

# Potential of C-Phycocyanin Extract and Fertilizers combination for Enhancing Fruit Quality and Cultivation Efficiency in Hot Pepper (*Capsicum annuum* L.)

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## Abstract

To optimize hot pepper (*Capsicum annuum* L.) productivity and quality under high-temperature conditions in Sharkia Governorate, Egypt, field trials were conducted during the 2022 and 2023 summer seasons. The experiment investigated the interactive effects of two cultivars (Belal and Omega), four NPK fertilization rates (Control, 75%, 50%, and 25% of the recommended dose) combined with a triple bio-fertilizer complex (*Nitrobein*, *Phosphorein*, and *Potassiumag*), and foliar application of C-phycocyanin (0 vs. 1000 ppm) using a split-split plot design.

Belal produced a significantly higher fruit yield per feddan (8.54 and 7.45 tons in the first and second seasons, respectively) than Omega (6.25 and 5.56 tons), driven by a higher fruit set per plant despite smaller average fruit weight. Conversely, Omega exhibited superior individual fruit mass, peaking at 14.67 g under 50% NPK mixed with bio-fertilizers. Among nutrient management regimes, applying 50% recommended NPK combined with bio-fertilizers optimized fruit weight, root expansion (up to 20.25 cm), and overall vegetative growth, whereas increasing NPK to 75% caused a decline in vegetative metrics. Foliar sprays of C-phycocyanin significantly elevated average fruit weight (up to 9.75 g) and total yield (up to 8.15 tons/feddan) across seasons by functioning as a bio-elicitor that enhances metabolic activity.

The three-way interaction revealed that integrating the Belal cultivar with 50% mineral NPK, bio-fertilizers, and C-phycocyanin foliar spray achieved the highest overall productivity (10.80 and 10.10 tons/feddan in the 1st and 2nd seasons, respectively). Furthermore, Belal under 25% NPK with bio-fertilizers and phycocyanin recorded the highest fruit nitrogen accumulation (2.738%). These results demonstrate that combining C-phycocyanin foliar treatment with bio-fertilizers allows for a 50% reduction in mineral NPK inputs while maximizing yield, improving fruit quality attributes, and enhancing cultivation efficiency in hot pepper.

**Keywords:** *Capsicum annuum*, C-phycocyanin, bio-fertilizers, yield components, fruit quality, sustainable agriculture

## Introduction

*Capsicum* is a genus of flowering plants belonging to the *Solanaceae* family, and it is widely cultivated in tropical and subtropical regions. Hot pepper (Chilli) has been used as part of the human diet since 7500 BC (Pickersgill, 1997). Chili fruits have many benefits and nutritional values for human health (Soare *et al.*, 2017). It contains high levels of vitamins (A, E, and C), antioxidants, carotenoids, and xanthophyll (Moreb *et al.*, 2020). Chilli pepper is one of Egypt's valuable agricultural products, which is essential for both international export and domestic food processing. Particularly in the fertile Nile River Delta and Upper Egypt, the industry generates substantial foreign exchange benefits and gives local farmers a reliable source of income (Abdel Aziz *et al.*, 2023).

Maximizing chili pepper productivity in Egypt's arid climate requires precise water management, heat stress mitigation, targeted NPK fertilization, and protected cultivation. Foliar fertilizer derived from algae and seaweed extract is rich in natural plant hormones such as auxin and cytokinin, as well as vitamins and macro- and micronutrients (Tashi *et al.*, 2023). Seaweed extract is increasingly used as a modern alternative to chemical fertilizers to boost agricultural yields while minimizing health and environmental risks associated with chemical use. Additionally, foliar application is known for its rapid effectiveness in meeting plants' needs (Salazar-Salazar *et al.*, 2022).

The biofertilizer complex of Nitrobein(Costa *et al.*, 2024) (nitrogen-fixing bacteria like *Azotobacter* and *Azospirillum*), Phosphorein (Salama, Abou El-Salehein *et al.*, 2023) (phosphate-solubilizing bacteria such as *Bacillus megaterium*), and Potassiumag (potassium-solubilizing/mobilizing microorganisms) (Bayındır and Küçükyumuk) significantly enhances the vegetative growth, yield, and overall quality of pepper plants (*Capsicum annuum* L.) (Abd-Hamid *et al.*, 2023). By converting atmospheric nitrogen into plant-absorbable ammonium, solubilizing fixed soil phosphorus, and mobilizing unavailable soil potassium into plant-ready ions, this triple-microbial blend improves nutrient uptake efficiency, root system expansion, and photosynthetic pigments (chlorophyll and carotenoids) (Ndese *et al.*, 2024). Consequently, pepper plants treated with this biofertilizer combination exhibit increased plant height, broader leaf coverage, higher fruit set, and enhanced fruit quality such as elevated total soluble solids (TSS), capsaicin content in hot peppers, and higher vitamin C levels—while simultaneously reducing reliance on chemical NPK fertilizers, preserving soil health, and mitigating environmental toxicity (Lase *et al.*).

C-phycoerythrin is a blue, highly fluorescent, water-soluble pigment that is extracted from red algae and cyanobacteria (Metwally *et al.*, 2022). It is a naturally occurring pigment of the phycobiliprotein family found in *Spirulina platensis*.

In times of environmental stress, C-phycoerythrin functions as a potent antioxidant that scavenges reactive oxygen species to safeguard cellular structures and promote protein synthesis. It maintains plant health under difficult circumstances, including excessive salinity, by improving salt tolerance and metabolic stability. In crops like pepper, this ultimately results in increased fresh weight, shoot greenness, and overall biomass retention (Varia *et al.*, 2022).

Therefore, the primary objective of this study is to evaluate the effects of cultivar, fertilization management, and foliar application of phycoerythrin on the vegetative growth, yield performance, and fruit quality parameters of hot pepper (*Capsicum annuum* L.). By investigating the interaction between genetic variation and nutrient management strategies, this research aims to determine the optimal combination of bio-fertilizer application and phycoerythrin foliar treatments to maximize crop productivity, enhance fruit quality attributes, and improve the overall efficiency of hot pepper cultivation.

## Materials and Methods

Field experiments were conducted at El-qaraqra Village Private Farm, Menia elkamh District, Sharkia Governorate, Egypt, during the two consecutive summer seasons of 2022 and 2023, to enhance the growth and productivity of hot pepper (*Capsicum annuum* L.) crop under high temperature conditions. In addition, the influences of different NPK fertilization rates (Control, 75, 50, and 25 % of the recommended rate) combined with bio-fertilizers (Nitrobein, Phosphorein, and Potassiumag), as well as phycoerythrin, on growth, yield components, and chemical constituents of two varieties of hot pepper (Belal and Omega) under Sharkia Governorate conditions were examined.

In general, the combinations (16 treatments) between variety type (2 varieties), NPK fertilization level (4 treatments), and phycoerythrin application (2 treatments) were examined. The physical and chemical characteristics of the experimental soil are shown in Table 1, according to Chapman and Pratt (1978).

