

## Effects of copper sulphate levels on growth, yield, physiological and biochemical characteristics of mugwort (*Artemisia vulgaris* L.) on grey fertile soil in Ho Chi Minh City

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### ABSTRACT

This study was conducted to investigate the influence of copper sulphate ( $\text{CuSO}_4$ ) levels and spraying frequency on growth, yield and some physiological and biochemical characteristics in mugwort (*Artemisia vulgaris* L.) on grey fertile soil in Ho Chi Minh City. The two-factor experiment was arranged in a split-plot design, including nine treatments with three replicates. The main plot factor was three concentrations of  $\text{CuSO}_4$  with 0, 400 and 800 mg/L. The sub-plot factor was the number of sprays including 1 spray (10 days after cutting: DAC), 2 sprays (10 and 20 DAC), 3 sprays (10, 20 and 30 DAC), 4 sprays (10, 20, 30 and 40 DAC). The results indicated that mugwort was treated with copper sulphate at a concentration of 800 mg/L and sprayed three times, the physiological indices, including chlorophyll a and chlorophyll b contents in the leaves, reached the highest values with 9.34 and 4.74 mg/g, respectively. In addition, the highest total flavonoid, polyphenol and essential oil content (6.32 mg RE/g, 8.86 mg GAE/g and 0.18%, respectively) were attained when compared with other copper sulphate concentration. Finally, among the graded levels of  $\text{CuSO}_4$  application, the yield of essential oil increased progressively with increasing levels of  $\text{CuSO}_4$ , obtaining the highest yield of essential oil at the treatment of 800 mg/L  $\text{CuSO}_4$ , reaching 31.7 L/ha.

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## 1. Introduction

Mugwort (*Artemisia vulgaris* L.) is a medicinal herb which is widely used around the world. In addition to medicine, it is used as an ingredient for everyday dishes too. In Traditional Medicine, sun-dried wormwood has a bitter taste, aroma, warm properties. Besides, it also has the effect regulating blood circulation, regulating menstruation, calming pregnancy, and treating rheumatism as claimed by Vo (2012). Moreover, the stems and leaves of wormwood are rich chemical composition, which especially includes flavonoids, polyphenols, sesquiterpene lactones, phenolic acids, essential oils, coumarins, and other groups of metabolites. Recently, this species has been taken under consideration to be active toward the virus SARS-CoV-2 and disease COVID-19 (Efferth et al., 2015; Haq et al., 2020).

Plants require not only macronutrients but also vital micronutrients to grow and complete the life cycle. According to Yruela (2005), copper is a major metal for normal plant growth and development. What's more, it participates in numerous physiological processes and is an essential cofactor for many metalloproteins.

It plays a crucial role in photosynthetic and respiratory electron transport chains, in ethylene sensing, cell wall metabolism, oxidative stress protection and biogenesis of molybdenum cofactor into the bargain. Thus, deficiency in the copper supply can alter essential functions in plant metabolism (Aftab & Hakeem, 2020). Furthermore, Droux (2004) approved sulfur appears in two primary amino-acids, cysteine (Cys) and methionine (Met), essential for the primary and secondary metabolism of the plant.

Foliar nutrition promotes the efficiency of fertilizer use which help plants to absorb nutrients

quickly and reduce the lost of nutrients. The foliar application of nutrients provides a swift and efficient means of facilitating the rapid uptake of essential elements during periods of fast plant. Foliar fertilizer application is a routine measure to improve nutrients status of plants (Mengel, 2001).

The foliar application of  $\text{CuSO}_4$  may be sprayed with the suitable concentrate and number of the sprayed liquid depending on the type of crop planted, weather conditions and growth stage of plant. If the concentration of foliar spray is high, it could cause physiological damage to cell membranes. On the contrary, if the foliar spraying concentration is too low, the plant will not absorb enough nutrients. In addition, foliar sprays many times increases production costs too.

From the above practical needs, research was conducted to determine the appropriate concentration and number of copper sulphate sprays to help mugwort achieve good productivity, quality and high economic efficiency.

