month-old red bell pepper plants of the Nirvin variety from Netherlands. The research involved 7 treatments at three levels (concentrations), resulting in 21 treatments. Each treatment was replicated 3 times, resulting in 84 two-plant plots, evaluated using a randomized complete block design. The treatments were as follows: biochar (BC) at 100, 200, and 300 grams per plant; wood vinegar (WV) at 1.25, 2.5, and 3.33 milliliters per liter of water; gibberellic acid (GA) at 25, 50, and 75 milligrams per liter of water; organic fertilizer (C) at 2.5, 5, and 7.5 milliliters per liter of water; humic acid (HA) at 150, 200, and 250 milligrams per liter of water; fulvic acid (FA) at 5, 6.5, and 8 grams per liter of water; and a combined treatment of fulvic acid with humic acid (FAHA), in the proportions of 5g + 150mg, 6.5g + 200mg, and 8g + 250mg per liter of water (with grams referring to fulvic acid and milligrams referring to humic acid). All treatments, except biochar (which was mixed with the soil), were applied as foliar sprays. A control group, where distilled water was sprayed on the plants, was also included. To measure the exact amounts of materials used in the study, a digital scale with milligram precision was used to weigh and categorize all materials. A 2.5 or 5 milliliter syringe was used to collect liquid materials. To prepare the solutions, water at an appropriate temperature (approximately 20°C) was mixed with the required amount of each substance in 100 milliliters of water. The mixture was then transferred to a 2-liter sprayer, making up to 1 liter of solution. Irrigation was done before the foliar spray, which was performed in three rounds at 15-day intervals during calm hours. Both the upper and lower surfaces of the leaves, which contain the plant's stomata, and the green branches were thoroughly sprayed until the solution droplets began to drip. One liter of the solution was sufficient for each treatment across four replicates (8 5-month-old pepper plants).

The traits studied included the number of flowers, fruit diameter, pedicel length, and vitamin C content in the fruit. The number of flowers was counted manually, while the fruit diameter and pedicel length were measured using calipers. To measure vitamin C, fruit samples from each replicate were individually packaged and sent to the Horticulture Laboratory of the Islamic Azad University, Isfahan (Khorasgan) campus for analysis.

Vitamin C Measurement Method

First, 0.5 grams of the pepper fruit tissue was ground in a mortar and then mixed with 5 milliliters of 5% TCA (trichloroacetic acid) for 5 minutes. The mixture was then filtered, and 3 milliliters of the filtrate were taken from each sample. The sample was centrifuged at 9000 rpm for 10 minutes at 4°C. Then, the extract was mixed in a 1:3 ratio with 2,6-dichloroindophenol and its absorbance was measured at a wavelength of 520 nm.

RESULTS

Counting and measuring of physiological traits, along with fruit sampling for vitamin C analysis, were conducted.

Measured Traits

The results obtained from statistical analyses, based on the LSD test, are presented in Table (1) and Figures (1), (2), (3), and (4).

Table 1. results of variance analysis of treatment effect on vitamin C and some growth characteristics of sweet pepper

Mean square					
petiole length	Fruit diameter	number of flowers	Vitamin C	Degree of freedom	Source of variation
0.051^{ns}	0.186^{ns}	90.14^{ns}	0.00004^{ns}	2	Block
0.689^{**}	0.399^{ns}	80.86^{**}	0.00101^{*}	21	Treatment
0.139	0.238	30.12	0.00052	42	Error
8.17	5.96	12.18	23.47		Coefficient of variance (CV)

*and **: respectively, becoming significant at the statistical level of 5 and 1 percent.

Vitamin C

The average vitamin C content under different treatment sprays is shown in Table (1) and Figure (1). The analysis of variance indicates that the effect of various treatments on the vitamin C content of red bell pepper fruit was significant at the 5% probability level (Table 1).

Additionally, a comparison of the means revealed that the highest vitamin C content in bell pepper fruit was observed in the fulvic acid treatment at a concentration of 8 grams per liter, with a value of 0.107 mg per 100 grams fresh weight. The lowest vitamin C content was found in the biochar treatment at a concentration of 100 grams per plant, the gibberellic acid treatment at a concentration of 50 milligrams per liter, both showing 0.023 mg, and the wood vinegar treatment at a concentration of 1.25 milliliters per liter, with 0.024 mg per 100 grams fresh weight. According to the results, other treatments did not show significant differences from the control treatment (Figure 1).