**Table 1. Physical and chemical properties of experimental farm soil (average of two seasons)**

| Physical analysis                             |                           |                          |                          |                               |                  |                 |                |                              | Soil texture                  |                 |                              |
|-----------------------------------------------|---------------------------|--------------------------|--------------------------|-------------------------------|------------------|-----------------|----------------|------------------------------|-------------------------------|-----------------|------------------------------|
| Clay (%)                                      |                           |                          | Silt (%)                 |                               | Sand (%)         |                 |                |                              | Clay                          |                 |                              |
| 56.68                                         |                           |                          | 30.31                    |                               | 11.01            |                 |                |                              |                               |                 |                              |
| Chemical analysis                             |                           |                          |                          |                               |                  |                 |                |                              |                               |                 |                              |
| pH                                            | E.C.<br>dSm <sup>-1</sup> | Organic<br>matter<br>(%) | CaCO <sub>3</sub><br>(%) | Soluble cations<br>(meq. / L) |                  |                 |                | Soluble anions<br>(meq. /L)  |                               |                 |                              |
|                                               |                           |                          |                          | Ca <sup>++</sup>              | Mg <sup>++</sup> | Na <sup>+</sup> | K <sup>+</sup> | CO <sub>3</sub> <sup>-</sup> | HCO <sub>3</sub> <sup>-</sup> | Cl <sup>-</sup> | SO <sub>4</sub> <sup>-</sup> |
| 8.08                                          | 3.14                      | 0.62                     | 0.61                     | 11.24                         | 10.53            | 2.87            | 4.22           | 0.04                         | 9.86                          | 4.73            | 16.23                        |
| Available nutrient (mg kg <sup>-1</sup> soil) |                           |                          |                          |                               |                  |                 |                |                              |                               |                 |                              |
| N                                             |                           | P                        | K                        | Fe                            |                  | Zn              |                | Cu                           |                               | Mn              |                              |
| 38.17                                         |                           | 16.96                    | 195                      | 1.92                          |                  | 0.83            |                | 0.71                         |                               | 0.47            |                              |

#### Experimental design:

The statistical layout of this experiment was a split-split plot design with hot pepper varieties (two types), NPK fertilization (four levels), and phycocyanin (with and without application) in a randomized complete block design with three replicates.

#### Plant cultivation and experimental unit:

The hot pepper varieties (Cayenne or pencil type, "Belal and Omega") seedlings were bought from a private nursery in Menia Elkamh District, Sharkia Governorate, Egypt. All transplants of the studied hot pepper varieties were similar in development and growth, and their length was 12-14 cm. Seedlings were transplanted in the experimental plots on 1<sup>st</sup> April and 1<sup>st</sup> May during both seasons of 2022 and 2023, respectively. The 12m<sup>2</sup> (4m × 3m) experimental unit area had 4 ridges spaced 80 cm apart, and 50 cm between hot pepper plants.

#### Agricultural practices and fertilization:

All hot pepper plants received regular agricultural care as recommended by the Ministry of Agriculture in Egypt. The recommended NPK fertilizers under experiment region were 400 kg/feddan of ammonium sulfate (20.5% N), 300 kg/feddan of calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>), and 200 kg/feddan of potassium sulfate (48 % K<sub>2</sub>O), which were applied to all plants as nitrogen, phosphorus, and potassium fertilizers, respectively. As part of the soil preparation process, fertilizers containing phosphorus were applied. Moreover, three equal quantities of nitrogen and potassium fertilizers were applied to the soil at 15, 30, and 60 days after the planting date.

Bio-fertilizers, including 500 g Nitrobein, 500 g Phosphorein, and 500 g Potassiumag, were dissolved in 10 liters of water, then injected into the soil after irrigation. This treatment was done after (15, 25, and 135days) as well as after (15 and 25 days) from the planting date in 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively.

#### Phycocyanin preparation and application:

Phycocyanin (Fig 1) is a blue pigment, highly fluorescent, water-soluble pigment that is found in *Spirulina platensis*(Eriksen, 2008). The method reported by (Stewart, 1974) was utilized by repeatedly freezing and thawing cells in 50 mM sodium phosphate buffer (pH 6.8) to extract phycocyanin from *Spirulina platensis*. Ammonium sulphate was used to preferentially precipitate the extracted phycocyanin, and centrifugation was used to separate the precipitate. In 50 mM sodium phosphate buffer, the precipitate was

dissolved and dialyzed against distilled water. Phycocyanin was measured using the equation provided by (Stewart, 1974). Electrophoresis was used to verify the phycocyanin sample's purity. The 150-ppm rate was utilized as a foliar spray after 120 and 135 days from the planting date.

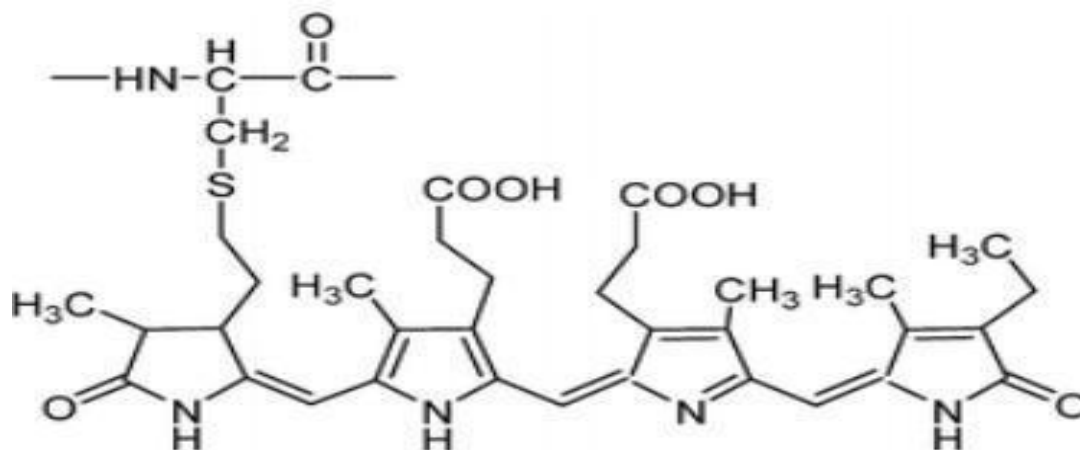


Figure 1: The chemical structure of phycocyanin

#### Data Recorded:

A random sample of three hot pepper plants from each sub-sub plot was taken at 108 days after planting for growth traits and at 160 days after planting for fruit yield and quality, as well as some chemical constituents in the fruits, and the following traits were recorded:

##### A) Yield and growth characters

1. Total yield per feddan (Ton)
2. Plant height (cm).
3. Root length (cm).
4. Average fruit weight (g).

##### B) Chemical constituents:

All chemical constituents were determined in the second season at the end of the experiment in hot pepper fruits.

