Phenolic Content and Antioxidant Activity of *Centella asiatica* L. in Response to Organic and Chemical Fertilizer

Tran Thanh Duc\(^1\), Hai Ly Hoang\(^1\)

**ABSTRACT**

**Background:** *Centella asiatica* L. is a traditional medicinal plant popular in several Asian countries. The cultivation of this herb is facing the problem of overuse of chemical fertilizers and quality deterioration. The aim of this study was to evaluate the effect of organic and non-organic fertilizer on growth, yield and phytochemical content of centella.

**Methods:** The set-up was performed in the field from June to November 2021. The treatments were: no fertilizer (control); inorganic fertilizer (the farmer’s recommended rate: 150 kg N ha\(^{-1}\):50 kg P ha\(^{-1}\): 50 kg K ha\(^{-1}\)); organic compost (10 tones ha\(^{-1}\); inorganic fertilizer (a half of recommendation rate: 75 kg N ha\(^{-1}\):25 kg P ha\(^{-1}\): 25 kg K ha\(^{-1}\)); organic compost (5 tones ha\(^{-1}\)) plus inorganic fertilizer (a half of recommendation rate: 100 kg N ha\(^{-1}\):25 kg P ha\(^{-1}\): 25 kg K ha\(^{-1}\)).

**Result:** The application of fertilizer increased the plant growth, biomass production and total chlorophyll content of centella. Nonetheless, the high amount of inorganic fertilizer led to a reduction in the phytochemical content and antioxidant activity in centella leaf. The combination of organic compost and inorganic fertilizer produced maximum growth and increased the total phenolic content and antioxidant activity.

**Key words:** Centella, Inorganic fertilizer, Organic fertilizer, Phenolic, Phytochemical.

**INTRODUCTION**

*Centella asiatica* L. is a herbaceous, perennial medicinal plant in the family Apiaceae. It has been used to treat a number of diseases such as skin diseases, anemia, nosebleeds, mental illness and dehydration in the traditional medicine system in Asian countries (Prakash et al., 2017). Centella has strong neuroprotective activities, anti-inflammatory, anti-cancer, hepatoprotective, anti-asthmatic, wound healing and anti-oxidative (Byka et al., 2013). Currently, this herb is being grown in many parts of the world because it has a growing demand in cosmetic and pharmaceutical (Kunjumon et al., 2022).

Centella consists of diversity of plant secondary metabolites such as phenolic, flavonoid, terpenes which contributed to their pharmaceutical values. Phenolics are the most abundant secondary metabolites in plant and are known for their antioxidant capacity and obvious effects in preventing certain cancers (Dai and Mumper, 2010). They play a major role in scavenging free radicals by acting as hydrogen or electron donors that stabilize and delocalize unpaired electrons or chelate metal ions, thereby preventing the generation of reactive oxygen species (Huang et al., 2019). The content and quality of secondary compounds in plants vary depending on environmental factors and cultivation techniques (Pant et al., 2021). In which, fertilizer is one of the important factors affecting the yield of phytochemicals and this is a controllable factor (Clemensen et al., 2019). The type of fertilizer directly affects the nutrient content in the plant and indirectly affects physiological processes, including the synthesis of secondary compounds (Hamedi et al., 2022).

The introduction of chemical fertilizers has produced a significant increase in crop yields worldwide and today chemical fertilizers are a necessary part of the farming process. However, the overuse of chemical fertilizers causes many adverse effects on human health and the environment (Savci, 2012). Currently, organic manure can be used as a safer alternative source to chemical fertilizers to improve soil chemical and physical properties, increase soil fertility and promote microbial activity (Shaji et al., 2021). A number of recent studies have shown that the application of organic fertilizers increases the quality of plants through the enhancement the content of secondary compounds in plants such as lettuce (Muscolo et al. 2022), spinach (Machado et al., 2020) and cassava (Omar et al., 2012). Sousa et al. (2008) indicated that higher concentration of phytochemical contents in plant can be explained by the role of organic fertilizers in the biosynthesis which induces the acetate pathway, resulting in higher production of flavonoids and phenolics. Young et al. (2000) also explained that the higher photopathogenic stress in organic farming may cause stress to plant which resulted in the enhancement in phenolics and flavonoid and their antioxidant activity.

