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## PHYTOHORMONES, BIO- AND MINERAL FERTILIZERS EFFECTS ON THE GROWTH AND SECONDARY COMPOUNDS OF CHAMOMILE (*MATRICARIA CHAMOMILLA* L.)

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### SUMMARY

Chamomile (*Matricaria chamomilla* L.) is one of the most renowned medicinal plants and a winter annual herbaceous long-day crop. The presented research on the chamomile (*M. chamomilla* L.) scrutinized the effects of using biostimulants (Bio health), mineral fertilizers (NPK), and phyto regulator (Kinetin) on the growth and active ingredients of chamomile plants and select the best combination of fertilizers and growth regulator for best results. The experiment commenced in a randomized complete block design (RCBD) with three replications. The experimental treatments included three factors; the first factor was the biofertilizer with two levels (0 and 10 g L<sup>-1</sup>), the second was NPK fertilizer addition with three levels (0, 300, and 500 kg ha<sup>-1</sup>), and the third was the foliar spray of kinetin with three concentrations (0, 50, and 100 mg L<sup>-1</sup>). The hydro-distilled essential oil of *Matricaria chamomilla* L. underwent analysis by GC. The highest floral inflorescence (384.33 floral inflorescence plant<sup>-1</sup>), inflorescence dry weight (0.11 g plant<sup>-1</sup>), volatile oil (1.54%), and turpentine (13.33%) were distinct with the combination and interaction of the three factors, biofertilizer (10 g L<sup>-1</sup>), NPK (300 kg ha<sup>-1</sup>), and kinetin (50 mg L<sup>-1</sup>).

**Keywords:** Biofertilizer, chamomile, NPK fertilizer, phyto regulator, turpentine, volatile oil

**Key findings:** The interaction and combined application of biological and mineral fertilizers along with kinetin provided fruitful and productive to meet the nutritional needs of the growth indicators and inflorescences, which boosted the medicinal importance of the chamomile plant.

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## INTRODUCTION

In addition to ecological and cultural aspects, medicinal plants are economically important for medicinal purposes. Hence, these plant substances are beneficial in treating various diseases and fighting against pathogens and infections. Since ancient times, medicinal plants have been a rich source of effective and safe ingredients that are safe to use as traditional medicines. Research revealed that more than 64% of the world's population relies on medicinal plants to treat their numerous health problems (Al-Asadi, 2018).

The chamomile (*Matricaria chamomilla* L.) is one of the most famous medicinal plants belonging to the Asteraceae family, a winter annual and herbaceous long-day plant characterized by an aromatic scent. Its use has been extensive since ancient times in most parts of the world for its medical and therapeutic significance because it contains volatile oils, phenolic compounds, flavonoids, coumarins, glycosides, tannins, resin, and mucilages (Haile *et al.*, 2022).

Chamomile also contains vital biological components, such as Chamazulene,  $\alpha$ -pinene, Limonene, Camphene, and terpinene, and is beneficial in the treatment of neuronal, brain-booster, rheumatism, pain, and headache (Tai *et al.*, 2020). It also helps treat internal infections and insulin resistance in patients with type-2 diabetes (Zemestani *et al.*, 2018). It also contributes to integrated nutrient management by preserving soil fertility and increasing crop productivity through required fertilizer management by following the mineral fertilizers with the biological and organic fertilizers application (Akbari *et al.*, 2022).

Bio-stimulants are one of the most successful technologies for achieving apparent enhancement in growth traits and the content of biologically active compounds in medicinal plants (Rafiee *et al.*, 2016). It is due to their applicable role in supplying the plant with nutrients, organic acids, and phytohormones, improving soil texture and composition, raising the efficiency of the plants, and promoting their growth through symbiotic living between

fungus and plants (Aboohanah *et al.*, 2019). The research on the secretion of glomalin, which are sticky glycoprotein compounds that work to hold the soil for minutes, increase the stability of their assemblies, and improve their ability to retain water and nutrients and prevent from losing them (Haselwandter *et al.*, 2020; Al-Silmawy and Abdul-Ratha, 2023).

Kinetin is one of the plant growth regulators that stimulates the growth parameters by stimulating the cell division, increasing their size, and eliminating apical dominance, which positively reflects on increasing the branches' number and flowers and delaying aging. It is also vital in attracting nutrients toward the plant parts treated with it, stimulating photosynthetic enzymes, and hindering chlorophyll demolition (Al-Asadi and Al-Khikani, 2019).