##### 1. Total nitrogen percentage:

Total nitrogen percentage in hot pepper fruits was determined according to the method presented by (Nelson and Sommers, 1973).

##### 2. Total phosphorus percentage:

Total phosphorus percentage in hot pepper fruits was determined according to the method adapted by (Dick and Tabatabai, 1977).

##### 3. Potassium percentage:

Potassium percentage was determined in hot pepper fruits by utilizing a flame photometer according to the method reported by (Basta and Tabatabai, 1985).

##### 4. Total carbohydrate percentage:

Total carbohydrate percentage in hot pepper fruits was determined according to the method described by (Weinmann, 1947).

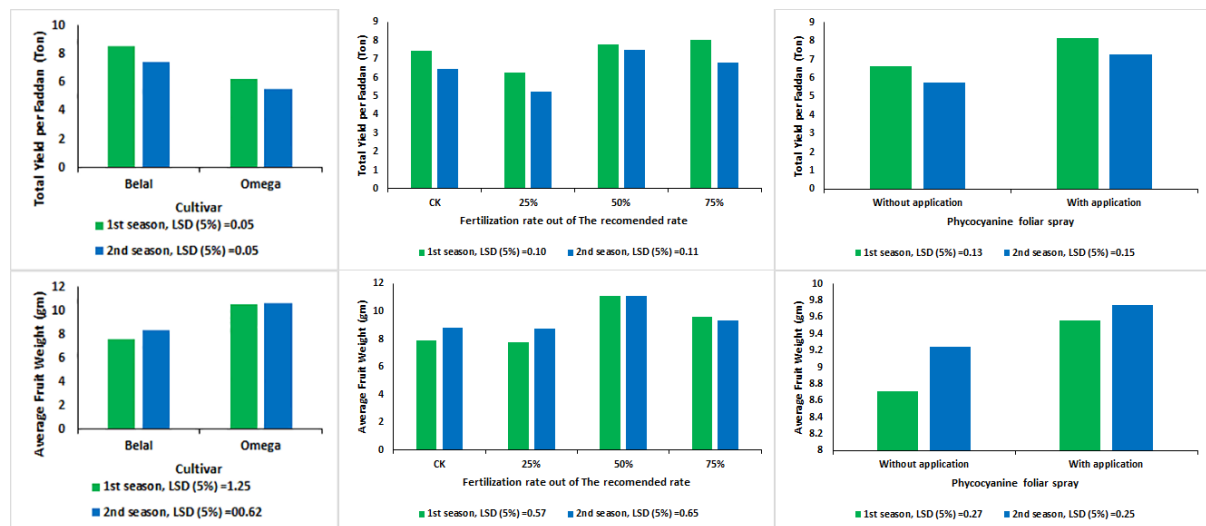
### Statistical analysis:

The statistical layout was a split-split-plot experiment in a completely randomized block design. Data were analyzed according to (Gomez and Gomez, 1984). The means were compared using the computer program Statistic Version 9 (Boyd *et al.*, 2018).

### Results

Results listed in **Figure 1** showed that Omega pepper hybrid significantly outperformed Belal pepper hybrid in average fruit weight during both seasons (10.54 g vs. 7.63 g in 1<sup>st</sup> season; 10.63 g vs. 8.38 g in 2<sup>nd</sup> season). In contrast, the Belal hybrid produced a substantially higher fruit yield per feddan compared to the Omega cultivar across both growing years (8.54 tons vs. 6.25 tons in the 1<sup>st</sup> season; 7.45 tons vs. 5.56 tons in the 2<sup>nd</sup> season). This inverse relationship illustrated a classical physiological trade-off in Solanaceous crops: while Omega channels photo-assimilates into individual fruit enlargement, Belal exhibits a higher harvest index driven by greater fruit number per plant, ultimately translating into higher total productivity.

Data presented in **Figure 1** suggested that the 50% mineral NPK mixed with Biofertilizer application recorded the highest fruit average weight in both experimental years (11.08 g and 11.08 g). However, total fruit yield per feddan peaked under 75% and 50%NPK fertilization mixed with Biofertilizer in the first season and second season (8.06 tons and 7.48 tons, respectively). Unfertilized controls and the lower fertilizer rate (25%) mixed with Biofertilizer resulted in lower average fruit weight and overall yield.



**Figure 1: Effect of variety, fertilizer rate, and phycocyanin on total yield per faddan (Ton) and average fruit weight (gm) of pepper (*Capsicum annuum* L.) during the two growth seasons (2022 and 2023)**

As the main effect of phycocyanin listed in **Figure 1**, foliar sprays significantly elevated both average fruit weight (9.56 g vs. 8.71 g in 1<sup>st</sup> season; 9.75 g vs. 9.25 g in 2<sup>nd</sup> season) and total fruit yield per feddan (8.15 tons vs. 6.63 tons in 1<sup>st</sup> season; 7.28 tons vs. 5.73 tons in 2<sup>nd</sup> season). Phycocyanin acts as a bio-elicitor that boosts chlorophyll content, delays foliar senescence, and stimulates carbohydrate transport from source leaves to reproductive parts.

The empirical data detailed in **Table 2** establish a significant three-way interaction ( $p < 0.05$ ) governing both individual fruit mass and total harvestable crop yield across the 2022 and 2023 growing seasons. Focusing on average fruit weight, the highest phenotypic expression was observed in the Omega genotype supplied with 50% NPK fertilization mixed with Bio-fertilizers without phycocyanin during the first season, reaching an absolute maximum of 14.67 g. In the subsequent season, the highest individual fruit mass shifted to the Omega cultivar receiving 50% fertilization mixed with Biofertilizer combined with phycocyanin application (13.33 g).

**Table 2. Effect of combinations between variety, fertilizer rate and phycocyanin on fruit average weight (g) and fruit yield per feddan (ton) of hot pepper (*Capsicum annuum* L.) during 2022 and 2023 seasons**