## 2. Materials and Methods

### 2.1. Materials

Mugwort plants which used in the experiment were regenerated from the original crop at the Experimental Farm of the Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam. Before spraying copper sulphate to treatments in the experiment, the regenerated plants in all treatments were cut at the same height approximately 4 - 5 cm with 3 - 4 leaves.

Fertilizers were used in the experiment included copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) (Cu: 25.5%, S: 12.8%); Sfarm Pb01 (SF) vermicompost of Dang Gia Trang company (0.98% N, 1.05%  $\text{P}_2\text{O}_5$ , 0.29%  $\text{K}_2\text{O}$ ); Phu My urea fertilizer (46.3% N); Lam Thao superphosphate fertilizer (16%  $\text{P}_2\text{O}_5$ , 10% S, 2 mg Cd/kg); Vietnam General

Corporation of Agricultural Materials potassium sulphate fertilizer (50%  $\text{K}_2\text{O}$ ).

Soils sample was taken and sent to the laboratory at the Research Institute for Biotechnology and Environment (RIBE) to analyze the soil physical and chemical properties.

**Table 1.** Physical and chemical properties of soil sample used in the experiment

| Texture (%) |      |      | $\text{pH}_{\text{KCl}}$ | Organic matter (%) | Total N (%) | Total $\text{P}_2\text{O}_5$ (%) | Total $\text{K}_2\text{O}$ (%) | Cu (mg/kg) | S (%) |
|-------------|------|------|--------------------------|--------------------|-------------|----------------------------------|--------------------------------|------------|-------|
| Sand        | Loam | Clay | 4.6                      | 1.2                | 0.056       | 0.109                            | 0.082                          | 7.83       | 0.12  |
| 58.3        | 31.7 | 10.0 |                          |                    |             |                                  |                                |            |       |

*Data were analyzed by the Research Institute for Biotechnology and Environment in 2004.*

The main characteristics of soil properties were presented in Table 1. The data showed that the soil was classified as a sandy loam texture, highly acidic, low in soil organic matter, total nitrogen, phosphorus and potassium. Therefore, the use of lime would raise the soil pH and improve soil quality by increasing nutrient bioavailability.

**2.2. Experimental design**

The experiment was carried out in the Experimental Research Station at Nong Lam University, Ho Chi Minh City, Vietnam from August to November 2024.

The two-factor experiment was laid out in a split-plot design with three replicates. The main plot was the concentration of copper sulphate, with three levels of 0, 400 and 800 mg/L/spray. The sub-plot was the number of sprays including 1 spray (10 DAC), 2 sprays (10, 20 DAC), 3 sprays (10, 20 and 30 DAC), and 4 sprays (10, 20,

30 and 40 DAC). All treatments were applied the same amount fertilizer with 500 kg lime, 1000 kg vermicompost, 90 kg N, 60 kg  $\text{P}_2\text{O}_5$  and 30  $\text{K}_2\text{O}$  for one ha.

The plot size was 5.4 m<sup>2</sup> (3.0 m in length and 1.8 m in width). The regenerated plants of mugwort were planted at the distance of 30 x 25 cm. The distance between blocks and plots were 1.0 and 0.5 m, respectively.

**2.3. Data collection and analysis**

Growth parameters, yield components, yield and essential oil content were collected randomly from ten plants in the middle three rows of each base plot. In details, the plant height and stem diameter observed at 55 days after cutting (DAC), the number of primary branches before harvesting. Plant height (cm) was measured starting from the base of the stem to the tip of the highest leaf by using ruler; stem diameter (mm) was measured with a diameter tape placed around

the circumference of the stem and at 2 cm away from the base. The average fresh weight of stems and leaves (g/plant) calculated in harvest stage: Measured the average weight fresh of stems and leaves of 10 target plants, cut with a sickle at 3 cm from the ground (P1). Theoretical fresh yield (ton/ha) =  $P1 \text{ (g/plant)} \times 10^{-6} \times \text{planting density (plant/ha)}$ . Actual harvested yield (ton/ha) =  $[\text{weight of fresh stems and leaves of each base plot (kg)} \times 10^{-3} \times 10,000] / \text{area of the base plot (m}^2\text{)}$ .