The study conducted by Al-Khazraji (2017) on anise plants showed that using NPK fertilizer and foliar application of kinetin provided the best results in growth parameters and effective biological compounds. Study findings by Bączek *et al.* (2019) revealed that the treatment with *Matricaria recutita* L. significantly outperformed and showed the best values for the number of floral inflorescences and their fresh and dry weights. Ahmad and Rabea (2022) attained the best results in the radical, vegetative, and floral growth and production of the biologically active compounds in the chamomile plant by treating it with mycorrhiza with foliar application of cytokinin. Ahmad *et al.* (2022) confirmed that fertilizing watercress with mineral NPK fertilizer enunciated a significant effect and recorded the highest values in vegetative growth parameters.

From the above discussion, the presented study sought the effects of bio- and mineral fertilizers and growth regulator (kinetin) and their interactions on the growth of the chamomile plant and further determine the best level of plant productivity and raise plant efficiency through the proposed nutritional program in enhancing the proportion of volatile oils and other bioactive compounds in chamomile (*M. chamomilla* L.).

## MATERIALS AND METHODS

The latest experiment on chamomile (*M. chamomilla* L.) commenced in the crop season 2022–2023 at the Botanical Garden, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, Iraq. The study aimed to determine the effects of the bio-mineral (NPK) fertilizers and growth regulator (kinetin) separately and their interaction on the growth parameters and the production of medically effective biological compounds in chamomile plants.

The experimental design was a randomized complete block design (RCBD) with three replications. The trial treatments included three factors. The first factor was the biofertilizer with two levels (0 and 10 g L<sup>-1</sup>) symbolized by B<sub>1</sub> and B<sub>2</sub>. The second factor was the addition of NPK fertilizer with three levels (0, 300, and 500 kg ha<sup>-1</sup>) marked as c<sub>1</sub>, c<sub>2</sub>, and c<sub>3</sub>. The third factor was the foliar application of kinetin with three concentrations (0, 50, and 100 mg L<sup>-1</sup>) labeled k<sub>1</sub>, k<sub>2</sub>, and k<sub>3</sub>. The biostimulant (Biohealth) injection ensued at the bottom of each plant having a distance of 10 cm from the plant, using a 10 ml medical syringe twice. The first was when five true leaves appeared (after 30 days of planting), while the second was after two weeks. The Biohealth came from the Belgian company (Unifert), and it was a granular mixture of several components, i.e., Trichoderma (5%), Bacillus subtilis strains (5%), Marine algae

(5%), Humic acid (75%), and water (10%). The second factor was mineral fertilizer NPK (20:20:20) added twice (before sowing and after 40 days of planting). The growth regulator (Kinetin) application was through foliar spraying in the early morning until purely wet with a hand spray twice. The first spray transpired 50 days after planting, and the second continued after two weeks after the first spray. The total number of experimental units was 54.

For soil analysis, 10 random soil samples collected progressed from different parts of the soil intended for cultivation, mixing well for homogenization, and then analyzed to know their chemical and physical properties (Table 1) (Page *et al.*, 1982). The chamomile (*M. chamomilla* L.) seeds cultivation proceeded in mid-October 2022 in three panels with dimensions of 3.5 m<sup>2</sup> × 1.5 m<sup>2</sup> for each panel. Each main panel underwent subdividing into 18 experimental units with an area of 0.4 m<sup>2</sup> × 0.4 m<sup>2</sup> for each experimental unit and a distance of 75 cm between each panel.

### Characteristics measured and statistical analysis

Recording the data on various growth-related characteristics of chamomile (*M. chamomilla* L.) comprised the dry matter in the vegetative total (%), the number of floral inflorescences per plant, dry weight of the floral inflorescence (g), and the main root diameter (mm plant<sup>-1</sup>).

**Table 1.** Physical and chemical properties of the experimental field soil.

| Properties       | Values | Units                 |
|------------------|--------|-----------------------|
| pH               | 7.60   |                       |
| EC               | 0.56   | dS m <sup>-1</sup>    |
| O.M              | 0.92   | Gm kg <sup>-1</sup>   |
| N                | 12.50  | mg kg <sup>-1</sup>   |
| P                | 4.10   | mg kg <sup>-1</sup>   |
| K                | 25.10  | mg kg <sup>-1</sup>   |
| Mg <sup>++</sup> | 0.55   | mmol. L <sup>-1</sup> |
| Na <sup>+</sup>  | 2.16   |                       |
| Ca <sup>++</sup> | 1.60   |                       |
| HCO <sub>3</sub> | 0.60   |                       |
| Cl               | 4.70   |                       |
| Silt             | 270.0  | g kg <sup>-1</sup>    |
| Clay             | 70.0   |                       |
| Sand             | 660.0  |                       |
| Texture          |        | Loamy sand            |

In the chamomile (*M. chamomilla* L.), the volatile oil extraction used the water distillation method, as reported by Chalchat *et al.* (1991). The volatile oil (%) estimates followed the equation reported by Guenther (1972).