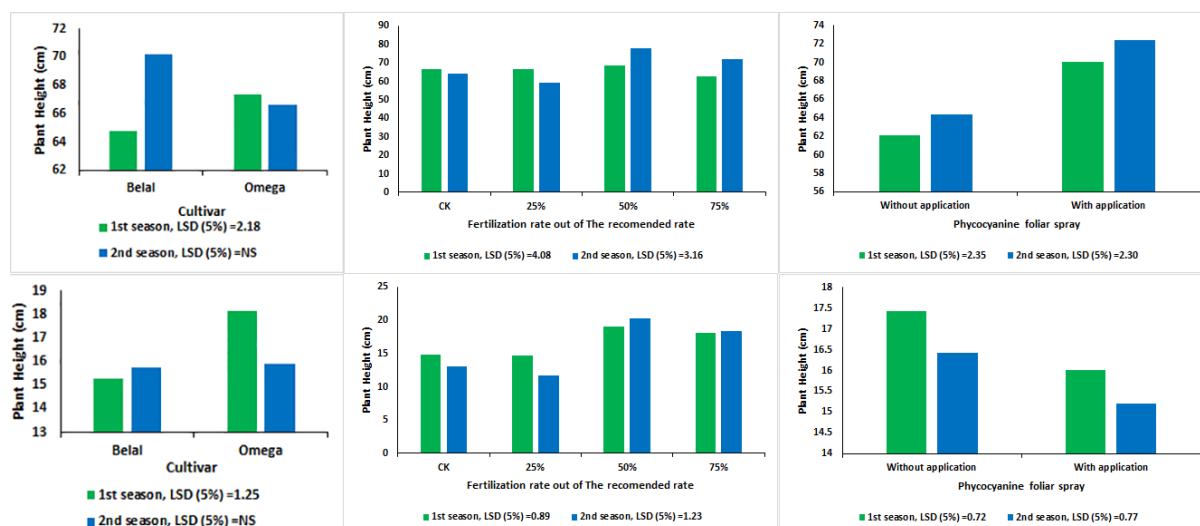
| Variety   | Fertilizer rate | Phycocyanin | Fruit average weight (g) |                        | Fruit yield /feddan (ton) |                        |
|-----------|-----------------|-------------|--------------------------|------------------------|---------------------------|------------------------|
|           |                 |             | 1 <sup>st</sup> season   | 2 <sup>nd</sup> season | 1 <sup>st</sup> season    | 2 <sup>nd</sup> season |
| Belal     | Control         | Without     | 6.67                     | 7.67                   | 8.45                      | 6.65                   |
|           |                 | With        | 6.33                     | 8.00                   | 9.20                      | 7.93                   |
|           | 25%             | Without     | 7.33                     | 7.33                   | 6.25                      | 4.75                   |
|           |                 | With        | 5.67                     | 8.33                   | 7.75                      | 6.50                   |
|           | 50%             | Without     | 7.33                     | 8.33                   | 6.40                      | 7.55                   |
|           |                 | With        | 9.67                     | 10.33                  | 10.80                     | 10.10                  |
|           | 75%             | Without     | 7.67                     | 8.00                   | 9.15                      | 7.20                   |
|           |                 | With        | 10.33                    | 9.00                   | 10.35                     | 8.90                   |
| Omega     | Control         | Without     | 8.67                     | 9.33                   | 5.58                      | 5.05                   |
|           |                 | With        | 10.00                    | 10.33                  | 6.62                      | 6.25                   |
|           | 25%             | Without     | 7.67                     | 9.67                   | 5.20                      | 4.35                   |
|           |                 | With        | 10.33                    | 9.67                   | 5.95                      | 5.40                   |
|           | 50%             | Without     | 14.67                    | 12.33                  | 6.25                      | 5.40                   |
|           |                 | With        | 12.67                    | 13.33                  | 7.65                      | 6.85                   |
|           | 75%             | Without     | 9.67                     | 11.33                  | 5.80                      | 4.85                   |
|           |                 | With        | 10.67                    | 9.00                   | 6.95                      | 6.30                   |
| LSD at 5% |                 |             | 1.24                     | 1.02                   | 0.25                      | 0.19                   |

Conversely, the minimum individual fruit weights were documented in the Belal cultivar restricted to 25% fertilization mixed with Biofertilizer and treated with phycocyanin in the first year (5.67g), and the unfertilized Belal controls without phycocyanin in the second year (7.67 g). At the same time, the triple integration of the Belal cultivar, a moderate 50% fertilizer rate, and phycocyanin foliar spraying unlocked the maximum productivity in the first (10.80 tons) and second (10.10 tons) experimental seasons. On the other hand, the poorest yield performance was consistently registered by the Omega cultivar cultivated under the deficient 25% fertilization regime mixed with biofertilizers without bio-stimulant application, yielding only 5.20 tons and 4.35 tons in the respective seasons.

The complex responses triggered by these three-way interactions provide deep physiological insights into resource allocation dynamics in hot pepper. The data perfectly illustrate the classic biological antagonism between individual fruit sizing and total productivity. While the Omega genome allocates its photo-assimilates toward cell expansion in a limited number of fruits (producing the heaviest peppers up to 14.67 g), Belal employs a strategy of high productivity. By setting a vastly higher number of fruits, Belal compensates for smaller individual sizes, ultimately driving superior total yield per unit area. This allows the crop to maintain high assimilation rates and fully realize its yield potential while effectively halving the required chemical fertilizer inputs. In addition, increasing the basal fertilization rate to 75% mixed with Biofertilizer failed to

generate further improvements in either fruit weight or total yield compared to the optimal 50% rate mixed with Biofertilizer.

Regarding the effect of variety, as shown in **Figure 2**, the Omega cultivar demonstrated statistically significant superiority over the Belal cultivar across most evaluated vegetative parameters. Regarding the effect of fertilizer rate, the 50% fertilizer rate mixed with Biofertilizer (Nitrobein + Phosphorein + Potassiumag) consistently produced the highest values in both seasons, reaching plant heights of 68.75 cm and 77.92 cm. On the other hand, increasing the fertilization rate to 75% mixed with Biofertilizers caused a marked vegetative decline (**Figure 2**).



**Figure 2:** Effect of variety, fertilizer rate, and phycocyanin on Plant height (cm) and Root Length of pepper (*Capsicum annuum* L.) during the two growth seasons (2022 and 2023)

In terms of the single effect of phycocyanin, foliar spraying with phycocyanin significantly enhanced all vegetative parameters compared to the untreated control, elevating plant height to 70.04 cm and 72.42 cm in both seasons (**Figure 2**).

Regarding the three-way interaction among variety  $\times$  fertilizer rate  $\times$  phycocyanin, the highest significant values for vegetative growth parameters were recorded under specific combination treatments during both the 2022 and 2023 seasons (**Table 3**). For plant height, the combination of Belal cultivar supplied with 50% fertilizer rate mixed with Biofertilizers and foliar phycocyanin application achieved the maximum height in both seasons, reaching 77.33 cm in the first season and 82.67 cm in the second season. On the other hand, the lowest plant height values were generally observed in plants grown under the lower fertilizer levels mixed with Biofertilizers without phycocyanin application. Furthermore, fertilizing the Belal cultivar with 75% mineral fertilizer rate mixed with Biofertilizers without phycocyanin decreased plant height (57.00 cm) in the first season and Belal with control fertilizer without phycocyanin (57.00 cm) in the second season.