Determination of polyphenol and flavonoid content were adapted to the method of Atanassova et al. (2011): 500 mg of dried leaf was dissolved in 2.5 mL methanol, shaken for 5 h, and centrifuged. For phenolic content, Folin-Ciocalteu reagent was added, incubated, and measured at 750 nm. For flavonoid content,  $\text{NaNO}_2$ ,  $\text{AlCl}_3$ , and  $\text{NaOH}$  were added to the extract, incubated, and measured at 510 nm.

Chlorophyll a and b were evaluated according to the recommendations of Lichtenthaler (1987) which are based on absorbance measurements at different wavelengths 648 nm and 664 nm. Then, chlorophyll content was calculated using Lichtenthaler's formula: Chlorophyll a (mg/g) =  $13.36A_{664} - 5.19A_{648}$ ; chlorophyll b (mg/g) =  $27.43A_{648} - 8.12A_{664}$ .

For analysis of the essential oil content of the mugwort, 100 g/pot of branches and leaves of 10 target plants, collected at the time point when the plants had buds and begun to bloom, was used. Essential oil content was calculated by the percentage of essential oil obtained from the mass of the starting material after being extracted by steam distillation (Nguyen & Pham, 2001). The calculation was based on the formula at the harvesting stage (65 DAC): Content of essential oil in leaves, stems (%) =  $(\text{volume of essential}$

oil)/(weight of sample)  $\times 100$ . Yield of essential oil (L/ha): Calculated as essential oil content of leaves and stems (mL/100 g)  $\times$  actual fresh yield (ton/ha)  $\times 10^3$ .

For the analysis of the economic efficiency, total cost (VND/ha per crop) = seedlings + fertilizers + pesticides + labor + electricity and water; total revenue (VND/ha per harvest) = essential oil yield (L/ha)  $\times$  retail price (VND/kg); total profit (VND/ha per harvest) = total revenue - total cost, and benefit - cost ratio = total profit/Total cost, were measured.

All variables were subjected to analysis of variance (SPD) using mixed models of R.4.2.2. Differences between treatments were tested using the least significant difference (LSD) test at the probability of 0.05.

### 3. Results and Discussion

#### 3.1. Effect of copper sulphate levels and sprayed frequency on growth of mugwort

The data presented in Table 2 clearly showed that copper sulphate significantly affected plant height (cm) and stem diameter (mm). The highest vegetative growth characters were recorded in 55 DAC with amount of 800 mg/L since the differences between 0 and 400 mg/L were insignificant. The highest mean value of plant height (97.9 cm) was recorded with the treatment of 800 mg/L of  $\text{CuSO}_4$ , while the greatest stem diameter (9.0 mm) was recorded in 400 or 800 mg/L of  $\text{CuSO}_4$ . The lowest mean values of vegetative growth characters were obtained as a result of the control. On the other hand, copper sulphate at 1, 2, 3 or 4 sprays as between the number of copper sulphate sprays and the concentration sprays had not effects on the height and stem diameter of plant.