$$\text{Volatile oil (\%)} = \frac{\text{weight of the resulting oil (g)}}{\text{weight of plant sample (g)}} \times 100$$

Analyzing the turpentine compound (%) in the volatile oil of chamomile (*M. chamomilla* L.) happened in the Laboratory of the Department of Environment and Water, Ministry of Science and Technology, Iraq, using a Japanese-origin gas chromatography device model Shimadzu 2010, the Flame ionization (FID), and employing a capillary separation column type (DM-5MS) with lengths (30 m × 0.25 μm × 0.25 mm) (SE-54). The temperatures of the detector and injection areas were 280 °C and 340 °C, respectively, with the temperature of the separation column gradually started from 100 °C–300 °C, with a high rate of 10 degrees minute<sup>-1</sup>. Inert nitrogen gas served as a carrier gas at 100 KPa.

All the recorded data on various growth traits and biochemical compounds sustained statistical analysis using the statistical program GenStat Version 7 as the required analysis of

variance (ANOVA). The treatment means further comparison and separation using the least significant difference (LSD<sub>0.05</sub>) test.

## RESULTS

### Dry matter of shoots

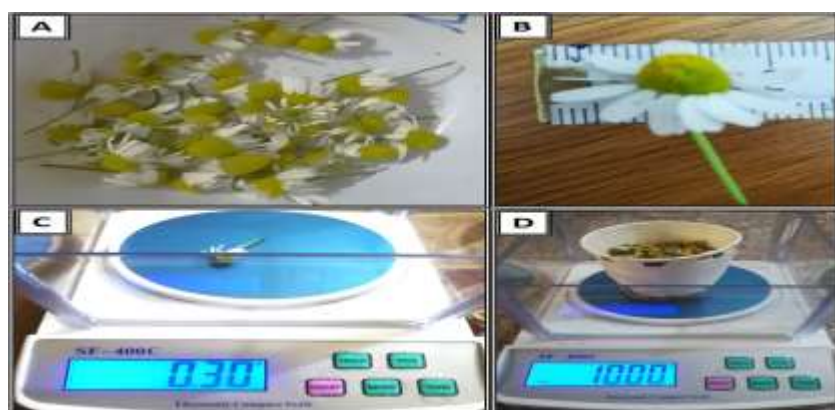
The results revealed that using biological fertilizers (10 g L<sup>-1</sup>) showed the highest dry matter percentage (18.26%) compared with the control treatment (16.95%) in chamomile (*M. chamomilla* L.) (Table 2). The NPK fertilizer (300 kg ha<sup>-1</sup>) emerged with 19.67%, while the lowest amount appeared in the control treatment (14.90%). The kinetin (50 mg L<sup>-1</sup>) was significantly superior and showed 19.32% dry matter compared with the control treatment (17.87%). The bilateral interaction of NPK fertilizer (300 kg ha<sup>-1</sup>) and kinetin (50 mg L<sup>-1</sup>) significantly outperformed and gave the maximum dry matter (22.00%) versus the control treatment (13.67%). In the triple interaction among the three factors (biological fertilizers 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>), the highest percentage of dry matter (22.80%) surfaced as compared with the control treatment (13.13%).

**Table 2.** Effect of Bio- and NPK fertilizers and kinetin on the dry matter of shoot (%) in chamomile plant.

| NPK                                       | Biostimulant                           | Kinetin            |   |  | C×B   | Mean C |
|---|--|--------------------|---|--|-------|--------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |       |        |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 13.13              | 14.87                                   | 14.80                                    | 14.27 | 14.90  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 14.20              | 16.40                                   | 16.00                                    | 15.53 |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 16.60              | 21.20                                   | 19.20                                    | 19.00 | 19.67  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 17.73              | 22.80                                   | 20.47                                    | 20.33 |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 15.00              | 19.80                                   | 17.93                                    | 17.58 | 18.24  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 17.07              | 20.87                                   | 18.80                                    | 18.91 |        |
| LSD <sub>0.05</sub>                       |  | 0.54               |   |  | 0.31  | 0.22   |
|   |  | C×K                |   |  |       |        |
| C <sub>1</sub> (0)                        |  | 13.67              | 15.63                                   | 15.40                                    | 0.38  |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 17.17              | 22.00                                   | 19.83                                    |       |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 16.03              | 20.33                                   | 18.37                                    |       |        |
| Mean B                                    |  | B×K                |   |  |       |        |
| B <sub>1</sub> (0)                        |  | 14.91              | 18.62                                   | 17.31                                    | 16.95 |        |
| B <sub>2</sub> (10 g L <sup>-1</sup> )    |  | 16.33              | 20.02                                   | 18.42                                    | 18.26 |        |
| LSD <sub>0.05</sub>                       |  | 0.31               |   |  | 0.18  |        |
|   |  | K Means            |   |  |       |        |
|   |  | 15.62              | 19.32                                   | 17.87                                    |       |        |
| LSD <sub>0.05</sub>                       |  | 0.22               |   |  |       |        |

