Journal of Kerbala for Agricultural Sciences Issue (4), Volume (11), (2024)

Impact of inorganic fertilizer doses on growth, yield, physical and chemical components of broccoli plants

Nyan Tahir Mohammed[1], Hawar Sleman Halshoy[2*], Naz Fayaq Saed[3], Heshw Wafa Rashid Ali[4], Nask Ibrahim Mohammed[1], Sawsan Mohammed Ali[2]

[1] Horticulture Department, Directorate of Agricultural Research, Sulaymaniyah, Kurdistan Region, Iraq.
[2] Horticulture Department, College of Agricultural Engineering Sciences, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq.
[3] Soil and Water Department, Directorate of Agricultural Research, Sulaymaniyah, Kurdistan Region, Iraq.
[4] Horticulture Department, College of Agricultura, University of Kirkuk, Kirkuk, Iraq.
***Corresponding author e-mail: hawar.hama@univsul.edu.iq, hawarhalsho@gmail.com**
https://doi.org/10.59658/jkas.v11i4.2791

Received: July 03, 2024

Accepted: Aug. 15, 2024

Published: Dec. 15, 2024

Abstract

Due to its high nutritional value and secondary metabolites, broccoli is one of the most significant vegetable crops globally. Thus, a greenhouse experiment was conducted to determine how the application of inorganic fertilizer impacts the plant growth and internal quality of broccoli plants. NPK fertilizer was applied at different levels (0, 300, 500, and 700 kg ha^{-1}) three times at 14-day intervals after 21 days of transplanting. Results showed that 700 kg ha^{-1} NPK significantly improved some morphological characteristics, yield components, and total polyphenolic content (TPC) (93.89 µg GAE g^{-1} FW). 500 kg ha^{-1} provided the best value for leaf chlorophyll intensity (82.96 SPAD), TSS (11.57 ˚Brix), ascorbic acid (107.33 mg $100g^{-1}$ FW), and the highest rate of nitrate accumulation (502.33 ppm). Moreover, 300 kg ha^{-1} NPK produced the maximum results for leaf dry matter (20.27%), lateral head weight (354.33 g), zinc (551.58 ppm), manganese (11.29 ppm), and iron (41.17 ppm). Conversely, the control plants recorded the minimum results in this study except for total antioxidant activity (TAA) (70.50%) and copper (7.05 ppm). The results of this research could assist growers in determining the optimal amounts of NPK application for their broccoli plants or other crops while maintaining quality in greenhouses

Keywords: *Brassica oleracea* var. italica, Phytochemicals, Vitamin C, Nitrate, Heavy Metals, NPK

Introduction

Broccoli (*Brassica oleracea* var. italica) is one of the edible vegetables belonging to the Brassicaceae family [1]. This crop is of significant importance because of its excellent nutritional and economic value [2, 3]. Broccoli gained increased attention because of its diverse applications and impressive nutritional benefits; well-known for its abundance of minerals and extensive selection of bioactive compounds (non-enzymatic) with health benefits [4, 5]. Phenolic acids, carotenoids, glucosinolates, vitamins, and selenium are among the bioactive substances that demand particular attention. Also, it had the greatest antioxidant capacities and was the richest supplier of kaempferol and quercetin glucosides, as well as chlorophyll [6]. Another benefit of broccoli is its ability to collect heavy metals. A daily intake of 150 g of broccoli meets an adult's vitamin E, A, B1, and C needs while also boosting the immune system [7, 8]. Despite the lack of insight into the process, the findings demonstrate that even a brief dietary therapy with broccoli sprouts may significantly reduce oxidative stress and cell death in the heart due to ischemia-reperfusion [9].

Moreover, providing adequate food for the quickly growing population is a significant obstacle, but it can be accomplished by boosting both the quantity and quality of crop production. However, food security objectives cannot be met unless vital plant nutrients are available [10]. Nowadays, crop productivity in the developing world is limited by various factors, and a significant challenge is the insufficient supply of essential crop nutrients in the appropriate amounts and forms [11]. Sustainable agriculture depends on the availability of plant nutrients, which play an important function in achieving optimal plant growth and production. These essential nutrients must be present in balanced proportions and sufficient quantities. Chemical fertilizers are one of the most effective sources of readily available nutrients that can help maintain a favorable nutrient balance [12].

In this regard, when cultivating broccoli in recently reclaimed soils, several challenges are encountered, including insufficient availability of essential nutrients. The compensation for this, the optimal level of macronutrient mineral fertilizer must be added to the soil to replenish and maintain its fertility, thereby promoting productivity. It has been mentioned that plants need macronutrients for growth and development. Nitrogen, for example, encourages vegetative growth [13], while phosphorus, on the other hand, promotes root growth and provides energy by producing ATP [14, 15]. Additionally, potassium is essential for osmotic control, enzyme activation, and glucose metabolism [16]. In addition, the growing of broccoli is influenced by various factors, with fertilizer application being the most significant. Adequate management of macronutrients, for instance, NPK is critical for achieving high yields and internal quality, as well as sufficient for the marketability of the crop of broccoli. Other researchers also indicated the different doses and fertilizer effects on broccoli characteristics [17]. Thus, the objective of this research is to investigate the impact of NPK at different doses on both the produce and internal quality of broccoli, as well as to identify the optimal NPK dosage for effective broccoli cultivation under greenhouse conditions.

Materials and Methods

Experimental design

The experimental study was carried out under greenhouse conditions at Bakrajo Agriculture Research Center (N35 ˚54’, E45 ˚ 35’), Directorate of Agriculture Research, Sulaymaniyah, Kurdistan Region, Iraq. Randomized Complete Block Design (RCBD) was used, and the multi-span structures were 18 m in width, 24 m in length, and 4 m in height. They were covered with clear color and a double layer of transparent polyethylene plastic. The research was started by plowing, leveling, and then plotting the soil inside the multi-span. The ground was divided into three terraces, and a uniform drip irrigation system was set on them. The soil texture was identified as silty-clay (175 g kg^{-1} sand, 405 g kg^{-1} silt, and 420 g kg^{-1} clay), with a pH of 7.84, electrical conductivity of 0.48 ds m^{-1}, organic matter content at 1.59%, calcium carbonate content at 24%, total nitrogen at 0.16%, available phosphorus at 37.14 ppm, and soluble potassium at 0.207 meq L^{-1}.

Plant Materials

The broccoli seedlings were planted with three replications per treatment. In each replication, 7 seedlings were planted in terraces 60 cm wide, and the distance between each two terraces was one meter. The replications were divided and arranged on four different levels of NPK balance (15:15:15) at 0, 300, 500, and 700 kg ha^{-1}. The fertilizer was applied three times at 14-day intervals via soil applications after 21 transplants. The content of fertilizer was total nitrogen 15% (NH_4 9% and NO_3 6%), total phosphorus (P_2O_5) 15% and total potassium (K_2O) 15%.