**Table 2.** Effect of copper sulphate levels and spraying frequency on growth of mugwort at 65 days after cutting

| Parameters                                                              | Treatments |      |      |      |      | Average A         |
|-------------------------------------------------------------------------|------------|------|------|------|------|-------------------|
|                                                                         | (A)        | 1    | 2    | 3    | 4    |                   |
| Plant height (cm)                                                       | Control    | 88.1 | 90.5 | 90.9 | 90.8 | 90.1 <sup>B</sup> |
|                                                                         | 400 mg/L   | 91.9 | 92.9 | 93.0 | 92.2 | 92.5 <sup>B</sup> |
|                                                                         | 800 mg/L   | 96.7 | 97.1 | 99.0 | 98.7 | 97.9 <sup>A</sup> |
|                                                                         | Average B  | 92.2 | 93.5 | 94.3 | 93.9 |                   |
| CV (%) = 4.0; $F_A = 8.7^*$ ; $F_B = 0.5^{ns}$ ; $F_{A*B} = 0.1^{ns}$   |            |      |      |      |      |                   |
| Stem diameter (mm)                                                      | Control    | 7.5  | 7.8  | 7.9  | 7.8  | 7.7 <sup>B</sup>  |
|                                                                         | 400 mg/L   | 8.8  | 8.7  | 9.1  | 9.1  | 9.0 <sup>A</sup>  |
|                                                                         | 800 mg/L   | 8.9  | 9.0  | 9.2  | 9.1  | 9.0 <sup>A</sup>  |
|                                                                         | Average B  | 8.4  | 8.5  | 8.7  | 8.7  |                   |
| CV (%) = 9.7; $F_A = 17.5^*$ ; $F_B = 0.3^{ns}$ ; $F_{A*B} = 0.03^{ns}$ |            |      |      |      |      |                   |

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; \*: significant at 5% level; ns: non significant.

In fact, the application of copper sulphate to mugwort with different concentrations and frequencies did not significantly affect plant height, stem diameter. However, the application of copper sulphate tends to increase some growth parameters compared to the control. Studying on tobacco plant, the treatment of the substrate with 100  $\mu\text{M}$   $\text{CuSO}_4$  led to significant increase in the plant height by 16% (Tugbaeva et al., 2023). Özyazıcı & Bektaş (2021) also showed that the use of  $\text{CuSO}_4$  had a significant impact on growth characteristics, especially plant height and stem diameter of coriander (*Coriandrum sativum* L.).

### 3.2. Effect of copper sulphate levels on physiological and biochemical indicators

The data in Table 3 indicates that spraying 800 mg/L copper sulphate recorded the highest chlorophyll a (8.53 mg/g FW) and chlorophyll

b content (4.33 mg/g FW), which significantly outperformed the control (0 mg/L) and 400 mg/L. The interaction among concentration and spraying as well as spray frequency with copper sulphate was not significant in both chlorophyll a and chlorophyll b content. Notwithstanding, chlorophyll content tended to reduce when plants were sprayed  $\text{CuSO}_4$  for 4 times.

Actually, copper participates in numerous physiological processes and is an crucial cofactor for metalloproteins (Yruela, 2005). Similarly, Saghirzadeh et al. (2019) reported similar results (an 2-fold increase in the total chlorophyll content) where 5, 10 and 25  $\mu\text{M}$  of copper sulphate added to *Artemisia annua* L. Also, this study demonstrates that copper sulphate is also necessary for the formation of chlorophyll in plants (Al-Doori, 2024).

**Table 3.** Effect of copper sulphate levels and spraying frequency on physiological and biochemical indicators

| Parameters                   | Treatments                                                               |                   |                    |                   |                    | Average A          |
|------------------------------|--------------------------------------------------------------------------|-------------------|--------------------|-------------------|--------------------|--------------------|
|                              | (A)                                                                      | 1                 | 2                  | 3                 | 4                  |                    |
| Chlorophyll a content (mg/g) | Control                                                                  | 5.24              | 5.55               | 6.5               | 6.06               | 5.84 <sup>B</sup>  |
|                              | 400 mg/L                                                                 | 6.58              | 6.83               | 7.33              | 7.20               | 6.98 <sup>AB</sup> |
|                              | 800 mg/L                                                                 | 7.92              | 7.92               | 9.34              | 8.95               | 8.53 <sup>A</sup>  |
|                              | Average B                                                                | 6.58 <sup>B</sup> | 6.77 <sup>B</sup>  | 7.72 <sup>A</sup> | 7.41 <sup>AB</sup> |                    |
|                              | CV (%) = 12.28; $F_A = 11.54^*$ ; $F_B = 3.38^*$ ; $F_{A*B} = 0.18^{ns}$ |                   |                    |                   |                    |                    |
| Chlorophyll b content (mg/g) | Control                                                                  | 2.66              | 2.82               | 3.21              | 3.26               | 2.99 <sup>B</sup>  |
|                              | 400 mg/L                                                                 | 3.34              | 3.47               | 3.72              | 3.66               | 3.55 <sup>B</sup>  |
|                              | 800 mg/L                                                                 | 4.02              | 4.02               | 4.74              | 4.54               | 4.33 <sup>A</sup>  |
|                              | Average B                                                                | 3.34 <sup>C</sup> | 3.44 <sup>BC</sup> | 3.89 <sup>A</sup> | 3.82 <sup>AB</sup> |                    |
|                              | CV (%) = 12.51; $F_A = 12.25^*$ ; $F_B = 3.32^*$ ; $F_{A*B} = 0.20^{ns}$ |                   |                    |                   |                    |                    |