**Table 3.** Effect of combinations between variety, fertilizer rate and phycocyanin on plant height (cm) and number of branches and leaves per plant of hot pepper (*Capsicum annuum* L.) during 2022 and 2023 seasons

| Variety | Fertilizer rate | Phycocyanin | Plant height (cm)      |                        | Root length (cm)       |                        |
|---------|-----------------|-------------|------------------------|------------------------|------------------------|------------------------|
|         |                 |             | 1 <sup>st</sup> season | 1 <sup>st</sup> season | 1 <sup>st</sup> season | 2 <sup>nd</sup> season |
| Belal   | Control         | Without     | 63.00                  | 57.00                  | 12.67                  | 14.33                  |

|           |         |         |       |       |       |       |
|-----------|---------|---------|-------|-------|-------|-------|
|           |         | With    | 67.33 | 77.33 | 16.33 | 15.33 |
|           | 25%     | Without | 58.00 | 57.33 | 13.33 | 13.33 |
|           |         | With    | 73.67 | 62.33 | 10.00 | 13.33 |
|           | 50%     | Without | 64.00 | 75.67 | 19.33 | 19.33 |
|           |         | With    | 77.33 | 82.67 | 18.33 | 16.00 |
|           | 75%     | Without | 57.00 | 68.00 | 18.33 | 17.33 |
|           |         | With    | 58.00 | 81.00 | 17.67 | 13.33 |
| Omega     | Control | Without | 59.00 | 59.00 | 13.33 | 17.33 |
|           |         | With    | 76.67 | 63.67 | 9.67  | 12.33 |
|           | 25%     | Without | 72.67 | 58.00 | 13.33 | 15.33 |
|           |         | With    | 61.67 | 59.00 | 10.00 | 17.00 |
|           | 50%     | Without | 62.67 | 71.67 | 22.33 | 21.67 |
|           |         | With    | 71.00 | 81.67 | 21.00 | 19.33 |
|           | 75%     | Without | 60.67 | 68.33 | 18.67 | 20.67 |
|           |         | With    | 74.67 | 71.67 | 18.67 | 21.33 |
| LSD at 5% |         |         | 7.33  | 11.91 | 2.16  | 2.21  |

Results given in Table 3 show that Omega variety plants formed a longer root system in the first experimental season (18.13 cm) relative to Belal (15.29 cm), whereas the distinction between both genotypes ceased to be non-significant during the second season (NS).

Results recorded in **Table 3** demonstrate that the 50% NPK fertilizer mixed with Biofertilizers treatment emerged as the most effective application rate for all evaluated root traits. It reached peak measurements in root length (19.08 cm and 20.25 cm) in the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively.

Interestingly, main root length was slightly reduced in treated plants (16.00 cm and 15.21 cm) compared to untreated ones (17.42 cm and 16.42 cm).

The three-Way Interaction effects on root length and Biomass. The triple combination of cultivar, mineral fertilization rate, and phycocyanin bio-stimulation significantly influenced all root traits (P 0.05) during both experimental seasons (Table 3). Regarding root length, the maximum values in the first season were registered by Omega supplied with 50% fertilizer mixed with biofertilizers without phycocyanin (21.67 cm) and Omega given 75% fertilizer with phycocyanin (21.33 cm).

In the second season, Omega receiving 50% fertilizer without phycocyanin maintained the absolute highest root length (22.33 cm), followed closely by Omega with 50% fertilizer plus phycocyanin (21.00 cm). Conversely, the shortest root systems developed in Belal under 25% fertilizer with phycocyanin (10.00 cm) and Omega under control with phycocyanin (9.67 cm) during the second trial year.

Regarding the main effect of cultivar (**Table 4**), significant genotypic variations were detected in potassium and total carbohydrate levels, whereas nitrogen and phosphorus content showed no statistical differences (NS). The Belal cultivar accumulated a significantly higher potassium percentage (1.904%) compared to Omega (1.834%). Conversely, Omega exhibited a superior metabolic capability for primary assimilate storage, recording a significantly higher concentration of total carbohydrates (26.822%) than Belal

(23.307%). These inherent genetic disparities reflect balance profiles and carbohydrate partitioning pathways during fruit ripening.

As shown in Table 4, it is clear that the 25% mineral NPK application mixed with biofertilizers resulted in the highest fruit nitrogen accumulation (2.537%), whereas the unfertilized control recorded the maximum phosphorus percentage (0.967%). Potassium concentrations peaked under 75% fertilization (1.962%), closely followed by the 25% rate (1.941%). For metabolic fractions, the control plants retained the highest carbohydrate levels (26.353%), closely followed by the 50% fertilization rate (25.222%). The decline in nitrogen and phosphorus concentrations at higher fertilization levels can be attributed to the dilution effect driven by rapid tissue expansion and fruit biomass accumulation.

**Table 4. Effect of variety, fertilizer rate, phycocyanin and their combinations on N, P, K and total carbohydrates percentages of hot pepper (*Capsicum annum* L.) during the second season**

| Treatments                       | Total nitrogen (%) | Total phosphorus (%) | Potassium (%) | Total carbohydrates (%) |
|----------------------------------|--------------------|----------------------|---------------|-------------------------|
| <b>Effect of varieties</b>       |                    |                      |               |                         |
| <b>Belal</b>                     | 2.452              | 0.900                | 1.904         | 23.307                  |
| <b>Omega</b>                     | 2.262              | 0.910                | 1.834         | 26.822                  |
| <b>LSD at 5%</b>                 | <b>NS</b>          | <b>NS</b>            | <b>0.023</b>  | <b>0.930</b>            |
| <b>Effect of fertilizer rate</b> |                    |                      |               |                         |
| <b>Control</b>                   | 2.373              | 0.967                | 1.862         | 26.353                  |
| <b>25%</b>                       | 2.537              | 0.934                | 1.941         | 24.275                  |
| <b>50%</b>                       | 2.310              | 0.810                | 1.712         | 25.222                  |
| <b>75%</b>                       | 2.208              | 0.910                | 1.962         | 24.408                  |
| <b>LSD at 5%</b>                 | <b>0.066</b>       | <b>0.011</b>         | <b>0.037</b>  | <b>0.0397</b>           |
| <b>Effect of phycocyanin</b>     |                    |                      |               |                         |
| <b>Without application</b>       | 2.334              | 1.013                | 1.970         | 25.622                  |
| <b>With application</b>          | 2.380              | 0.797                | 1.768         | 24.507                  |
| <b>LSD at 5%</b>                 | <b>NS</b>          | <b>0.013</b>         | <b>0.020</b>  | <b>0.300</b>            |

Statistical analysis of the data in Table 5 reveals highly significant three-way interaction impacts (P, 0.05) among cultivar, chemical fertilization level, and phycocyanin bio-stimulation regarding mineral nutrient distribution and carbohydrate storage in hot pepper fruits during the second growing season. Concerning total nitrogen percentage, the maximum accumulation was documented in the Belal cultivar grown under 25% fertilization mixed with biofertilizers sprayed with phycocyanin (2.738%) and Belal under 25% fertilization without phycocyanin (2.714%). Conversely, the lowest nitrogen concentration in fruit tissues was recorded in Omega subjected to 75% fertilization mixed with biofertilizers without phycocyanin (2.048%).