Data collection

During this study, several parameters were measured by using different instruments, such as plant height (cm), stem diameter (mm), plant leaf number, plant branch number, leaf dry matter (%), lateral head diameter, main head diameter (mm), lateral head weight (g), main head weight (g), plant fruit weight (kg $plant^{-1}$), fruit dry matter (%). Leaf chlorophyll intensity (SPAD) was measured as SPAD units using a Monitor chlorophyll meter (SPAD 502 PLUS). In addition, leaf and fruit dry matter content (DM %) was measured by taking the fresh weight of plant material (Wb) and dried at 65˚C in a forced-air oven for 72 hr until the weight was stable, then it was weighed again (Wa) and dry matter percentage was calculated [18] using the following equation $DM\% = \frac{Wa}{Wb} \times 100$.

Vitamin C determination

The content was measured by titration, where 10 mL of the fruit extract was titrated against 0.1 N of dichlorophenolindophenol (DCPIP), using a methyl-red-indicator. The volume of DCPIP was recorded and used in the following equation to calculate the vitamin C contents [19]. The result was expressed as ascorbic acid mg $100g^{-1}$ FW.

$$\text{Ascorbic acid mg } 100\text{g} - 1 \text{ FW} = \frac{\text{Volume of DCPIP (mL) x normality (0.1) x equivalent weight x dilution factor}}{\text{Volume or weight of the fruit extract}}$$

Plant fruit extracts

For determining TPC and TAA in the fruit, the method of [20] was used for preparing the fruit sample extracts by the following methods: fruit samples were snap-frozen by using liquid nitrogen. Pestle and mortar were used to pulverize frozen samples. After that, 1 g of ground powders were taken and placed in 15 mL tubes, then 10 mL of 80% methanol was added into the tubes and shaken in a water bath for 3 hours at 38 ˚C. The mixture was centrifuged at 5000 rpm for 15 minutes at 4 °C. The upper layer (extracts) was transferred into new tubes and they were kept in a refrigerator at 4 °C as a crude extracted solution for non-enzymatic antioxidants analysis.

Total polyphenol content (TPC)

The TPC of each extract was calculated using a modified version of the Folin-Ciocalteu technique [21]. In brief, 4 mL of the Folin-Cioculate reagent (Thomasbaker, India) was added to 100 µL of each extract, and the mixture was left to react at room temperature for 5 minutes. Then, 50 minutes were spent in the dark at 38 °C after adding 2 mL of a 20% Na_2CO_3 solution. For the blank, we used the identical protocol as before; only we substituted 100 µL of distilled water for the sample extracts at each stage. A spectrophotometer (Thermo Electron, UK) was used to measure the amount of light absorbed by the reaction mixture at a wavelength of 765 nm. The standard solutions of gallic acid (0.1-1mg mL^{-1}) were prepared to create a standard curve and linear regression between the absorbance values at 765 nm and the gallic acid concentrations. The TPC was calculated and expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh weight of the fruit samples (mg GAE g^{-1} FW) by using the following equation: $\text{TPC (mg GAE g} - 1 \text{ FW} = \text{C} \times \frac{\text{V}}{\text{W}}$

Where: C: is the gallic acid concentration (mg mL^{-1}) calculated using the standard curve, V: is the volume of the extracts (mL), and W: is the fresh weight of the samples (g).

Total Antioxidant Activity (TAA)

The TAA of the fruit extracts was determined according to the DPPH (1-diphenyl-2-picrylhydrazyl) method, which was described by [22]. According to this method, 30 µL of the fruit extracts was added to 1.7 mL of methanolic solution of DPPH. The mixture was shaken vigorously and then incubated for 30 minutes at room temperature in the dark condition. The absorbance at 517 nm was registered. TAA was measured as the inhibition percentage of the DPPH radical using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{ABS 517 of control} - \text{ABS 517 of sample}}{\text{WABS 517 of control}} \times 100$$

Nitrate accumulation in the fruits of broccoli plants was determined according to [23]. Mineral quantification, such as heavy metals zinc (Zn), copper (Cu), manganese (Mn), and iron (Fe) were quantified according to [24]. The total soluble solids content of samples was determined by a Digital Refractometer and stated as °Brix [25].

Statistical analysis

To determine whether the plant growth, yield, physical and chemical values were significantly different between the plant treatments, we performed an ANOVA test, followed by one-way comparisons between control and treated plants using XLSTAT statistical analysis software (version 2019.2.2). Duncan’s new multiple range test at ($p \leq 0.05$) was used to compare the means. Furthermore, principal component analysis (PCA) and multiple correlation tests were implemented.

Results and Discussions

Plant growth

The purpose of this experiment was to investigate the influence of inorganic fertilizer on broccoli quality characteristics. Different concentrations of nitrogen, phosphorus, and potassium (NPK) affected the above-ground parts of the broccoli plant (Figure 1). The results showed that the maximum values for plant height (30.43 cm), stem diameter (50.36 mm), plant branch number (5.62), and plant leaf number (56.38) were achieved with the application of 700 kg ha^{-1} NPK, while the lowest values (26.91 cm, 33.85 mm, 3.66, and 42.48, respectively) were noted in control plants (Figures 1A, B, C, D). The leaf chlorophyll intensity (82.96 SPAD) increased substantially with the 500 kg ha^{-1} application compared to the other treatments and the control (Figure 1E). Leaf dry matter significantly improved with the 300 kg ha^{-1} application, achieving the best value (20.27%) (Figure 1F).