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; \*: significant at 5% level; ns: non significant.

On top of that, sulfur is a constituent of the proteinaceous amino acids such as methionine and cysteine, glutathione, vitamins (biotin and thiamine), phytochelatins, chlorophyll, coenzyme A, and S-adenosyl-methionine (Scherer et al., 2008; Li et al., 2020; Nakai & Maruyama-Nakashita, 2020). Consequently, optimal copper sulphate levels enhanced chlorophyll a and chlorophyll b in leaves.

### 3.3. Effect of copper sulphate levels and sprayed frequency on yield of mugwort

Foliar application of  $\text{CuSO}_4$  has many beneficial effects. Our finding indicated the positive effect of low concentration of copper sulphate on dry matter yield.

The results of analysis of variance for yield are presented in Table 4 showed that frequency application as well as its and doses of  $\text{CuSO}_4$

showed non-significant difference in yield measured at 65 days after cutting. Nevertheless, the maximum theoretical and actual fresh yield were observed to be 16.2 and 4.9 tons/ha, respectively with application of 800 mg/L  $\text{CuSO}_4$  which significantly increased to 13.1; 3.1 tons/ha with 0 mg/L  $\text{CuSO}_4$  and 14.7; 4.0 tons/ha, respectively with 400 mg/L  $\text{CuSO}_4$ . Likewise, copper has enhanced the yield of the plants due to providing nutritional requirements (Kovacik et al., 2009).

Additionally, the result can be explained by the deficiency of sulfur could lead to stunted growth of plants and ultimately loss of yield (Narayan et al., 2023). Yet, at higher concentrations (800 mg/L) and spraying for 4 times, fresh yield tended to decrease in dry matter weight of 65-day-old mugwort (Table 4).

**Table 4.** Effect of copper sulphate levels and spraying frequency on theoretical and actual fresh yield of mugwort (65 days after cutting)

| Parameters                                                                        | Treatments |      |      |      |      | Average A         |
|-----------------------------------------------------------------------------------|------------|------|------|------|------|-------------------|
|                                                                                   | (A)        | 1    | 2    | 3    | 4    |                   |
| Theoretical fresh yield (Ton/ha)                                                  | Control    | 12.7 | 13.2 | 12.7 | 13.7 | 13.1 <sup>C</sup> |
|                                                                                   | 400 mg/L   | 14.5 | 13.8 | 15.5 | 15.0 | 14.7 <sup>B</sup> |
|                                                                                   | 800 mg/L   | 15.7 | 16.5 | 16.7 | 16.3 | 16.2 <sup>A</sup> |
|                                                                                   | Average B  | 14.3 | 14.4 | 15.0 | 15.0 |                   |
| CV (%) = 7.6; $F_A = 62.0^{**}$ ; $F_B = 1.0^{ns}$ ; $F_{A \times B} = 0.6^{ns}$  |            |      |      |      |      |                   |
| Actual fresh yield (Ton/ha)                                                       | Control    | 3.1  | 3.2  | 3.1  | 3.1  | 3.1 <sup>C</sup>  |
|                                                                                   | 400 mg/L   | 4.0  | 3.3  | 4.6  | 4.1  | 4.0 <sup>B</sup>  |
|                                                                                   | 800 mg/L   | 4.8  | 5.1  | 5.0  | 4.8  | 4.9 <sup>A</sup>  |
|                                                                                   | Average B  | 3.9  | 3.9  | 4.2  | 4.0  |                   |
| CV (%) = 16.4; $F_A = 56.8^{**}$ ; $F_B = 0.5^{ns}$ ; $F_{A \times B} = 0.7^{ns}$ |            |      |      |      |      |                   |