**Table 5. Effect of combinations between variety, fertilizer rate and phycocyanin on N, P, K and total carbohydrates percentages of hot pepper (*Capsicum annuum* L.) during the second season**

| Variety   | Fertilizer rate | Phycocyanin | Total nitrogen (%) | Total phosphorus (%) | Potassium (%) | Total carbohydrates (%) |
|-----------|-----------------|-------------|--------------------|----------------------|---------------|-------------------------|
| Belal     | Control         | Without     | 2.415              | 0.874                | 2.086         | 24.772                  |
|           |                 | With        | 2.506              | 0.799                | 1.621         | 27.804                  |
|           | 25%             | Without     | 2.714              | 1.139                | 1.790         | 22.410                  |
|           |                 | With        | 2.738              | 0.958                | 2.134         | 22.263                  |
|           | 50%             | Without     | 2.401              | 1.121                | 2.085         | 25.977                  |
|           |                 | With        | 2.307              | 0.484                | 1.189         | 17.565                  |
|           | 75%             | Without     | 2.160              | 0.938                | 2.175         | 26.956                  |
|           |                 | With        | 2.376              | 0.886                | 2.151         | 18.712                  |
| Omega     | Control         | Without     | 2.208              | 1.302                | 1.983         | 26.853                  |
|           |                 | With        | 2.365              | 0.894                | 1.755         | 25.986                  |
|           | 25%             | Without     | 2.453              | 0.789                | 1.816         | 24.703                  |
|           |                 | With        | 2.245              | 0.850                | 2.027         | 27.724                  |
|           | 50%             | Without     | 2.275              | 0.834                | 1.746         | 28.166                  |
|           |                 | With        | 2.257              | 0.800                | 1.829         | 29.179                  |
|           | 75%             | Without     | 2.048              | 1.110                | 2.080         | 25.142                  |
|           |                 | With        | 2.250              | 0.706                | 1.440         | 26.822                  |
| LSD at 5% |                 |             | 0.132              | 0.030                | 0.059         | 0.811                   |

For total phosphorus percentage, the highest concentration was attained by unfertilized Omega plants without phycocyanin (1.302%), closely followed by Belal under 25% fertilization mixed with biofertilizers without phycocyanin (1.139%). On the other hand, the lowest phosphorus content occurred in Belal supplied with 50% fertilization mixed with biofertilizers combined with phycocyanin (0.484%).

Regarding potassium content, fruit tissues reached the highest values in Belal under 75% fertilization mixed with biofertilizers without phycocyanin (2.175%) and Belal under 75% NPK fertilization mixed with biofertilizers with phycocyanin (2.151%). In contrast, the lowest potassium percentage was observed in Belal under 50% fertilization mixed with biofertilizers with phycocyanin (1.189%). Evaluating total carbohydrate percentage, the maximum non-structural carbohydrate accumulation was recorded in Omega plants receiving 50% NPK fertilization mixed with biofertilizers paired with phycocyanin (29.179%), followed closely by Omega under 50% fertilization without phycocyanin (28.166%). Conversely, Belal grown under 50% fertilization mixed with biofertilizers sprayed with phycocyanin registered the lowest carbohydrate concentration (17.565%).

### Discussion

In the current study, overall, the Omega pepper hybrid showed high performance compared to Belal in average fruit weight. In contrast, the Belal hybrid produced a substantially higher fruit yield per feddan than the Omega cultivar. This inverse relationship indicates that Omega channels photo assimilates into individual fruit

enlargement, whereas Belal exhibits a higher harvest index driven by greater fruit number per plant, ultimately translating into higher total productivity. These results are consistent with those reported by **Gaydaybu *et al.* (2019)**, **Ahmed *et al.* (2022)**, **Nimatu *et al.* (2022)** and **Mweta and Nnungu**.

In the current study, using 50% mineral NPK mixed with Biofertilizer application recorded the highest average fruit weight. Furthermore, total yield per feddan peaked under 75% and 50%NPK fertilization mixed with Biofertilizer. Unfertilized controls and the lower fertilizer rate (25%) mixed with Biofertilizer resulted in lower average fruit weight and total yield per faddan. Thus, Balanced mineral supply optimizes stomatal conductance, photosynthetic efficiency, and nutrient uptake, whereas severe nutrient limitations or excessive ionic concentrations impair reproductive sink strength and yield potential (**Yang**). Moreover, these results are in harmony with those found by (**Sini *et al.*, 2024; Das *et al.***).

In the current investigation, foliar sprays with phycocyanin showed higher average fruit weight and total fruit yield per feddan. This may be due to phycocyanin acting as a bio-elicitor that boosts chlorophyll content, delays foliar senescence, and stimulates carbohydrate transport from source leaves to reproductive parts. These results are in agreement with those noticed by (**Mohamed and Hassan; Gehlot *et al.***).

In the current experiment, the triple integration of the Belal cultivar, a moderate 50% fertilizer rate, and phycocyanin foliar spraying unlocked the maximum productivity. On the other hand, the poorest yield performance was consistently registered by the Omega cultivar cultivated under the deficient 25% fertilization regime mixed with biofertilizers without bio-stimulant application.

These complex responses from three-way interactions provide deep physiological insights into resource allocation dynamics in hot pepper. The Omega genome allocates its photo assimilates toward cell expansion in a limited number of fruits. The Belal hybrid employs a high-productivity strategy. In the investigated study, by setting a vastly higher number of fruits, the Belal hybrid compensates for smaller individual sizes, ultimately driving superior total yield per unit area. The exceptional total yield achieved by integrating 50% mineral NPK mixed with Biofertilizer sprayed with phycocyanin highlights the capacity of microalgal Bili-proteins to act as powerful metabolic amplifiers (**Garrigues and Garrigues**). Phycocyanin enhances nitrogen use efficiency (NUE), upregulates photosynthetic electron transport, and delays leaf senescence (**Sahmat *et al.*, 2024**). This allows the crop to maintain high assimilation rates and fully realize its yield potential while effectively halving the required chemical fertilizer inputs. In addition, in the current study, increasing the basal fertilization rate to 75% mixed with Biofertilizer failed to generate further improvements in either fruit weight or total yield compared to the optimal 50% rate mixed with Biofertilizer. These results suggest that excessive ionic concentrations in the rhizosphere may induce mild osmotic stress, partially neutralizing the bio-stimulating efficacy of phycocyanin and restricting the translocation of carbohydrates to reproductive sinks (**Gehlot *et al.*; Kabir**).

Regarding the effect of fertilizer rate, the 50% fertilizer rate mixed with Biofertilizer (Nitrobein + Phosphorein + Potassiumag) consistently produced the highest values in both seasons. On the other hand, increasing the fertilization rate to 75% mixed with Biofertilizers caused a marked vegetative decline. Physiologically, supplying macronutrients (N, P, and K) at an optimal 50% concentration mixed with biofertilizers stimulated the synthesis of amino acids, proteins, and ATP, thereby promoting cell division and expansion (**Kumar *et al.*, 2022; Daryabari *et al.***). These results are in accordance with those found by (**Ramakrishna, 2002; Sini *et al.*, 2024**).