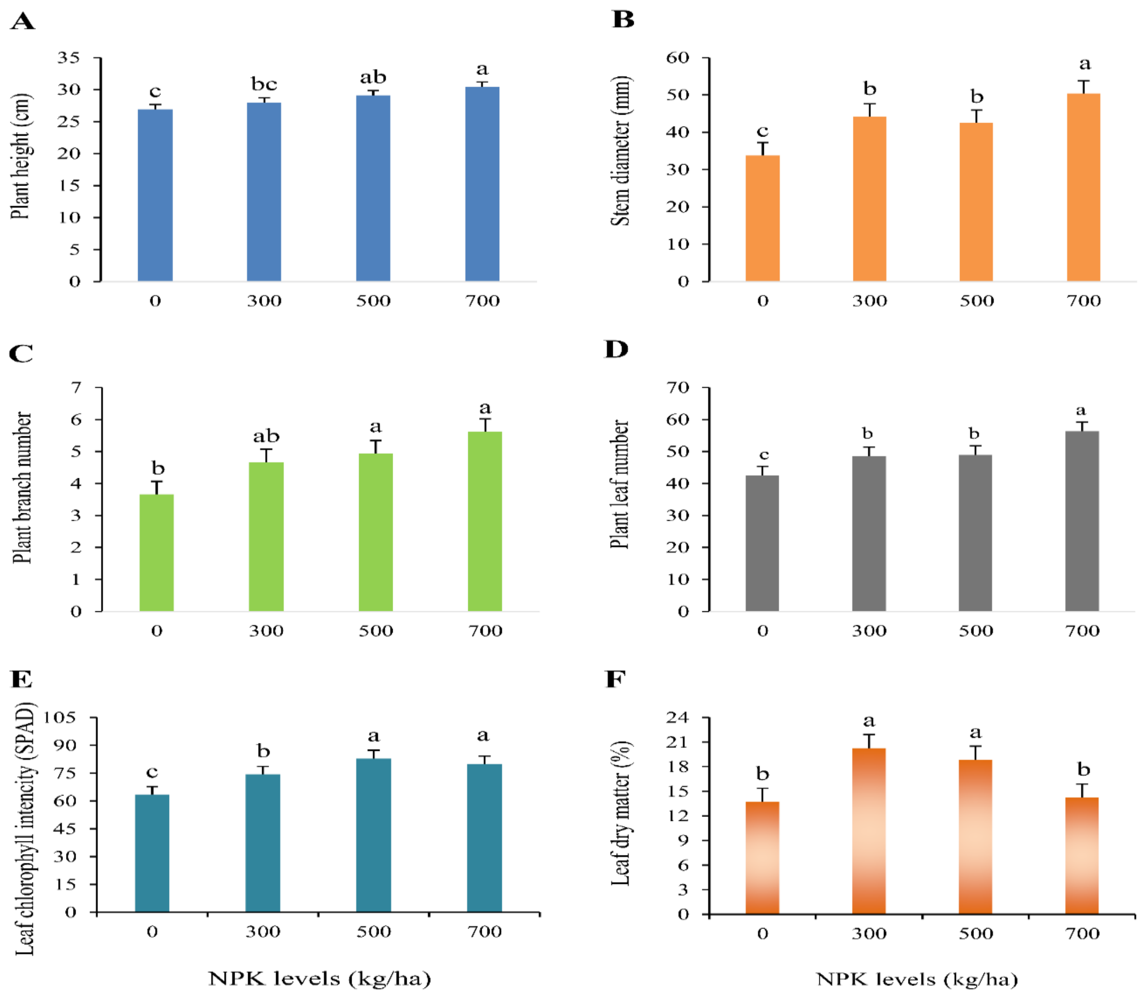

Figure (1): Effect of NPK different doses on the variables of broccoli plants. According to Duncan's new multiple range test ($p \leq 0.05$), Data are given as mean, and error bars indicate standard deviations, n=3. Bars with different letters indicate a significant difference between the treatments.

Plant yields

The data (Figure 2) represent the effect of different levels of NPK on broccoli fruit components. The results revealed that 700 kg ha^{-1} produced the best fruit characteristics, including lateral head diameter (12.33 cm), main head diameter (33.00 cm), main head weight (1234.67 g), plant fruit weight (1.55 kg ha^{-1}), and fruit dry matter (12.94%). Conversely, the lowest values (9.00 cm, 23.81 cm, 910.67 g, 1.08 kg ha^{-1}, and 10.50%, respectively) were observed under the control treatment (Figures 2A, B, D, E, F). However, the lateral head weight (354.33 g) was recorded as the maximum weight with the 300 kg ha^{-1} application, but it was not statistically superior to the 500 kg ha^{-1} application, and both were significantly different from the control (Figure 2C).

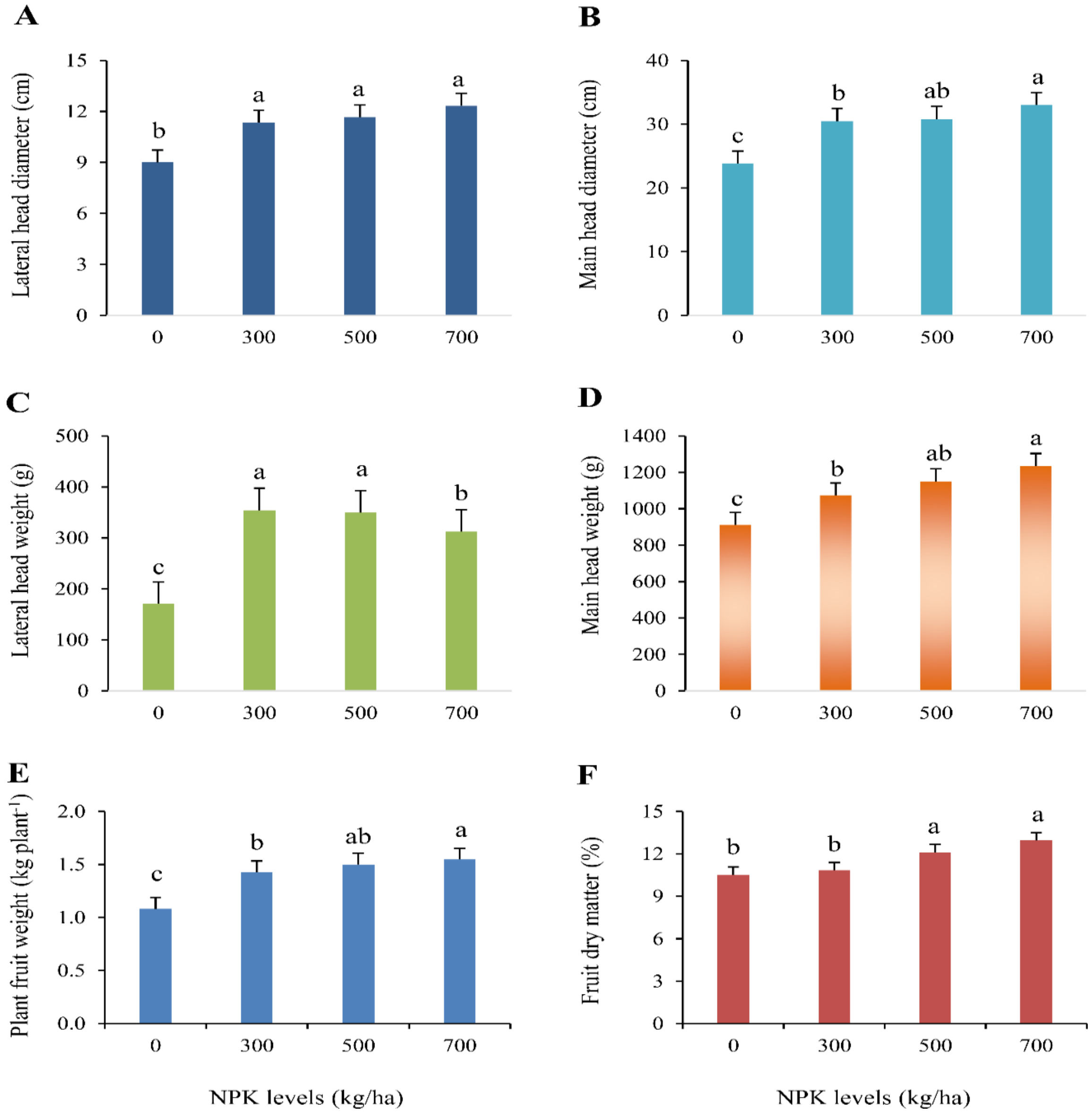

Figure (2): Effect of NPK different doses on the variables of broccoli plants. According to Duncan's new multiple range test ($p \leq 0.05$), Data are given as mean, and error bars indicate standard deviations, n=3. Bars with different letters indicate a significant difference between the treatments.