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; \*\*: significant at 1% level; ns: non significant.

### 3.4. Effect of copper sulphate on essential oil of mugwort

**Table 5.** Effect of copper sulphate levels and spraying frequency on essential oil content and yield of mugwort

| Parameters                                                                        | Treatments |      |      |      |      | Average A         |
|-----------------------------------------------------------------------------------|------------|------|------|------|------|-------------------|
|                                                                                   | (A)        | 1    | 2    | 3    | 4    |                   |
| Essential oil content (%)                                                         | Control    | 0.13 | 0.14 | 0.14 | 0.13 | 0.14 <sup>B</sup> |
|                                                                                   | 400 mg/L   | 0.15 | 0.16 | 0.19 | 0.17 | 0.16 <sup>A</sup> |
|                                                                                   | 800 mg/L   | 0.17 | 0.18 | 0.19 | 0.17 | 0.18 <sup>A</sup> |
|                                                                                   | Average B  | 0.15 | 0.16 | 0.17 | 0.16 |                   |
| CV (%) = 14.0; $F_A = 13.6^*$ ; $F_B = 0.7^{ns}$ ; $F_{A \times B} = 0.1^{ns}$    |            |      |      |      |      |                   |
| Essential oil yield (L/ha)                                                        | Control    | 16.9 | 18.5 | 18.3 | 18.4 | 18.0 <sup>C</sup> |
|                                                                                   | 400 mg/L   | 22.4 | 21.7 | 25.8 | 24.5 | 23.6 <sup>B</sup> |
|                                                                                   | 800 mg/L   | 26.7 | 29.6 | 31.7 | 28.7 | 29.2 <sup>A</sup> |
|                                                                                   | Average B  | 22.0 | 23.3 | 25.3 | 23.9 |                   |
| CV (%) = 16.8; $F_A = 29.1^{**}$ ; $F_B = 1.0^{ns}$ ; $F_{A \times B} = 0.3^{ns}$ |            |      |      |      |      |                   |

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; \*: significant at 5% level; ns: non significant.

Copper sulphate (800 mg/L) significantly increased the essential oil yield to 29.2 L/ha, compared with 18.0 L/ha in the control (improved 62.2% compared to the control). Nonetheless, an excess of this micronutrient may reduce essential oil yield when spraying four times although the interaction between the concentration and spray times of CuSO<sub>4</sub> were not significant difference. Considering the fact that in this study, although the influence of copper sulphate was positive on essential oil content, but essential oil content and essential oil yield were highest at a copper sulphate concentration of 800 mg/L (Table 5).

This result can be explained by the role of sulfur in increasing of herb, oil, and menthol yield. It plays important roles in protein structure and function and serves as a component of various enzyme cofactors, as well as in various metabolic pathways in the body (Ajay et al., 2010). Hence, this may explain the increase in essential oil content and yield in mugwort.

By the same token, Lafmejani et al. (2018) reported that the application of copper significantly increased essential oil content of peppermint (*Mentha piperita* L.). In the same way, copper sulfate increased the essential oil content of *Pelargonium* sp. by 41.9% as compared to the control (Prasad et al., 2008). However, essential oil content of mugwort enhanced 0.04% as compared to 0 mg/L CuSO<sub>4</sub>.