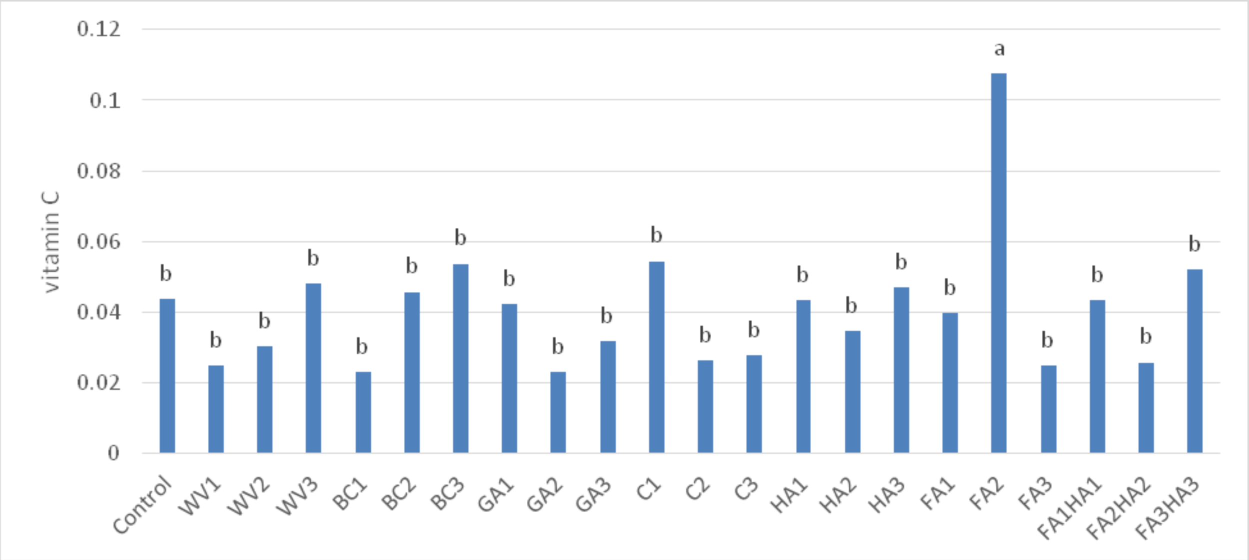

Figure 1. Comparison of the average amount of vitamin C in bell peppers in terms of mg/100g FW in different treatments

WV: Wood vinager, BC: Biochar, GA: Gibberellic acid, C: Crecer, HA: Humic acid, FA: Fulvic acid, FAHA: Fulvic acid+ Humic acid

The amount of vitamin C in vegetables for human consumption varies greatly. In one study, it was observed that the highest amount of vitamin C (more than 2 mg per gram FW) in red peppers was recorded, indicating very high levels of ascorbic acid among 66 vegetables (Isabelle *et al*., 2010). However, the production of ascorbic acid, provitamins, proteins, and some minerals is influenced by genotypes and color stages. Ascorbic acid increased in some varieties with the development of color, but in others, it remained unchanged or decreased. Black, purple, and white peppers contained lower levels of ascorbic acid than green, yellow, red, brown, or orange peppers (Simonne *et al*., 1997). Bell peppers vary in color depending on their maturity, ranging from green to yellow, orange, and red. Differences in color also lead to variations in vitamin C content (Nerdy, 2018). Research results showed that the application of fulvic acid as a biostimulant at a concentration of 5.5 milliliters per liter, by promoting fruit maturation, increased vitamin C in bell pepper plants (Kanabar *et al*., 2024). An increase in vitamin C after the application of fulvic acid in pomegranate has also been reported (Sarkar *et al*., 2019). Higher levels of ascorbic acid during the red stage are associated with carbohydrate metabolism (Martínez *et al*., 2005). This mechanism plays a crucial role in providing energy and the necessary compounds for ascorbic acid production. Studies have shown that as fruit matures, ascorbic acid synthesis increases, which is influenced by genetic and environmental factors (Zheng *et al*., 2022; Liao *et al*., 2023). On the other hand, ascorbic acid is one of the water-soluble antioxidants that plays an important role in neutralizing free radicals, particularly hydrogen peroxide. It also directly neutralizes reactive oxygen species such as superoxides and assists in regenerating alpha-tocopherol and other fat-soluble antioxidants (Noctor & Foyer, 1998). Humic acid compounds and their derivatives can generate free radicals that act as signaling messengers within the cell, inducing physiological effects (Cordeiro *et al*., 2011). Therefore, it is likely that humic acid compounds and derivatives such as fulvic acid can induce oxidative stress, leading to an increase in antioxidants such as vitamin C (Sarkar *et al*., 2019). Considering the biosynthesis pathway of ascorbic acid in higher plants (Barata-Soares *et al*., 2004) and the involvement of specific enzymes like glucose-6-phosphate isomerase in this process, it is likely that fulvic acid enhances the activity of these enzymes. Thus, it can be inferred that the application of fulvic acid probably increases vitamin C production by stimulating fruit maturation in bell peppers, which aligns with the findings of this study. Overall, the results suggest that the application of fulvic acid is effective in increasing the vitamin C content in bell pepper fruit.