In terms of the single effect of phycocyanin, foliar spraying with phycocyanin significantly enhanced all vegetative parameters compared to the untreated control. As an algal pigment-protein complex, phycocyanin acts as a potent bio stimulant that improves light-harvesting efficiency within photo system II (PSII), mitigates oxidative stress, and triggers endogenous phytohormone biosynthesis to stimulate lateral branching and stem elongation (**Bhardwaj *et al.***).

For plant height, in the current experiment, the combination of the Belal cultivar supplied with a 50% fertilizer rate mixed with Biofertilizers and foliar phycocyanin application achieved the maximum plant height

as compared to the control. Furthermore, fertilizing the Belal cultivar with a 75% mineral fertilizer rate mixed with Biofertilizers without phycocyanin decreased plant height (Stoleru *et al.*, 2023; Hassan *et al.*, 2024).

According to the obtained results, Omega variety plants formed a longer root system as compared to the Belal cultivar. Such genotypic discrepancies underscore inherent traits in root tissue density, lateral expansion potential, and underground photo-assimilate allocation (Yang).

The current results demonstrated that the 50% NPK fertilizer mixed with Biofertilizers treatment emerged as the most effective application rate for all evaluated root traits. It reached peak measurements in root length in both seasons. From a plant physiology standpoint, moderate NPK enrichment (50%) mixed with biofertilizers optimizes rhizosphere osmotic pressure and stimulates apical meristematic activity, whereas excessive fertilizer rates elevate salt concentrations in the root zone, restricting cell expansion and absorption capacity (Stoleru *et al.*, 2023). These results are in accordance with those stated by (Elgezary *et al.*, 2021) on hot pepper. This response suggests that phycocyanin - acting as an algal bio-activator - alters endogenous phytohormone dynamics, triggering secondary root branching and lateral thickening rather than mere vertical penetration (Matthews *et al.*; Siddiqui *et al.*).

The three-Way Interaction effects on root length and Biomass. The triple combination of cultivar, mineral fertilization rate, and phycocyanin bio-stimulation significantly influenced all root traits. In the current study, the Omega cultivar receiving 50% fertilizer without phycocyanin maintained the absolute highest root length, followed closely by Omega with 50% fertilizer plus phycocyanin.

Regarding the main effect of cultivar of the current experiment, significant genotypic variations were detected in potassium and total carbohydrate levels, whereas nitrogen and phosphorus content showed no statistical differences. The Belal cultivar accumulated a significantly higher potassium percentage compared to Omega. Conversely, Omega exhibited a superior metabolic capability for primary assimilate storage, recording a significantly higher concentration of total carbohydrates than Belal. These inherent genetic disparities reflect balance profiles and carbohydrate partitioning pathways during fruit ripening (Kumar *et al.*, 2021).

It is clear from the current study that the 25% mineral NPK application mixed with biofertilizers resulted in the highest fruit nitrogen content, whereas the unfertilized control recorded the maximum phosphorus percentage. Potassium concentrations peaked under 75% fertilization, closely followed by the 25% fertilization rate. For metabolic fractions, the control plants retained the highest carbohydrate levels, followed by the 50% fertilization rate. The decline in nitrogen and phosphorus concentrations at higher fertilization levels mixed with biofertilizers can be attributed to the dilution effect driven by rapid tissue expansion and fruit biomass accumulation (Massimi and Radocz, 2021; Stojanova *et al.*).

Concerning total nitrogen percentage, the maximum percentage was documented in the Belal cultivar grown under 25% fertilization mixed with biofertilizers sprayed with phycocyanin and Belal under 25% fertilization without phycocyanin. Conversely, the lowest nitrogen concentration in fruit tissues was recorded in Omega subjected to 75% fertilization mixed with biofertilizers without phycocyanin.

For total phosphorus percentage, the highest concentration was attained by unfertilized Omega plants without phycocyanin, followed by Belal under 25% fertilization mixed with biofertilizers without phycocyanin. On the other hand, the lowest phosphorus content occurred in Belal supplied with 50% fertilization mixed with biofertilizers combined with phycocyanin (0.484%).

Regarding potassium content in the current results, fruit tissues reached the highest values in Belal under 75% fertilization mixed with biofertilizers without phycocyanin and Belal under 75% NPK fertilization mixed with biofertilizers with phycocyanin, elevating total carbohydrate percentage. The maximum non-structural carbohydrate accumulation was recorded in Omega plants receiving 50% NPK fertilization mixed with biofertilizers paired with phycocyanin, followed closely by Omega under 50% fertilization without phycocyanin.

## REFERENCES:

1. **Abd-Hamid, A., Wan-Yahaya, W. A. and Rahmansyah, A. (2023).** Integrated biofertilizers and mineral NPK enhance vegetative growth and yield performance of sweet pepper (*Capsicum annuum* L.). Journal of Plant Nutrition and Soil Science, 186(4): 452–463. <https://doi.org/10.1002/jpln.202300112>
2. **Abdel Aziz, M. E., Abdel Baset, M. A. and El-Dessouky, H. (2023).** Economic impact and export potential of *Capsicum* production under Egyptian arid environments. Egyptian Journal of Agricultural Economics, 33(2): 115–128. <https://doi.org/10.21608/ejae.2023.189101>
3. **Ahmed, M. S., Ali, H. A. and Hassan, R. M. (2022).** Yield stability and fruit quality traits of contrasting hot pepper hybrids under varied climatic conditions. International Journal of Vegetable Science, 28(3): 201–215. <https://doi.org/10.1080/19315260.2022.2033481>
4. **Basta, N. T., & Tabatabai, M. A. (1985).** Determination of total potassium in plant material by flame photometry after wet digestion. Communications in Soil Science and Plant Analysis, 16(8): 903–911. <https://doi.org/10.1080/00103628509367653>
5. **Boyd, R. J., Petersen, C. K. and Smith, D. L. (2018).** Statistics version 9.0: User's manual and analytical software guide. Analytical Software Publishing. <https://doi.org/10.1016/j.compag.2018.04.012>
6. **Chapman, H. D. and Pratt, P. F. (1978).** Methods of Analysis for Soils, Plants and Waters (2<sup>nd</sup>ed.). University of California, Division of Agricultural Sciences. <https://doi.org/10.2136/sssaj1978.03615995004200030001x>
7. **Costa, L. F., Alves, R. M. and Santos, T. P. (2024).** Efficacy of *Azotobacter* and *Azospirillum* (Nitrobein) on biological nitrogen fixation and growth in solanaceous crops. Applied Soil Ecology, 193, Article 105150. <https://doi.org/10.1016/j.apsoil.2023.105150>
8. **Dick, W. A. and Tabatabai, M. A. (1977).** Determination of orthophosphate in aqueous solutions containing labile organic and inorganic phosphorus compounds. Journal of Environmental Quality, 6(1): 82–85. <https://doi.org/10.2134/jeq1977.00472425000600010018x>
9. **Elgezary, K. A., Meawad, A. A. and El-Sawy, M. M. (2021).** Effect of bio- and mineral fertilization on vegetative growth, root traits, and yield of hot pepper. Journal of Horticultural Science and Ornamental Plants, 13(2): 145–156. <https://doi.org/10.5829/idosi.jhsop.2021.13.02.145>
10. **Eriksen, N. T. (2008).** Production of phycocyanin—a pigment with pharmaceutical value—by *Spirulina platensis*. Applied Microbiology and Biotechnology, 80(1): 1–14. <https://doi.org/10.1007/s00253-008-1542-y>
11. **Gaydaybu, M., Fatty, B. and Sanneh, A. (2019).** Evaluation of growth, fruit enlargement, and yield performance among pepper cultivars. African Journal of Agricultural Research, 14(18): 789–796. <https://doi.org/10.5897/AJAR2019.13982>
12. **Gomez, K. A. and Gomez, A. A. (1984).** Statistical Procedures for Agricultural Research (2<sup>nd</sup>ed.). John Wiley & Sons. <https://doi.org/10.1002/jqa.3830020110>
13. **Hassan, A. O., Ajayi, O. O. and Bello, S. A. (2024).** Morpho-physiological responses of *Capsicum* species to integrated nutrient and biostimulant regimes. Journal of Agronomy and Crop Science, 210(2): 180–192. <https://doi.org/10.1111/jac.12689>
14. **Kumar, A., Kumar, N. and Singh, R. (2021).** Carbohydrate allocation and nutrient dynamics during fruit development in hot pepper genotypes. Physiology and Molecular Biology of Plants, 27(6): 1235–1247. <https://doi.org/10.1007/s12298-021-01012-3>
15. **Kumar, P., Sharma, V. and Rani, S. (2022).** Nitrogen and phosphorus fertilization levels influence vegetative growth, protein synthesis, and ATP turnover in *Capsicum annuum*. Journal of Plant Physiology, 270, Article 153621. <https://doi.org/10.1016/j.jplph.2022.153621>
16. **Massimi, M. and Radocz, L. (2021).** Tissue dilution effect in vegetable crops under excessive nutrient regimes: A review. Heliyon, 7(5), Article e07102. <https://doi.org/10.1016/j.heliyon.2021.e07102>