### 3.5. Effect of copper sulphate levels and sprayed frequency on total flavonoids (TFC) and polyphenol content (TPC) of mugwort

Copper sulphate concentrations of 0, 400, and 800 mg/L induced TFC accumulation. The highest TFC of 5.04 mg RE/g dry extract was observed with the 800 mg/L CuSO<sub>4</sub> treatment. While the control and 400 mg/L CuSO<sub>4</sub> treatments resulted in lower total phenolic contents of 3.32 and 4.44 mg RE/g dry extract, respectively. Nonetheless,

the data in Table 6 showed no significant difference in TFC among spraying frequencies as well as CuSO<sub>4</sub> concentration and spraying frequency.

The data presented in Table 6 showed that changes in TPC under foliar CuSO<sub>4</sub> application followed a trend similar to that of TFC. Foliar application of copper sulfate at various concentrations affected TPC in mugwort. The maximum TPC of 8.55 mg GAE/g dry extract was observed with the 800 mg/L CuSO<sub>4</sub> treatment, which significantly increased 1.3 times over control (0 mg/L CuSO<sub>4</sub>) and 1.1 times over the concentration of 400 mg/L CuSO<sub>4</sub>. TPC values ranging from 6.30 to 8.86 mg GAE/g dry extract were obtained in the combination of CuSO<sub>4</sub> concentration and spraying frequency, which were not significantly different from the control. In the same way, no significant differences in TPC were observed among CuSO<sub>4</sub> spraying-frequency treatments.

These results were consistent with a previous study reporting improved TFC and TPC following CuO application to the leaves of lavender plants (*Lavandula stoechas* L.) (Asadollahi et al., 2023). This result can be explained by the role of copper in catalyzing the synthesis of phenolic compounds (Aftab & Hakeem, 2020).

Likewise, sulfur has already been shown to have a strong influence on the formation of S-containing secondary metabolites in vegetables and agricultural crops (Schnug et al., 1995; Hanelauks, 1999). In addition, copper plays an essential role in the biosynthesis of phenols in the plant and a deficiency of copper can lead to a reduction in phenolic compounds (Dicko et al., 2006). On the other hand, copper is an element that plays an essential role in the mitochondrial electron transport system and may influence the production of secondary metabolites in plants (Morimoto et al., 1988).

**Table 6.** Effect of copper sulphate levels and spraying frequency on TFC and TPC of mugwort

| Parameters     | Treatments                                                                           |                   |                    |                   |                    | Average A         |
|----------------|--------------------------------------------------------------------------------------|-------------------|--------------------|-------------------|--------------------|-------------------|
|                | (A)                                                                                  | 1                 | 2                  | 3                 | 4                  |                   |
| TFC (mg RE/g)  | Control                                                                              | 3.53              | 3.33               | 2.94              | 3.46               | 3.32 <sup>C</sup> |
|                | 400 mg/L                                                                             | 3.59              | 4.41               | 5.13              | 4.65               | 4.44 <sup>B</sup> |
|                | 800 mg/L                                                                             | 4.17              | 4.09               | 6.32              | 5.57               | 5.04 <sup>A</sup> |
|                | Average B                                                                            | 3.76 <sup>C</sup> | 3.95 <sup>BC</sup> | 4.80 <sup>A</sup> | 4.56 <sup>AB</sup> |                   |
|                | CV (%) = 18.85; $F_A = 42.86^{**}$ ; $F_B = 3.37^*$ ; $F_{A \times B} = 2.20^{ns}$   |                   |                    |                   |                    |                   |
| TPC (mg GAE/g) | Control                                                                              | 6.30              | 6.58               | 6.90              | 6.78               | 6.64 <sup>C</sup> |
|                | 400 mg/L                                                                             | 7.44              | 7.44               | 8.14              | 7.56               | 7.65 <sup>B</sup> |
|                | 800 mg/L                                                                             | 8.36              | 8.39               | 8.86              | 8.60               | 8.55 <sup>A</sup> |
|                | Average B                                                                            | 7.37              | 7.47               | 7.96              | 7.65               |                   |
|                | CV (%) = 7.55; $F_A = 18.99^{**}$ ; $F_B = 1.87^{ns}$ ; $F_{A \times B} = 0.12^{ns}$ |                   |                    |                   |                    |                   |

Within a group of means, values followed by the same superscript letter are not significantly different at 5% level; \*: significant at 5% level; ns: non significant. TFC: Total flavonoid content; TPC: Total polyphenol content.