Flower Count

The average flower count under different treatments is shown in Table (1) and Figure (2). The analysis of variance revealed that the effect of the various treatments on flower count was significant at the 1% level (Table 1).

A comparison of the mean data showed that the highest number of flowers (34.33) was observed in the gibberellic acid treatment at a concentration of 75 milligrams per liter, while the lowest number (12) was found in the control treatment (distilled water). Most treatments showed a significant increase in flower count compared to the control (Figure 2).

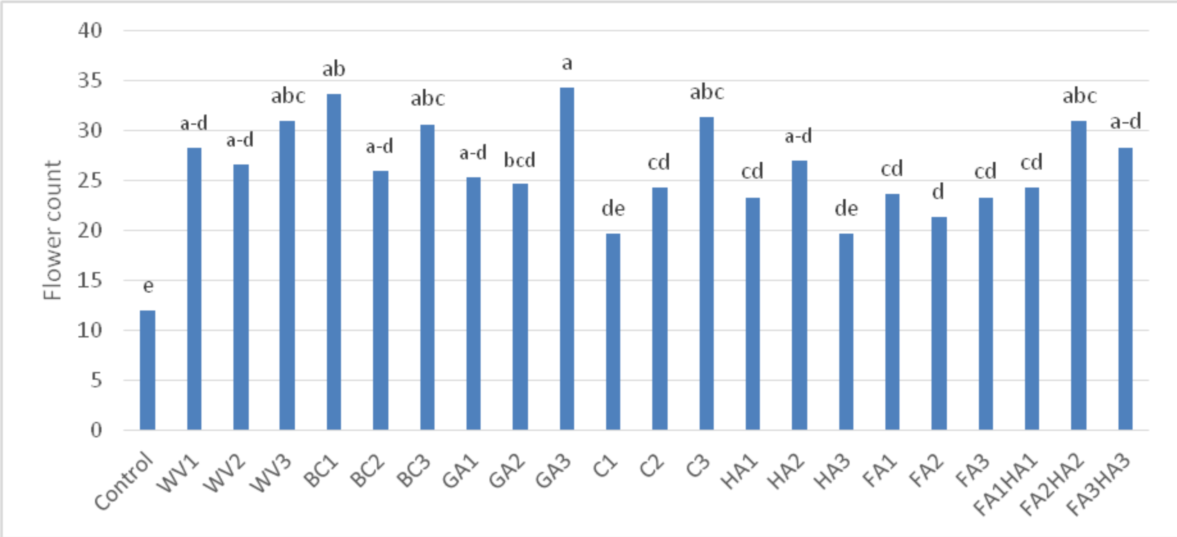

Figure 2. Comparison of the flower count in different treatments

WV: Wood vinager, BC: Biochar, GA: Gibberellic acid, C: Crecer, HA: Humic acid, FA: Fulvic acid, FAHA: Fulvic acid+ Humic acid

The positive effect of gibberellic acid on increasing the yield and flower count of bell peppers has been proven (Batalang, 2008; Belakbir *et al*., 1998). Reports indicate that plant growth regulators are organic compounds that alter plant physiology. They play an important role in enhancing plant growth and quality, increasing stem length, and flower production. Furthermore, they influence vegetative and fruit production (Ouzounidou *et al*., 2008). Another study showed that growth regulators increased the number, size, and weight of sweet pepper fruits (Sarkar, 2015). Gibberellic acid, by utilizing more nutrients for reproductive growth, such as flowering and fruit production, improving photosynthesis efficiency, reducing respiration rate, and increasing sugar accumulation and transfer, enhances vegetative, reproductive growth, and overall yield (Georgi *et al*., 2010; Ouzounidou *et al*., 2008). These findings align with the results obtained in this study.