**Table 3.** Effect of Bio- and NPK fertilizers and kinetin on the number of inflorescences in chamomile plant (inflorescence plant<sup>-1</sup>).

| NPK                                       | Biostimulant                           | Kinetin            |   |  | C×M    | Mean C |
|---|--|--------------------|---|--|--------|--------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |        |        |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 71.90              | 145.67                                  | 112.00                                   | 109.86 | 132.59 |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 89.00              | 197.00                                  | 180.00                                   | 155.33 |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 196.33             | 346.00                                  | 312.67                                   | 285.00 | 307.11 |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 250.00             | 384.33                                  | 353.33                                   | 329.22 |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 146.67             | 291.67                                  | 254.00                                   | 230.78 | 259.56 |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 210.00             | 336.67                                  | 318.33                                   | 288.33 |        |
| LSD <sub>0.05</sub>                       |  | 17.133             |   |  | 9.892  | 6.995  |
|   |  | C×K                |   |  |        |        |
| C <sub>1</sub> (0)                        |  | 80.45              | 171.33                                  | 146.00                                   | 12.115 |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 223.17             | 365.17                                  | 333.00                                   |        |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 178.33             | 314.17                                  | 286.17                                   |        |        |
| Mean B                                    |  | B×K                |   |  |        |        |
| B <sub>1</sub> (0)                        |  | 138.30             | 261.11                                  | 226.22                                   | 208.54 |        |
| B <sub>2</sub> (10 g L <sup>-1</sup> )    |  | 183.00             | 306.00                                  | 283.89                                   | 257.63 |        |
| LSD <sub>0.05</sub>                       |  | 9.892              |   |  | 5.711  |        |
|   |  | K Means            |   |  |        |        |
|   |  | 160.65             | 283.56                                  | 255.06                                   |        |        |
| LSD <sub>0.05</sub>                       |  | 6.995              |   |  |        |        |



**Figure 1.** A) Flowers after harvesting, B) Flower Diameter around 23 mm, C and D) Dry weight of flower.

### Floral inflorescence

The results indicated that the treatment of biostimulant (10 g L<sup>-1</sup>) showed the utmost value of inflorescences per plant (257.63) compared with the control treatment (208.54 inflorescences plant<sup>-1</sup>) in chamomile (*M. chamomilla* L.) (Table 3). The highest value of inflorescence (307.11) was also evident with NPK fertilizer (300 kg ha<sup>-1</sup>), whereas the lowest resulted in the control treatment (132.59 inflorescences plant<sup>-1</sup>). The kinetin (50 mg L<sup>-1</sup>)

showed more inflorescences plant<sup>-1</sup> (283.56) than the control treatment (160.65 inflorescences plant<sup>-1</sup>) (Figure 1A). The interaction of NPK fertilizer (300 kg ha<sup>-1</sup>) and kinetin (50 mg L<sup>-1</sup>) exhibited significantly higher inflorescences plant<sup>-1</sup> (365.17) than the control treatment (80.45 inflorescences plant<sup>-1</sup>). The triple interaction (biological fertilizers 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) was significantly superior (384.33) compared with the control treatment (71.90 inflorescences plant<sup>-1</sup>) (Table 3).

### Dry weight of inflorescence

The results enunciated that biostimulant (10 g L<sup>-1</sup>) showed the highest dry weight of inflorescence plant<sup>-1</sup> (0.09 g plant<sup>-1</sup>) compared with the control treatment (0.08 g plant<sup>-1</sup>) in chamomile (*M. chamomilla* L.) (Table 4). The NPK fertilizer (300 kg ha<sup>-1</sup>) provided the maximum value of dry weight of inflorescence plant<sup>-1</sup> (0.09 g plant<sup>-1</sup>) compared with the control treatment (0.07 g plant<sup>-1</sup>). The kinetin (50 mg L<sup>-1</sup>) also exhibited significant superiority and provided the maximum dry weight of inflorescence plant<sup>-1</sup> (0.10 g plant<sup>-1</sup>) versus the control treatment (0.06 g plant<sup>-1</sup>) (Figure 1C). For the dry weight of inflorescence plant<sup>-1</sup>, the bilateral interaction of the NPK fertilizer (300 kg ha<sup>-1</sup>) and kinetin (50 mg L<sup>-1</sup>) was significantly superior (0.11 g plant<sup>-1</sup>) compared with the control treatment (0.05 g plant<sup>-1</sup>). The highest and lowest amount was evident at 0.12 and 0.05 g plant<sup>-1</sup>. The triple interaction (biofertilizers 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) also showed the highest dry weight of inflorescence plant<sup>-1</sup> (0.12 g plant<sup>-1</sup>), whereas the lowest was with the control treatment (0.05 g plant<sup>-1</sup>) (Table 4).