Chemical analysis

Quality characteristics associated with the chemical composition of broccoli heads are displayed in Table 1. Our research confirmed that different NPK application doses noticeably influenced TSS, ascorbic acid, and nitrate accumulation values. The TSS in the 500 kg ha^{-1} treatment was significantly different from the other concentrations and obtained the highest value (11.57 ˚Brix), while the control received the minimum value at reaching 9.10 ˚Brix. Regarding ascorbic acid, the 500 kg ha^{-1} NPK treatment increased ascorbic acid to 107.33 mg 100g^{-1} FW, whereas it declined with the control, which observed the lowest content (76.00 mg 100g^{-1} FW). Moreover, the control had the lowest nitrate accumulation (187.33 ppm), but the 500 kg ha^{-1} application increased it to the maximum value (502.33 ppm). The utilizing NPK had a substantial influence on the TPC and TAA of this vegetable. Each increase in NPK dosages up to a maximum of 700 kg ha^{-1} resulted in significantly higher TPC (93.89 µg GAE g^{-1} FW), while the control treatment recorded the lowest level (61.10 µg GAE g-1 FW). Additionally, the control treatment recorded the highest inhibition percentage of the DPPH radical (70.50%), which was more active than other treatments, with the lowest value (69.94%) achieved by the 300 kg ha^{-1} dose of NPK.

Table (1): Effect of NPK different doses on the variables of broccoli plants

Treatments		**TSS (˚Brix)**	**Ascorbic Acid (mg 100g^{-1} FW)**	**Nitrate accumulation (ppm)**	**TPC (µg GAE g^{-1} FW)**	**TAA (%)**
NPK (kg ha^{-1})	0	9.10 ± 0.10 c	76.00 ± 12.49 b	187.33 ± 15.99 c	61.10 ± 0.34 d	70.50 ± 0.04 a
	300	10.40 ± 1.12 b	86.00 ± 1.15 ab	483.33 ± 17.50 a	69.86 ± 0.61 c	67.06 ± 0.05 d
	500	11.57 ± 0.10 a	107.33 ± 19.50 a	502.33 ± 10.00 a	78.01 ± 2.62 b	70.35 ± 0.03 b
	700	10.20 ± 0.10 b	97.67 ± 18.33ab	345.00 ± 13.65 b	93.89 ± 0.74 a	69.94 ± 0.05 c

*According to Duncan's new multiple range test ($p \leq 0.05$). Data are given as mean, and error bars indicate standard deviations, n=3. A column with different letters indicates a significant difference between the treatments.

The NPK level applications impacted some heavy metals in fruit broccoli plants, such as zinc, iron, manganese, and copper (Figure 3). The level of 300 kg ha^{-1} had a significant effect on zinc and iron, producing the maximum rates (551.58 ppm and 46.17 ppm, respectively). In contrast, the values decreased dramatically when the NPK was increased to the highest level of 700 kg ha^{-1}. Likewise, the concentration of 300 kg ha^{-1} was superior to all the others in terms of manganese (11.29 ppm), while the control offered the lowest (5.83 ppm). The plants under control treatment reached the maximum values of copper (7.05 ppm), but the minimum was obtained with the application of 700 kg ha^{-1}, although it did not statistically differ from 500 kg ha^{-1}.

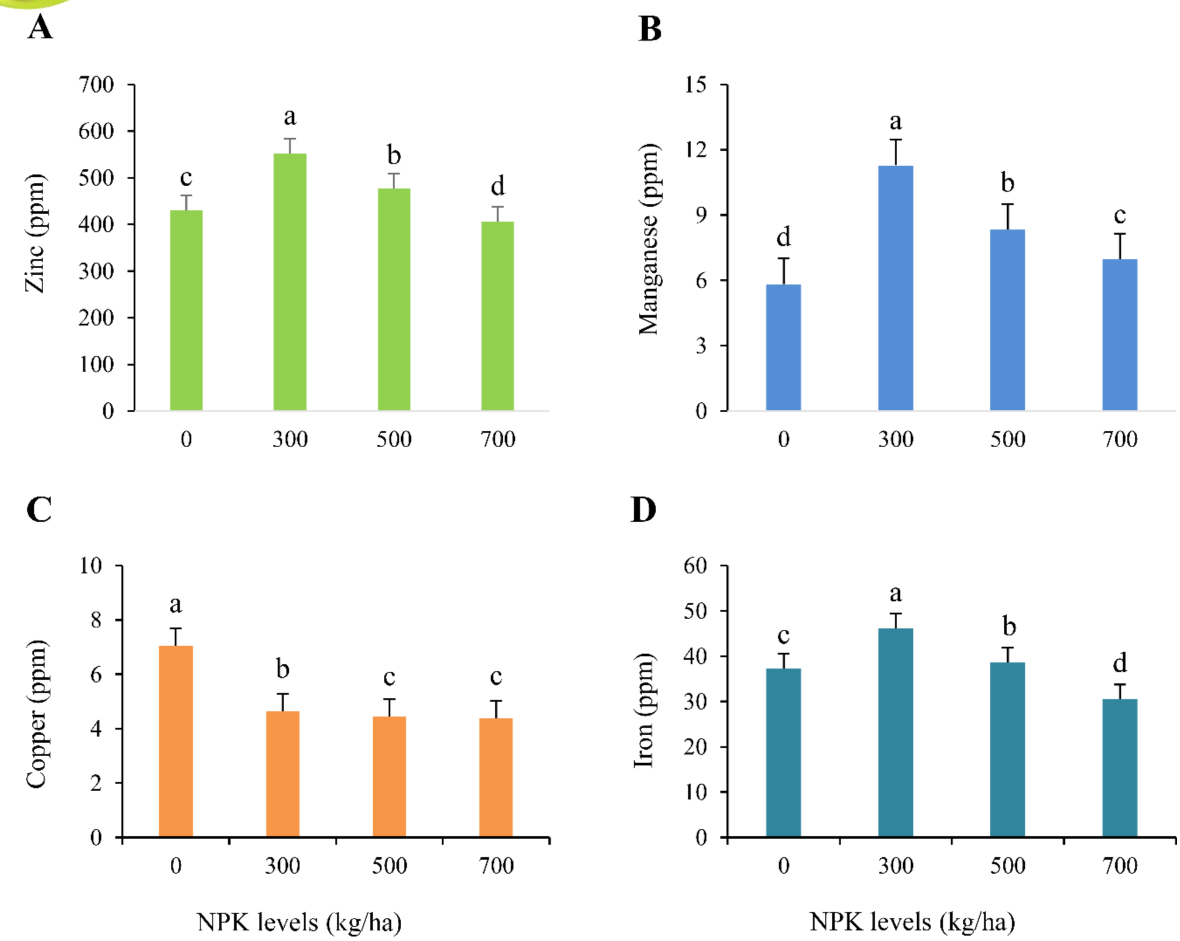

Figure (3): Effect of NPK different doses on the variables of broccoli plants. According to Duncan's new multiple range test ($p \leq 0.05$), Data are given as mean, and error bars indicate standard deviations, n=3. Bars with different letters indicate a significant difference between the treatments.