Fruit Diameter

The average fruit diameter under different treatments is shown in Table (1) and Figure (3). The analysis of variance revealed that the effect of various treatments on fruit diameter was insignificant across the different treatments (Table 1).

A comparison of the mean results indicated that the largest fruit diameter was observed in the fulvic acid treatment at a concentration of 5 grams per liter (8.69 cm) and the wood vinegar treatment at a concentration of 2.5 milliliters per liter (8.675 cm). The smallest diameter was found in the corsair treatment at a concentration of 2.5 milliliters per liter (7.51 cm) and in the fulvic acid treatment at a concentration of 5.6 grams per liter (7.52 cm). Most treatments did not show significant differences compared to the control (Figure 3).

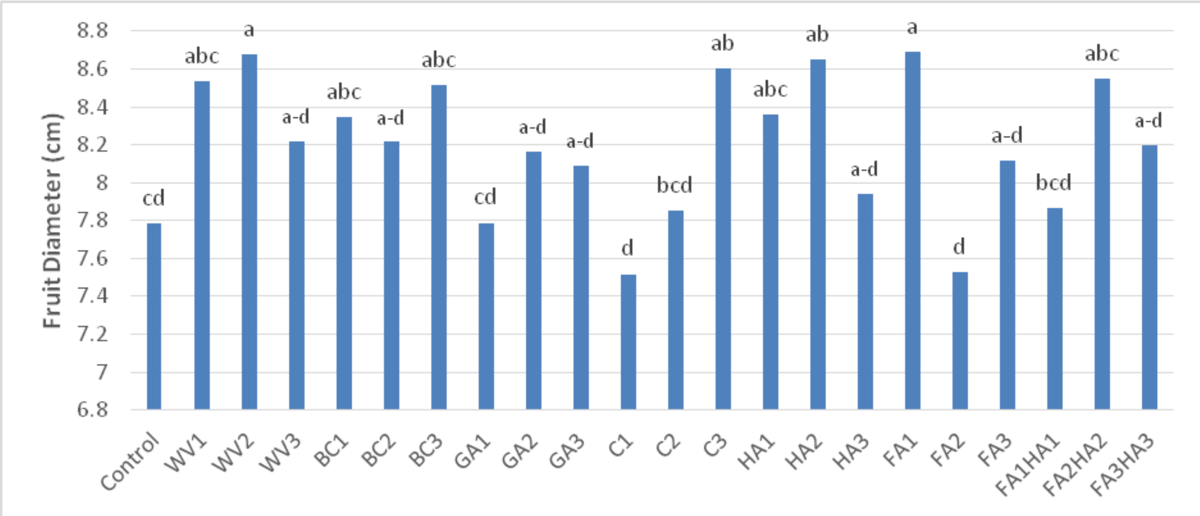

Figure 3. Comparison of average fruit diameter in different treatments (cm)

WV: Wood vinager, BC: Biochar, GA: Gibberellic acid, C: Crecer, HA: Humic acid, FA: Fulvic acid, FAHA: Fulvic acid+ Humic acid

According to researchers, the application of fulvic acid in peppers resulted in an increase in fruit diameter (Naderi *et al*., 2022). Additionally, researchers reported the positive effect of fulvic acid on increasing fruit diameter in peppers based on a study conducted in Tunisia (Kanabar & Nandwani, 2023). The above results align with the data obtained in this study. Fulvic acid has a low molecular weight and is biologically active due to its small molecular size. It facilitates the dissolution and mobility of minerals within its molecular structure, making it easier for these minerals to bind with elements. Humic acid and its derivatives are rich in carboxyl, hydroxyl, and carbonyl groups, as well as phenols, quinones, and semi-quinones (Bravo, 1998; Yoshino & Murakami, 1998), and directly stimulate growth by increasing the absorption of certain micronutrients such as iron, zinc, and manganese (Chen & Aviad, 1990).

Peduncle Length

The average peduncle length under different treatments is shown in Table (1) and Figure (4). The analysis of variance revealed that the effect of various treatments on peduncle length was significant at the 1% level (Table 1).

A comparison of the mean data showed that the longest peduncle length was observed in the gibberellic acid treatment at a concentration of 50 milligrams per liter (5.76 cm), while the shortest length was found in the biochar treatment with a concentration of 100 grams per plant (3.84 cm). No significant differences were observed in most of the treatments (Figure 4).