### Main root diameter

For the main root diameter in the chamomile (*M. chamomilla* L.) plant, the biostimulant (10 g L<sup>-1</sup>) showed the highest value (7.56 mm plant<sup>-1</sup>) compared with the control treatment (6.85 mm plant<sup>-1</sup>) (Table 5). The NPK fertilizer (300 kg ha<sup>-1</sup>) significantly exceeded the root diameter by recording the utmost value (8.71 mm plant<sup>-1</sup>) versus the control treatment (5.00 mm plant<sup>-1</sup>). The growth regulator (kinetin 50 mg L<sup>-1</sup>) provided the highest value for the main root diameter (8.41 mm plant<sup>-1</sup>), whereas the lowest was in the control treatment (5.73 mm plant<sup>-1</sup>). The bilateral interaction (NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) also gave the maximum value for the main root diameter (10.00 mm plant<sup>-1</sup>), and the lowest was at the control treatment (3.96 mm plant<sup>-1</sup>). The triple interaction (biofertilizer 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>)

revealed significant superior performance, exhibiting the topmost value for main root diameter (10.10 mm plant<sup>-1</sup>), while the minimum value was in the control treatment for the said trait (3.50 mm plant<sup>-1</sup>) (Table 5).

### Volatile oil (%)

The outcomes showed that the biostimulant (10 g L<sup>-1</sup>) significantly outperformed and provided the highest volatile oil (1.36%) compared with its control treatment (1.30%) in chamomile (*M. chamomilla* L.) (Table 6). The maximum volatile oil percentage (1.45%) occurred when applied with NPK fertilizer (300 kg ha<sup>-1</sup>), whereas the lowest value for the said trait was with the control treatment (1.15%). The kinetin (50 mg L<sup>-1</sup>) also showed the highest volatile oil content (1.41%) compared to the control treatment, which recorded 1.22%. The coefficients of bilateral interaction (NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) enunciated the utmost volatile percentage (1.54%) versus the control treatment (1.05%). The triple interaction treatment (biofertilizer 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) exhibited the highest percentage of volatile oil (1.54%), and the control treatment provided the lowest oil content (1.02%) (Table 6 and Figure 1D).

### Turpentine (%)

For turpentine content in the volatile oil of chamomile (*M. chamomilla* L.), the results showed that the biostimulant (10 g L<sup>-1</sup>) significantly exceeded and gave the highest percentage (12.05%) compared with the control treatment (11.53%) (Table 7). The NPK fertilizer (300 kg ha<sup>-1</sup>) also appeared with the maximum turpentine content (12.66%) compared with the control treatment (10.67%). The growth regulator kinetin (50 mg L<sup>-1</sup>) significantly exceeded other factors by showing the highest value for the said trait (12.55%) versus the control treatment (11.04%). The interaction of NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup> also gave the utmost value of turpentine (13.74%) compared with its control treatment (10.27%). The triple

interaction (biofertilizer 10 g L<sup>-1</sup> + NPK fertilizer 300 kg ha<sup>-1</sup> + kinetin 50 mg L<sup>-1</sup>) further revealed the utmost content of turpentine (13.96%) compared with the lowest value recorded by the control treatment (10.16%) (Table 7 and Figure 2).

## DISCUSSION

Based on the presented findings, it is apparent that the studied factors represented by the

biostimulant inoculum and NPK fertilizer significantly affected most of the measured characteristics of the chamomile plant, and the study factors influenced in one direction in raising the plant efficiency (Wang *et al.*, 2015). These bio- and NPK fertilizers also boosted the growth parameters synthesized through accumulating photosynthetic products that form carbon structures in the biosynthesis of terpene compounds (Alaarage and Alamery, 2023).