Principle Component Analysis (PCA)

PCA was applied to determine the association between the variables. The first two main components (PC_1 and PC_2) together explained 93.24% of the observed variation and were expressed in two dimensions (Figure 4). PC_1, plotted on the horizontal axis, demonstrated the highest proportion of the variance at 65.11%, while PC_2, plotted on the vertical axis, illustrated a further 28.13% of the total variation. The PCA results indicated that NPK strongly affects the studied variables. The 700 kg ha^{-1} application significantly impacted plant height (PH), stem diameter (SD), plant branch number (PBN), plant leaf number (PLN), lateral head diameter (LHD), main head diameter (MHD), plant fruit weight (PFW), fruit dry matter (FDM), and TPC. Additionally, the 500 kg ha^{-1} application significantly affected leaf chlorophyll intensity (LCI), TSS, ascorbic acid (AA), and nitrate accumulation (Nit). The 300 kg ha^{-1} application strongly correlated with leaf dry matter (LDM), lateral head weight (LHW), zinc (Zn), manganese (Mn), and iron (Fe). NPK applications partly influenced TAA and copper (Cu).

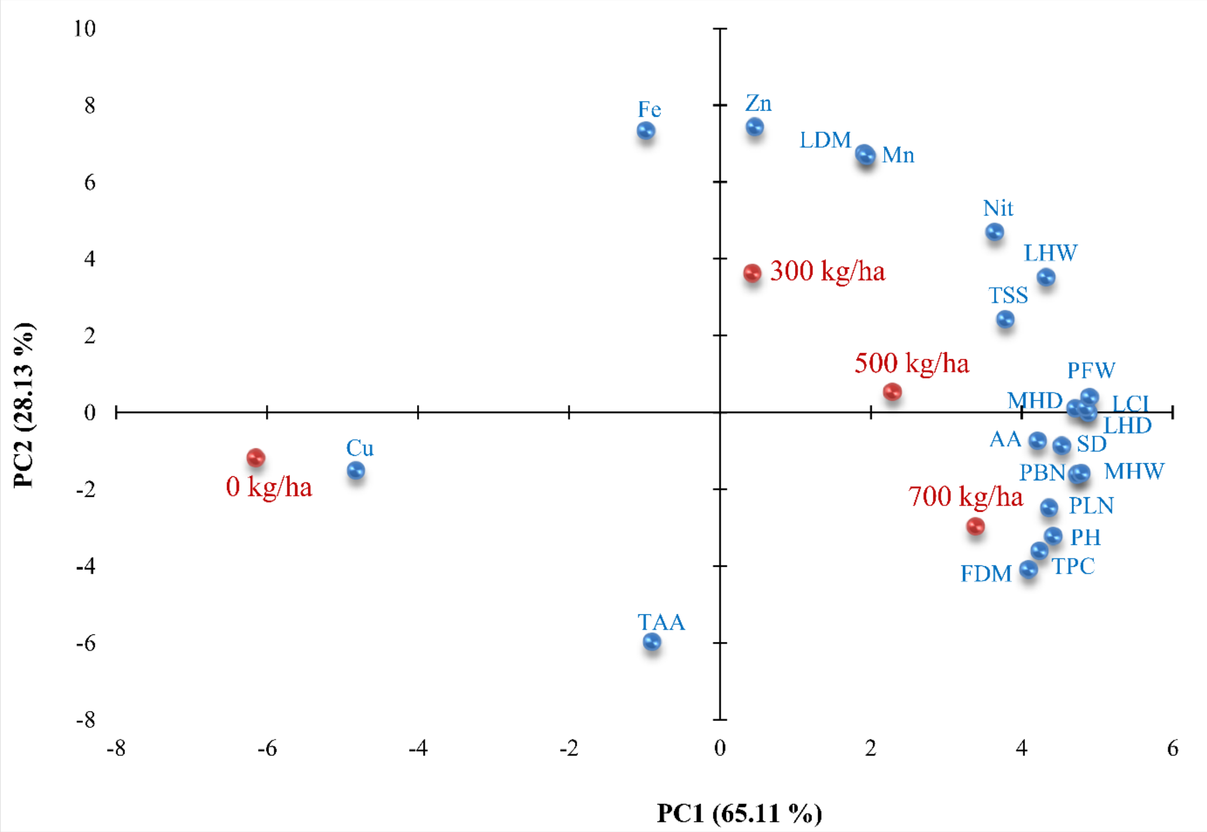

Figure (4): PCA biplot illustrating the relationship between NPK levels and the study variables of the broccoli plants.

Multivariate Analysis

Pearson's correlation was conducted to illustrate the relationships between the studied variables (Figure 5). The results revealed a strong correlation between plant height and plant branch number (r^2 = 0.97), plant leaf number (r^2 = 0.96), main head weight (r^2 = 0.97), fruit dry matter (r^2 = 0.98), and total phenolic content (r^2 = 0.99). Stem diameter was positively associated with plant branch number (r^2 = 0.97), plant leaf number (r^2 = 0.97), lateral head diameter (r^2 = 0.95), and main head diameter (r^2 = 0.97). Additionally, a significant positive correlation was observed between plant leaf number and main head weight (r^2 = 0.95) and total phenolic content (r^2 = 0.97). Leaf chlorophyll intensity was positively correlated with plant fruit weight (r^2 = 0.95) and ascorbic acid (r^2 = 0.96). There was a strong positive association between lateral head diameter and main head weight diameter (r^2 = 1.00), main head weight (r^2 = 0.98), and plant fruit weight (r^2 = 1.00). Furthermore, a significant positive association was observed between main head diameter and lateral head weight (r^2 = 0.97) and main head weight (r^2 = 0.99), as well as between lateral head weight and nitrate accumulation (r^2 = 0.96). Main head weight showed positive correlations with plant fruit weight (r^2 = 0.96) and total phenolic content (r^2 = 0.95), while fruit dry matter was positively correlated with total phenolic content (r^2 = 0.97) and zinc with iron (r^2 = 0.96). However, a significant negative correlation was recorded between lateral head diameter (r^2 = -0.98), main head diameter (r^2 = -0.97), lateral head weight (r^2 = -0.96), and plant fruit weight (r^2 = -0.99) with copper.