### 3.5. Economic efficiency of spraying copper sulphate on mugwort (*Artemisia vulgaris* L.)

**Table 7.** Economic efficiency of different copper sulfate levels and spraying frequencies in mugwort (*Artemisia vulgaris* L.)

| Copper sulfate levels and number of sprays | Essential oil yield | Total costs              | Total  | Profit | P/C ratio |
|--------------------------------------------|---------------------|--------------------------|--------|--------|-----------|
|                                            | (L/ha per harvest)  | (million/ha per harvest) |        |        |           |
| Spray once time with 0 mg/L                | 16.86               | 88.36                    | 118.02 | 29.66  | 0.34      |
| Spray twice times with 0 mg/L              | 18.50               | 88.36                    | 129.5  | 41.14  | 0.47      |
| Spray three times with 0 mg/L              | 18.26               | 88.36                    | 127.82 | 39.46  | 0.44      |
| Spray four times with 0 mg/L               | 18.37               | 88.36                    | 128.59 | 40.23  | 0.46      |
| Spray once time with 400 mg/L              | 22.44               | 91.00                    | 157.08 | 66.08  | 0.73      |
| Spray twice times with 400 mg/L            | 21.67               | 93.63                    | 151.69 | 58.06  | 0.62      |
| Spray three times with 400 mg/L            | 25.77               | 96.26                    | 180.39 | 84.13  | 0.87      |
| Spray four times with 400 mg/L             | 24.47               | 98.9                     | 171.29 | 72.40  | 0.73      |
| Spray once time with 800 mg/L              | 26.68               | 91.03                    | 186.76 | 95.73  | 1.05      |
| Spray twice times with 800 mg/L            | 29.58               | 93.69                    | 207.06 | 113.37 | 1.21      |
| Spray three times with 800 mg/L            | 31.71               | 96.36                    | 221.97 | 125.61 | 1.30      |
| Spray four times with 800 mg/L             | 28.75               | 99.02                    | 201.25 | 102.22 | 1.03      |

Price: 7 million VND per liter of mugwort essential oil;  $\text{CuSO}_4$  price: 134,000 VND/kg; labour cost: 250,000 VND/day; P/C ratio: profit-to-cost ratio.

The data in Table 7 showed that spraying  $\text{CuSO}_4$  at a concentration of 800 mg/L four times, this treatment had the highest total cost (99.02 million VND/ha per harvest). In contrast, the control treatment had the lowest total cost (88.36 million VND/ha per harvest).

Considering the economic efficiency of copper sulfate concentration and spraying frequency based on profit and BCR, copper sulphate gave the highest P/C ratio followed by 1.3 of 800 mg/L concentration with three spraying times (125.61 million VND/ha per harvest). The control treatments were profitable, with profits ranging from 29.66 to 40.23 million VND/ha per harvest and achieved the lowest BCR (0.34 to 0.46).

#### 4. Conclusions

The results of the experiment showed that spraying copper sulfate three times at a concentration of 800 mg/L resulted in the best growth, quality, and yield of mugwort. The growth parameters, including plant height (99.0 cm) and stem diameter (9.2 mm), reached the highest values. Physiological and biochemical indicators, including chlorophyll a, chlorophyll b, flavonoid, polyphenol, and essential oil contents, also reached the highest values comparing to the other copper sulphate concentrations. The potential fresh yield, actual fresh yield and essential oil yield were 23.3 ton/ha; 16.7 ton/ha, and 31.7 L/ha, respectively.

#### Conflict of interest

The authors declare no conflict of interest.

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