**Table 4.** Effect of Bio- and NPK fertilizers and kinetin on the dry weight of inflorescence in chamomile plant (g plant<sup>-1</sup>).

| NPK                                       | Biostimulant                           | Kinetin            |   |  | C×B   | Mean C |
|---|--|--------------------|---|--|-------|--------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |       |        |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 0.05               | 0.08                                    | 0.07                                     | 0.06  | 0.07   |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 0.06               | 0.09                                    | 0.08                                     | 0.08  |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 0.06               | 0.10                                    | 0.08                                     | 0.08  | 0.09   |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 0.08               | 0.12                                    | 0.08                                     | 0.09  |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 0.05               | 0.10                                    | 0.09                                     | 0.08  | 0.09   |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 0.08               | 0.11                                    | 0.10                                     | 0.10  |        |
| LSD <sub>0.05</sub>                       |  | 0.013              |   |  | 0.008 | 0.005  |
|   |  | C×K                |   |  |       |        |
| C <sub>1</sub> (0)                        |  | 0.05               | 0.08                                    | 0.07                                     | 0.009 |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 0.07               | 0.11                                    | 0.08                                     |       |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 0.07               | 0.10                                    | 0.10                                     |       |        |
| Mean B                                    |  | B×K                |   |  |       |        |
| B <sub>1</sub> (0)                        |  | 0.05               | 0.09                                    | 0.08                                     | 0.08  |        |
| B <sub>2</sub> (7 g L <sup>-1</sup> )     |  | 0.07               | 0.11                                    | 0.09                                     | 0.09  |        |
| LSD <sub>0.05</sub>                       |  | 0.008              |   |  | 0.002 |        |
|   |  | K Means            |   |  |       |        |
|   |  | 0.06               | 0.10                                    | 0.08                                     |       |        |
| LSD <sub>0.05</sub>                       |  | 0.005              |   |  |       |        |

**Table 5.** Effect of Bio- and NPK fertilizers and kinetin on the main root diameter in chamomile plant (mm plant<sup>-1</sup>).

| NPK                                       | Biostimulant                           | Kinetin            |   |  | C×B   | Mean C |
|---|--|--------------------|---|--|-------|--------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |       |        |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 3.50               | 5.29                                    | 4.91                                     | 4.5   | 5.0    |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 4.43               | 6.16                                    | 5.69                                     | 5.43  |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 6.66               | 9.91                                    | 8.86                                     | 8.48  | 8.71   |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 7.23               | 10.10                                   | 9.53                                     | 8.95  |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 5.73               | 9.23                                    | 7.53                                     | 7.50  | 7.90   |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 6.86               | 9.76                                    | 8.30                                     | 8.30  |        |
| LSD <sub>0.05</sub>                       |  | 0.062              |   |  | 0.036 | 0.025  |
|   |  | C×K                |   |  |       |        |
| C <sub>1</sub> (0)                        |  | 3.96               | 5.72                                    | 5.30                                     | 0.044 |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 6.95               | 10.00                                   | 9.20                                     |       |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 6.29               | 9.49                                    | 7.91                                     |       |        |
| Mean B                                    |  | B×K                |   |  |       |        |
| B <sub>1</sub> (0)                        |  | 5.29               | 8.14                                    | 7.10                                     | 6.85  |        |
| B <sub>2</sub> (10 g L <sup>-1</sup> )    |  | 6.17               | 8.67                                    | 7.84                                     | 7.56  |        |
| LSD <sub>0.05</sub>                       |  | 0.036              |   |  | 0.021 |        |
|   |  | K Means            |   |  |       |        |
|   |  | 5.73               | 8.41                                    | 7.47                                     |       |        |
| LSD <sub>0.05</sub>                       |  | 0.025              |   |  |       |        |

**Table 6.** Effect of Bio- and NPK fertilizers and kinetin on the volatile oil (%) in chamomile plant.

| NPK                                       | Biostimulant                           | Kinetin            |   |  | Interaction<br>C×B | Mean<br>C |
|---|--|--------------------|---|--|--------------------|-----------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |                    |           |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 1.02               | 1.16                                    | 1.14                                     | 1.11               | 1.15      |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 1.09               | 1.25                                    | 1.24                                     | 1.19               |           |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 1.30               | 1.53                                    | 1.45                                     | 1.43               | 1.45      |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 1.37               | 1.54                                    | 1.49                                     | 1.47               |           |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 1.21               | 1.47                                    | 1.39                                     | 1.36               | 1.39      |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 1.33               | 1.51                                    | 1.42                                     | 1.42               |           |
| LSD <sub>0.05</sub>                       |  | 0.029              |   |  | 0.017              | 0.012     |
| C <sub>1</sub> (0)                        |  | 1.05               | 1.21                                    | 1.19                                     | 0.020              |           |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 1.34               | 1.54                                    | 1.47                                     |                    |           |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 1.27               | 1.49                                    | 1.41                                     |                    |           |
| Mean B                                    |  | B×K                |   |  |                    |           |
| B <sub>1</sub> (0)                        |  | 1.18               | 1.39                                    | 1.33                                     | 1.30               |           |
| B <sub>2</sub> (10 g L <sup>-1</sup> )    |  | 1.26               | 1.44                                    | 1.38                                     | 1.36               |           |
| LSD <sub>0.05</sub>                       |  | 0.017              |   |  | 0.010              |           |
|   |  | K Means            |   |  |                    |           |
|   |  | 1.22               | 1.41                                    | 1.36                                     |                    |           |
| LSD <sub>0.05</sub>                       |  | 0.012              |   |  |                    |           |

**Table 7.** Effect of Bio- and NPK fertilizers and kinetin on the turpentine (%) in the volatile oil in chamomile plant.