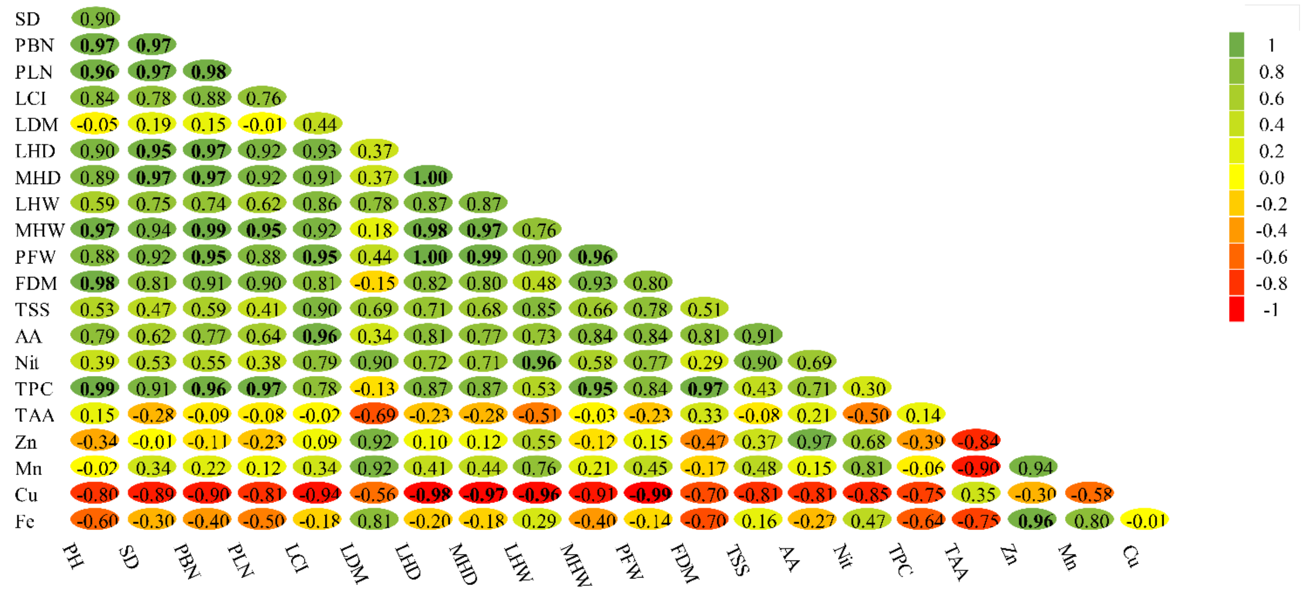

Figure (5): Pearson's correlation between all the studied variables was analyzed, taking into account the direction of the correlation and the significance threshold level.

Plant growth

According to the data in this study, applying NPK fertilizer significantly influenced the morphology of broccoli plants (Figure 1). These findings may be attributed to the role of NPK in the plant tissue of broccoli. Nitrogen primarily functions to produce structural proteins and phytohormones, which are crucial for various metabolic activities in plants; this enhances plant height, stem diameter, plant branch number, and plant leaf number. Phosphorus plays a vital role in capturing and utilizing energy for plant growth. Vegetative attributes are also linked to optimal potassium intake, as it is essential for glucose metabolism and water retention. In this context, NPK plays an essential role in plant development, as the concentrations of NPK stimulate plant growth effectively [12, 26] in broccoli [27]. Moreover, several researchers have reported that mineral fertilizers generally enhance vegetative growth [28, 29]. Nitrogen is an essential constituent of protein and plays a significant role in cell enlargement and division, thereby helping plant growth. In addition, nitrogen is crucial for the process of photosynthesis and facilitates the condensation of its byproducts within plant tissues [30, 31, 32] This was also confirmed by [33], who obtained a higher value for fresh and dry weight, leaf number, and leaf area when mono ammonium phosphate and phosphoric acid were applied as an alternate supply of phosphorus. These results are due to its high phosphorus content, water solubility as well as pH value. Melatonin was tested on two broccoli varieties in salinity. Melatonin applied to broccoli improved its morphology and chlorophyll in leaves and heads [34]. To promote broccoli development and production and to increase its ability to withstand salt stress, H_2S was applied as a foliar spray, and carotenoids, as well as chlorophyll, were significantly increased [35].

Plant yields

The formation and size head of the broccoli plant direct relatively relates to a higher yield (Figure 2). This outcome may be attributed to the effective use of carbohydrate dosages, which are created as a consequence of nitrogen being applied during the process of protein synthesis. Phosphorus is essential for the growth of roots as well as the acceleration of maturation owing to the recovery of chemical processes. In addition, potassium is essential for the proper functioning of a variety of plant body processes, including the metabolism of carbohydrates and the activation of enzymes, by facilitating the proper translocation of sugars, starches, and nitrogen compounds. Many researchers reported that the increase in yield was positively correlated with the increase in NPK fertilizer dosage applications [36, 37]. This is achieved through the supply of optimal levels of NPK [38, 39]. Inorganic fertilization has a positive effect on the plant. The improvement in head weight, head yield, and quality characteristics with increased doses of inorganic fertilizers can be ascribed to the better movement and availability of essential nutrients, which ultimately leads to better head development, increased head weight, and ultimately higher total yield [40].

Chemical analysis

Our results are in agreement with [40], who obtained a higher value for TSS and ascorbic acid content when inorganic fertilizer was applied; data was presented in Table 1. Inorganic minerals also have a positive effect on vitamin C in the head of broccoli [41]. The effect of several biomodulators on growth and chemicals in broccoli inflorescence was studied, and results showed an increase in ascorbic acid, carotenoids, and total soluble carbohydrates [42]. According to [41], the application of inorganic fertilizer treatments presented the best value to the total phenolic contents (Table 1). [43] concluded that the total phenolic content of broccoli was the same both before and after germination. After sprouting, broccoli developed a greater ability to fight free radicals. When broccoli sprouts were subjected to simulated digestion, both the total phenolic content and the antioxidant activity fell dramatically throughout the stomach phase. Also, The application of inorganic fertilizers is due to an increase in heavy metal rates in broccoli plants [44, 45] (Figure 3). Inorganic fertilizers significantly influence the growth, yield, and nutritional value of various plant varieties, enhancing their productivity and quality through the provision of essential nutrients, including cabbage [46], cauliflower [47] and lettuce [48] plants.

Plant growth and development require essential nutrients, including NPK fertilizer. This research indicated that the morphology, quality, and nutritional content of broccoli plants were significantly affected by NPK applications compared to control treatments. In particular, 700 kg ha^{-1} had a significant impact on the morphology, quality, and yield of broccoli. However, 500 kg ha^{-1} resulted in the highest nitrate accumulation and some physiochemical traits. Additionally, the application of 300 kg ha^{-1} produced the maximum levels of certain heavy metals. The experiment has the potential to enhance the productivity of broccoli and other crops grown under greenhouse conditions. However, further research is necessary to explore the effects on other vegetable crops and to examine varying NPK doses, as farmers often apply NPK as a primary fertilizer to produce marketable yields during the growing season.