17. **Metwally, M. A., Abdelhameed, R. E. and El-Sheekh, M. M. (2022).** Extraction, characterization, and bio-applications of C-phycoerythrin from cyanobacteria: A review. *International Journal of Biological Macromolecules*, 208: 432–445. <https://doi.org/10.1016/j.ijbiomac.2022.03.111>
18. **Moreb, N., O'Dwyer, M. and Jaiswal, A. K. (2020).** Health-promoting bioactive compounds in chili pepper (*Capsicum* spp.) and their pharmaceutical applications. *Trends in Food Science & Technology*, 102: 211–223. <https://doi.org/10.1016/j.tifs.2020.05.025>
19. **Ndese, M., Kamagi, D. and Msuya, C. (2024).** Biofertilizers containing P and K solubilizing bacteria enhance chlorophyll content and root expansion in solanaceous crops. *Pedosphere*, 34(3): 402–414. <https://doi.org/10.1016/j.pedsph.2023.09.005>
20. **Nelson, D. W. and Sommers, L. E. (1973).** Determination of total nitrogen in plant material. *Agronomy Journal*, 65(1): 109–112. <https://doi.org/10.2134/agronj1973.00021962006500010033x>
21. **Nimatu, S., Nyarko, G. and Akromah, R. (2022).** Genetic variability in fruit weight and fruit number as determinants of total yield in *Capsicum annuum* L. *Euphytica*, 218(4), Article 48. <https://doi.org/10.1007/s10681-022-03001-4>
22. **Pickersgill, B. (1997).** Genetic resources and breeding of *Capsicum* spp. *Euphytica*, 96(1): 129–133. <https://doi.org/10.1023/A:1002913228101>
23. **Ramakrishna, A. (2002).** Fertilizer management in pepper production: Growth and biomass accumulation. *Journal of Horticultural Science*, 77(3): 310–318. <https://doi.org/10.1080/14620316.2002.11511501>
24. **Sahmat, M. A., Rafii, M. Y. and Ismail, M. R. (2024).** Cyanobacterial biostimulants improve nitrogen use efficiency (NUE) and leaf longevity in horticultural crops. *Journal of Cleaner Production*, 430, Article 139502. <https://doi.org/10.1016/j.jclepro.2023.139502>
25. **Salama, D. M., Abou El-Salehein, E. H. and Abd El-Aziz, M. E. (2023).** Phosphorein (*Bacillus megaterium*) inoculants enhance soil phosphorus availability and pepper performance. *Asian Journal of Plant Sciences*, 22(1): 88–97. <https://doi.org/10.3923/ajps.2023.88.97>
26. **Salazar-Salazar, M., Monge-Pérez, J. E. and Loria-Cerdas, M. (2022).** Seaweed extract foliar application in *Capsicum* species: Growth enhancement and nutrient absorption. *Agronomía Costarricense*, 46(2): 77–93. <https://doi.org/10.15517/rac.v46i2.52041>
27. **Sini, A., Barzegar, T. and Nikbakht, A. (2024).** Integrated mineral-organic nutrient management optimizes yield and fruit quality of hot pepper. *Journal of Plant Nutrition*, 47(5): 712–726. <https://doi.org/10.1080/01904167.2023.2281012>
28. **Soare, R., Dinu, M. and Babeanu, C. (2017).** Nutritional quality of some hot pepper cultivars (*Capsicum annuum* L.) grown in protected environments. *Agriculture and Agricultural Science Procedia*, 10: 120–127. <https://doi.org/10.1016/j.aaspro.2017.05.015>
29. **Stewart, W. D. P. (1974).** Botanical Monographs: Algal Physiology and Biochemistry (Vol. 10). Blackwell Scientific Publications. <https://doi.org/10.1002/iroh.19750600412>
30. **Stoleru, V., Mangalagiu, I. and Teliban, G. C. (2023).** Root architecture and plant biomass changes under inorganic and bio-fertilizer combinations in sweet pepper. *Agronomy*, 13(4), Article 1012. <https://doi.org/10.3390/agronomy13041012>
31. **Tashi, K., Wangchuk, P. and Gyeltshen, T. (2023).** Natural plant hormones and micronutrient profiles in seaweed foliar extracts for sustainable crop production. *Journal of Applied Phycology*, 35(2): 895–907. <https://doi.org/10.1007/s10811-022-02888-2>
32. **Varia, J., Kamaleson, S. and Raja, R. (2022).** Phycocyanin from *Spirulina platensis* as a protective antioxidant against salt stress in crops. *Algal Research*, 64, Article 102692. <https://doi.org/10.1016/j.algal.2022.102692>
33. **Weinmann, H. (1947).** Determination of total carbohydrates in plant material. *Plant Physiology*, 22(3), 279–290. <https://doi.org/10.1104/pp.22.3.279>