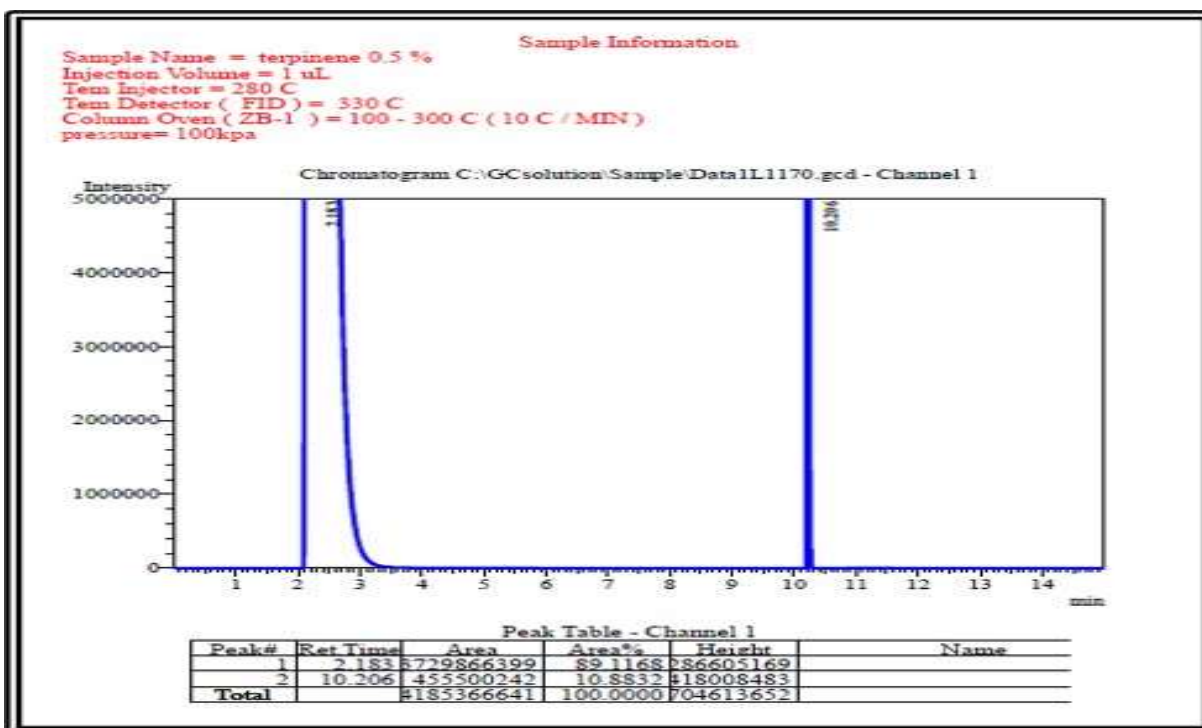
| NPK                                       | Biostimulant                           | Kinetin            |   |  | Interaction<br>C×B | Mean C |
|---|--|--------------------|---|--|--------------------|--------|
|   |  | K <sub>1</sub> (0) | K <sub>2</sub> (50 mg L <sup>-1</sup> ) | K <sub>3</sub> (100 mg L <sup>-1</sup> ) |                    |        |
| C <sub>1</sub> (0)                        | B <sub>1</sub> (0)                     | 10.16              | 10.59                                   | 10.47                                    | 10.40              | 10.67  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 10.38              | 11.32                                   | 11.12                                    | 10.94              |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) | B <sub>1</sub> (0)                     | 11.53              | 13.53                                   | 12.27                                    | 12.44              | 12.66  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 11.79              | 13.96                                   | 12.89                                    | 12.88              |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) | M <sub>1</sub> (0)                     | 10.80              | 12.60                                   | 11.83                                    | 11.74              | 12.04  |
|   | B <sub>2</sub> (10 g L <sup>-1</sup> ) | 11.59              | 13.33                                   | 12.10                                    | 12.34              |        |
| LSD <sub>0.05</sub>                       |  | 0.063              |   |  | 0.037              | 0.026  |
| C <sub>1</sub> (0)                        |  | 10.27              | 10.95                                   | 10.80                                    | 0.045              |        |
| C <sub>2</sub> (300 kg ha <sup>-1</sup> ) |  | 11.66              | 13.74                                   | 12.58                                    |                    |        |
| C <sub>3</sub> (500 kg ha <sup>-1</sup> ) |  | 11.19              | 12.96                                   | 11.96                                    |                    |        |
| Mean B                                    |  | B×K                |   |  |                    |        |
| B <sub>1</sub> (0)                        |  | 10.83              | 12.24                                   | 11.52                                    | 11.53              |        |
| B <sub>2</sub> (10 g L <sup>-1</sup> )    |  | 11.25              | 12.87                                   | 12.04                                    | 12.05              |        |
| LSD <sub>0.05</sub>                       |  | 0.037              |   |  | 0.021              |        |
|   |  | K Means            |   |  |                    |        |
|   |  | 11.04              | 12.55                                   | 11.78                                    |                    |        |
| LSD <sub>0.05</sub>                       |  | 0.026              |   |  |                    |        |