References

1) Islam, M. S., Hossain, M. F., & Pervin, S. (2023). Enhancing broccoli (Brassica oleracea var. italica) growth, yield and water productivity through irrigation and mulching techniques in local climate. *Asian Journal of Agricultural and Horticultural Research, 10*(4), 440–454.

2) Yoldas, F., Ceylan, S., Yagmur, B., & Mordogan, N. (2008). Effects of nitrogen fertilizer on yield quality and nutrient content in broccoli. *Journal of Plant Nutrition, 31*(7), 1333–1343.

3) Dos Santos, L. W. O., da Silva Ribeiro, J. E., Lopes, A. S., Targino, V. A., dos Anjos Neto, A. P., de Azevedo Soares, V., Henschel, J. M., Batista, D. S., & Dias, T. J. (2022). Effect of nitrogen:potassium fertilization ratios and biostimulant application on broccoli plants. *Journal of Soil Science and Plant Nutrition, 22*(4), 4857–4867.

4) Moreno, D. A., Carvajal, M., López-Berenguer, C., & García-Viguera, C. (2006). Chemical and biological characterisation of nutraceutical compounds of broccoli. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1508–1522.

5) Sardar, H., Irshad, M., Anjum, M. A., Hussain, S., Ali, S., Ahmad, R., & Ejaz, S. (2022). Foliar application of micronutrients improves the growth, yield, mineral contents, and nutritional quality of broccoli (Brassica oleracea L.). *Turkish Journal of Agriculture and Forestry, 46*(6), 791–801.

6) Halshoy, H., Mahmood, A., & Tofiq, G. (2023). Effect of plant biostimulants on growth, yield and some mineral composition of broccoli plants (Brassica oleracea var. Italica). *Tikrit Journal for Agricultural Sciences, 23*(1), 130–140.

7) Gudiño, I., Martín, A., Casquete, R., Prieto, M. H., Ayuso, M. C., & Córdoba, M. d. G. (2022). Evaluation of broccoli (Brassica oleracea var. italica) crop by-products as sources of bioactive compounds. *Scientia Horticulturae, 304*, 111284.

8) Borowski, J., Szajdek, A., Borowska, E. J., Ciska, E., & Zieliński, H. (2008). Content of selected bioactive components and antioxidant properties of broccoli (Brassica oleracea L.). *European Food Research and Technology, 226*, 459–465.

9) Akhlaghi, M., & Bandy, B. (2010). Dietary broccoli sprouts protect against myocardial oxidative damage and cell death during ischemia-reperfusion. *Plant Foods for Human Nutrition, 65*, 193–199.

10) Ali, S., Khan, A. R., Mairaj, G., Arif, M., Fida, M., & Bibi, S. (2008). Assessment of different crop nutrient management practices for yield improvement. *Australian Journal of Crop Science, 2*(3), 150–157.

11) Fischer, R., & Connor, D. (2018). Issues for cropping and agricultural science in the next 20 years. *Field Crops Research, 222*, 121–142.

12) Singh, M. K., Chand, T., Kumar, M., Singh, K. V., Lodhi, S. K., Singh, V. P., & Sirohi, V. S. (2015). Response of different doses of NPK and boron on growth and yield of broccoli (Brassica oleracea L. var. italica). *International Journal of Bio-resource and Stress Management*, *6*, 108-112.

13) 13) Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany, 105*(7), 1141-1157.

14) Bechtaoui, N., Rabiu, M. K., Raklami, A., Oufdou, K., Hafidi, M., & Jemo, M. (2021). Phosphate-dependent regulation of growth and stresses management in plants. *Frontiers in Plant Science, 12*, 679916.

15) Sofi, S. O., Talabani, S. K., Sleman, S. M., Faraj, S. H. G., & Halshoy, H. S. (2024). Impact of bio-based and synthetic phosphorus application on growth, yield, and protein profile of two chickpea genotypes. *Agricultural Research*, 1-12.

16) Nieves-Cordones, M., Al Shiblawi, F. R., & Sentenac, H. (2016). Roles and transport of sodium and potassium in plants. In *The alkali metal ions: Their role for life* (pp. 291-324).

17) Peralta-Antonio, N., Watthier, M., Santos, R. H. S., Martinez, H. E. P., & Vergütz, L. (2019). Broccoli nutrition and changes of soil solution with green manure and mineral fertilization. *Journal of Soil Science and Plant Nutrition, 19*, 816-829.

18) Ibrahim, A., Maduro-Dias, C., Halshoy, H., Borba, A., & Nunes, H. (2024). Optimizing nutritional value and biological profile of non-conventional forages for animal feeding by utilizing sodium hydroxide. *Multidisciplinary Science Journal*, *6*(11).

19) Halshoy, H. S., & Sadik, S. K. (2024). Impact of training methods and biostimulant applications on sweet pepper (Capsicum annuum) yield and nutritional values: Under greenhouse condition. *Horticultural Plant Journal*, 2024.

20) Ahmed, G. O., Halshoy, H. S., Mahmood, C. H., & Hama, J. R. (2024). Titanium nanoparticle and humic acid applications improve seed germination, growth development, and phytochemical contents of lettuce (Lactuca sativa) plants. *BioNanoScience*, 1-12.

21) Halshoy, H. S., Sulaiman, S. M., Haidar Arkwazee, H. A., & Mahmood, A. K. (2023). Impact of urea supplements and cultivars on yield, nitrate accumulation and some phytochemical properties in lettuce grown under greenhouse conditions. *Kirkuk University Journal For Agricultural Sciences, 14*(2), 35-53.

22) Ghazghazi, H., Aouadhi, C., Riahi, L., Maaroufi, A., & Hasnaoui, B. (2014). Fatty acids composition of Tunisian Ziziphus lotus L. (Desf.) fruits and variation in biological activities between leaf and fruit extracts. *Natural Product Research*, *28*(14), 1106-1110.

23) ISO F. (1984). Vegetables and derived products-Determination of nitrite and nitrate content-Molecular absorption spectrometric method. International Organization for Standardization, Geneva (Switzerland), ISO, 6635:1984.

24) Rodrigues, A. B., Almeida-Apolonio, A. A. D., Alfredo, T. M., Dantas, F. G. D. S., Campos, J. F., Cardoso, C. A. L., de Picoli Souza, K., & Oliveira, K. M. P. D. (2019). Chemical composition, antimicrobial activity, and antioxidant activity of Ocotea minarum (Nees & Mart.) Mez. *Oxidative Medicine and Cellular Longevity*, 2019, 5736919.