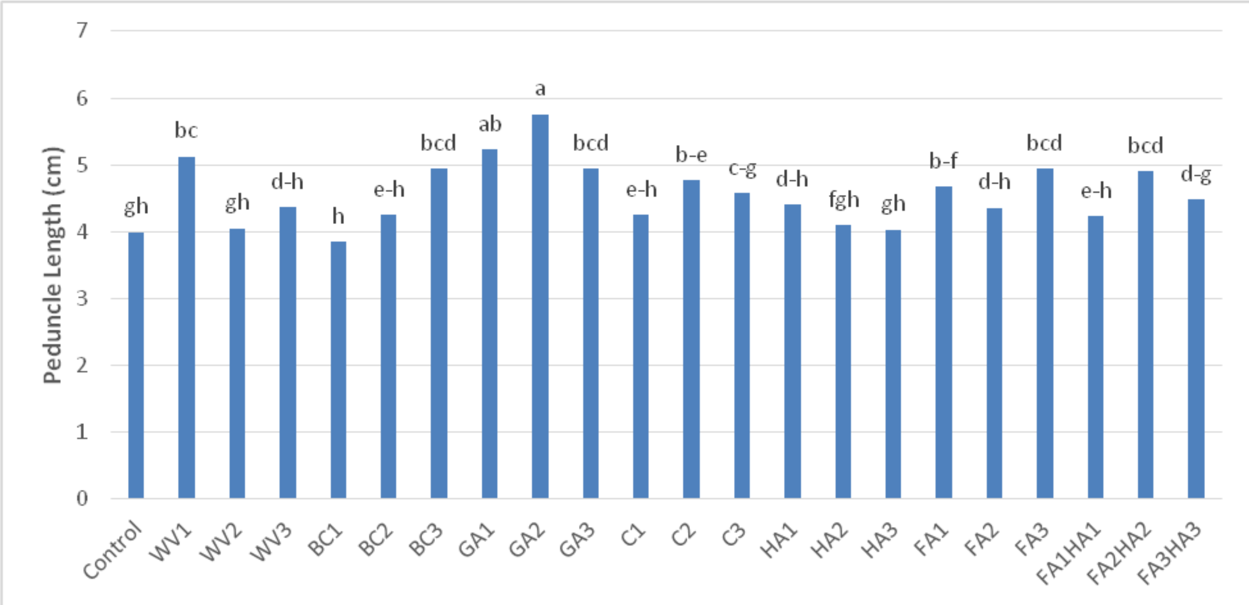

Figure 4. comparison of the average Peduncle Length in different treatments (cm)

WV: Wood vinager, BC: Biochar, GA: Gibberellic acid, C: Crecer, HA: Humic acid, FA: Fulvic acid, FAHA: Fulvic acid+ Humic acid

Reports indicate that the application of gibberellic acid on bell peppers enhances plant growth and development, leaf area, internodal distance, fruit growth, fruit quality, and reduces damage caused by biological stress factors (Georgi *et al*., 2010; Belakbir *et al*., 1998). An increase in internodal distance following the use of organic fertilizers has been reported in plants such as fenugreek (Alaghemand *et al*., 2017) and bread wheat (Navabpour *et al*., 2018). One of the main reasons for increased plant height after the application of various fertilizers is typically related to the increase in internodal distance, which may be due to the stimulation of plant growth hormone production as a result of fertilizer application (Nagananda *et al*., 2010). Research shows that elements like phosphorus and nitrogen are effective in increasing internodal distance (Navabpour *et al*., 2018). Since the organic fertilizer “Crecer” contains appropriate amounts of essential nutrients, it can influence the increase in internodal distance, which aligns with findings from other researchers.

CONCLUSION

Based on the results of this study, it was determined that growth stimulants affect the number of flowers, fruit diameter, pedicel length, and vitamin C content in bell peppers. The application of growth stimulants led to increased growth and development, yield, and vitamin C levels, likely due to the enhanced nutrient availability and improved metabolic processes in the plant. The best results in terms of physiological traits and vitamin C content were achieved following the application of fulvic acid and gibberellic acid. Although the results of other treatments and the increasing trends in most traits compared to the control treatment were significant, indicating their positive impact on growth and vitamin C content in bell peppers. Therefore, the use of growth stimulants to enhance the quantity and vitamin C content of bell peppers is recommended.

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