The reason may be due to the familiarity and efficiency of the symbiotic relationship between the roots of the chamomile plant and the biostimulant and the appropriate application method, the amount of inoculum encouraged in the early stages of growth, and the activity of the biostimulant and its effect in stimulating the secretion of cytokinins, auxins, and gibberellins (Bhat *et al.*, 2017). Additionally, it boosted the enzymes polyphenol oxidase, peroxidase, and cellulase

to produce biologically active compounds (Latif and Mustafa, 2019).

Increased vegetative growth parameters improve plants' ability to respond and encourage flowering, especially gibberellin and its effect on increasing the hormonal level, and control the direction of nutrient transmission to flowering, which is the storage place in the plants (Aboohanah *et al.*, 2019). The role of nitrogen in forming the porphyrin ring for the synthesis of chlorophyll pigment





**Figure 2.** Turpentine compounds (%) in the volatile oil.

(Chitra and Vinothini, 2021) and the formation of about 80% of the chlorophyll components has increased this pigment, which is one of the crucial factors for plant growth and development (Ali and Alshugeairy, 2023).

In addition to the synthesis of proteins, enzymes and amino acids also participate in synthesizing nitrogen bases, such as pyrimidine and purine (Taiz *et al.*, 2014), which, in turn, enter into the synthesis of nucleic acids that stimulate the production of cytokinins (Kieber and Schaller, 2018). It also has an association with phosphorus in the construction of cell membranes (Barita *et al.*, 2019), energy compounds and enzymatic accompaniments, such as NADH<sub>2</sub> and NADPH<sub>2</sub> that is vital in the process of redox occurring in the process of photosynthesis, respiration, and representation of carbohydrates and fatty acids (Salman, 2022).

Potassium activates several synthetic enzymes, redox and reduction enzymes, hydrogenase enzymes, transferases, and kinases enzymes (Xu *et al.*, 2020). It also activates more than 120 enzymes within the

plant, actively managing the opening and closing of stomata and regulating the osmotic stress in the plants. It is also essential in transporting the products of the photosynthesis operation from the leaves as a source to their storage places (Tahir *et al.*, 2023).

The effect of interaction with the foliar application of kinetin and its broader role in encouraging vegetative and floral growth has a reflection through its influences in stimulating cell division, inhibiting apical sovereignty, and enhancing energy production by increasing the activity of the invertase enzyme. Then, it increases the leaf content of glucose phosphate and adenosine phosphate compounds and its role in attracting various inorganic ions and organic molecules, such as sugar, amino acids, and the majority of wood and bark juices to the area where it found (Ahmad and Rabea, 2022).

It also encouraged chlorophyll synthesis and chloroplast development, preventing leaf loss, inhibiting the activity of the Pentose phosphate cycle's dehydrogenase enzyme, and reducing the activity of the

ribonuclease enzyme (Turk *et al.*, 2020). The binding of cytokinin with ribosomes may regulate the process of metabolism and synthesis of protein, which may be the enzymes needed by the cell in the process of division and its entry into the synthesis of t-RNA, as well as increasing the rate of translation of DNA and the construction of proteins (Nasrin *et al.*, 2022).

## CONCLUSIONS

Fertilization should include some of the nutrients required by crop plants. Adding the biofertilizers only without mixing with minerals and plant phyto regulators revealed better performance. The presented results confirmed the response of the chamomile plant to the biostimulant, the addition of NPK fertilizers, and the foliar application of phyto regulator (kinetin) using their appropriate concentrations, which showed the best standards of vegetative, root, and flowering traits and an enhancement in biological compounds in the flower inflorescences.

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