25) Mohammed, H. A., Aljabary, A. M. A. O., Halshoy, H. S., Hama, J. R., Rashid, H. A., & Rashid, H. W. (2023). Soil-borne microbes, natural stimulants, and post-harvest treatments alter quality and phytochemicals of tomato fruit. *International Journal of Vegetable Science*, *29*(6), 544-556.

26) Mohanta, R., Nandi, A., Mishra, S., Pattnaik, A., Hossain, M., & Padhiary, A. (2018). Effects of integrated nutrient management on growth, yield, quality and economics of sprouting broccoli (Brassica oleracea var. italica) cv. Shayali. *Journal of Pharmacognosy and Phytochemistry*, *7*(1), 2229-2232.

27) Mohapatra, S., Munsi, P., & Mahapatra, P. (2013). Effect of integrated nutrient management on growth, yield and economics of broccoli (Brassica oleracea L. var. italica plenck.). *Vegetable Science*, *40*(1), 69-72.

28) Zaki, M., Tantawy, A., Saleh, S., & Helmy, Y. I. (2012). Effect of bio-fertilization and different levels of nitrogen sources on growth, yield components and head quality of two broccoli cultivars. *Journal of Applied Sciences Research*, *8*(8), 3943-3960.

29) Howladar, S. M., Rady, M. M., & Osman, A. S. (2013). Effect of bio-nitrogen as a partial alternative to mineral-nitrogen fertiliser on growth, nitrate and nitrite contents, and yield quality in Brassica oleracea L. *International Journal of Agricultural Science and Engineering*, *7*(7), 578-583.

30) El-Shakry, M. (2005). Effect of bio-fertilizers, nitrogen sources and levels on vegetative growth characters, yield and quality, and oil yield of sweet fennel (Ph. D. thesis, Faculty of Agriculture, Cairo University, Egypt).

31) Abou El-Magd, M., Zaki, M., & Abo Sedera, S. (2014). Effect of bio-nitrogen as a partial alternative to mineral-nitrogen fertilizer on growth, yield and head quality of broccoli (Brassica oleracea L. var. italica). World Applied Sciences Journal, 31(5), 681-691.

32) Halshoy, H. S., Sulaiman, S. M., Arkwazee, H. A. H., & Mahmood, A. K. (2023). Impact of urea supplements and cultivars on yield, nitrate accumulation and some phytochemical properties in lettuce grown under greenhouse conditions. *Kirkuk University Journal for Agricultural Sciences (KUJAS), 14*(2).

33) Mohamed, M. H., Ali, M., Eid, R. S., El-Desouky, H. S., Petropoulos, S. A., Sami, R., Al-Mushhin, A. A., Ismail, K. A., & Zewail, R. M. (2021). Phosphorus and biofertilizer application effects on growth parameters, yield and chemical constituents of broccoli. *Agronomy*, *11*(11), 2210.

34) Sardar, H., Ramzan, M. A., Naz, S., Ali, S., Ejaz, S., Ahmad, R., & Altaf, M. A. (2023). Exogenous application of melatonin improves the growth and productivity of two broccoli (Brassica oleracea L.) cultivars under salt stress. *Journal of Plant Growth Regulation*, *42*(8), 5152-5166.

35) Shalaby, O. A. E-S., Farag, R., & Ibrahim, M. F. (2023). Effect of hydrogen sulfide and hydrogen peroxide on growth, yield and nutrient content of broccoli plants grown under saline conditions. *Scientia Horticulturae, 316*, 112035.

36) Bahadur, A., Singh, J., & Singh, K. (2004). Response of cabbage to organic manures and biofertilizers. *Indian Journal of Horticulture*, *61*(3), 278-279.

37) Islam, M. R., Hoque, T. S., Khan, R. N. A., Farzana, S., Ahmed, M., & Khodabakhshloo, N. (2021). Influence of different integrated nutrient management strategies on growth, yield and nutritional qualities of cauliflower. *Agricultural Research, 10*, 656-664.

38) Supe, V., & Marbhal, S. (2008). Effect of organic manures with graded levels of nitrogen on growth and yield of cabbage (Brassica oleracea var. capitata L.). *Asian Journal of Horticulture, 3*(1), 48-50.

39) Shah, D. A., Narayan, R., Ahmad, N., Narayan, S., & Wani, K. P. (2010). Influence of boron and zinc on growth, yield and quality of knolkhol cv. Early White Vienna. *Indian Journal of Horticulture, 67*(4), 323-328.

40) Mal, D., Chatterjee, R., & Nimbalkar, K. (2014). Effect of vermicompost and inorganic fertilizers on growth, yield and quality of sprouting broccoli (Brassica oleracea L. var. italica Plenck). *International Journal of Bio-resource and Stress Management*, 507-512.

41) Magd, E., Sawan, M., & Zaki, O. (2010). Productivity and quality of two broccoli cultivars as affected by different levels of nitrogen fertilizers. *Australian Journal of Basic and Applied Sciences*, *4*(12), 6125-6133.

42) Sakr, M. T., Ibrahim, H. M., ElAwady, A. E., & AboELMakarm, A. A. (2021). Growth, yield and biochemical constituents as well as post-harvest quality of water-

stressed broccoli (Brassica oleracea L. var. italica) as affected by certain biomodulators. *Scientia Horticulturae, 275*, 109605.

43) Miyahira, R. F., de Lima Pena, F., Fabiano, G. A., de Oliveira Lopes, J., Ponte, L. G. S., da Cunha, D. T., Bezerra, R. M. N., & Antunes, A. E. C. (2022). Changes in phenolic compound and antioxidant activity of germinated broccoli, wheat, and lentils during simulated gastrointestinal digestion. *Plant Foods for Human Nutrition, 77*(2), 233-240.

44) Mukherjee, A. B., & Kabata Pendias, A. (2007). Trace elements from soil to human. Springer: Berlin/Heidelberg, Germany.

45) Nahar, S., Karim, M. M., Polash, M. A. S., Juthee, S. A., Fakir, M. S. A., & Hossain, M. A. (2021). Influence of organic and inorganic sources of nutrients on the growth and seed yield of Chinese broccoli (Brassica oleracea L. var. alboglabra Baily). *Farming and Management, 6*(1), 8-14.

46) Chaudhary, S., Yadav, S., Mahto, D., Sharma, R., & Kumar, M. (2018). Response of growth, yield attributes and yield of cabbage (Brassica oleracea var. capitata) to different organic and inorganic sources of nutrients in Magadha plain of Bihar. *International Journal of Current Microbiology and Applied Sciences*, 4748-4756.

47) Devkota, S., Rayamajhi, K., & Shrestha, J. (2021). Effects of different doses of organic and inorganic fertilizers on cauliflower yield and soil properties. *Journal of Agriculture and Natural Resources, 4*(2), 11-20.

48) Kirova, E. B., Geneva, M. P., Kostadinov, K., & Filipov, S. (2022). Improving yield and quality-related physiological characteristics of lettuce by integrated inorganic and organic fertilizers management. *Agriculturae Conspectus Scientificus, 87*(2), 127-134.