\(^1\)University of Agriculture and Forestry, Hue University, Vietnam.

**Corresponding Author:** Hai Ly Hoang, University of Agriculture and Forestry, Hue University, Vietnam.

**Email:** hoanghaily@hueuni.edu.vn

**How to cite this article:** Duc, T.T. and Hoang, H.L. (2023). Phenolic Content and Antioxidant Activity of *Centella asiatica* L. in Response to Organic and Chemical Fertilizer. Indian Journal of Agricultural Research. doi:10.18805/IJARe.AF-780.
Despite numerous studies report the responses of plants to fertilizer, there is limited research available on responses of centella to organic and inorganic fertilizer. The present study therefore determined the influence of organic and inorganic fertilizer on the growth and biomass production of centella and their effect on phytochemical content and antioxidant activity.

**MATERIALS AND METHODS**

The experiment was carried out in the field at Quang Tho commune, Quang Dien district, Thua Thien Hue province, Vietnam from February to November 2021. Centella seeds were provided by Lucky seed company and germinated in trays containing of coconut fiber and sand. At the fourth leaf stage, 14 days after sowing, seedlings were transplanted to the field. The soil presented 1.5% organic matter content, a pH of 7.0, an EC, of 0.250 dS m⁻¹, 40 mg NO₃⁻ kg⁻¹, 160 mg K kg⁻¹, 162 mg P kg⁻¹, 7.57 meq Ca²⁺/100 g and 1.67 meq Mg²⁺.

The experiment design was randomized complete block design with three replicates. The four treatments were carried out: (T1) no fertilizer (control); (T2) inorganic fertilizer (the farmer’s recommended rate; 150 kg N ha⁻¹; 50 kg P ha⁻¹; 50 kg K ha⁻¹); (T3) organic compost (10 tones ha⁻¹); (T4) inorganic fertilizer (a half of recommendation rate; 75 kg N ha⁻¹; 25 kg P ha⁻¹; 25 kg K ha⁻¹); (T5) organic compost (5 tones ha⁻¹) plus inorganic fertilizer (a half of recommendation rate; 100 kg N ha⁻¹; 25 kg P ha⁻¹; 25 kg K ha⁻¹).

The manure was prepared according to the method of Bhattacharya et al. (2017) which contained a mixture of protein rich materials including animal manures, leaves and stems of leguminous plants. Fourteen days prior to transplanting, organic fertilizer was added to the field and mixed with upper 20 cm of the soil. The physicochemical of the compost are presented in Table 1. Chemical fertilizer was added one week after transplanting in three equal fertilizer application (at 7, 14, 21 days after transplanting). The irrigation water presented a low ECₑₚ (0.1 dS m⁻¹).

The plants were harvested at 90 days after transplanting. Plant traits include number of leaf, leaf area, specific leaf area, petiole length, rosette diameter were measured. Fresh weight was determined after harvesting. Petiole and leaves dry weights were measured after oven-drying at 80°C for 3 days.

In order to determine the total chlorophyll content, 100 g of leaf blade was ground in 8 mL 80% acetone (v/v) using a prechilled mortar and pestle. The extractant was filtered and the final volume was made up to 10 mL by adding diluted acetone. The absorbance of the extract was read at 663 and 645 nm on a spectrophotometer and the total chlorophyll content was calculated using the equation of Lichtenthaler (1987).

The total phenolic content was determined following the method of Velioglu et al. (1996). 1 mL of solutions of different extracts was mixed with 5 mL diluted Folin-Ciocalteu reagent and 4 mL of 1 M aqueous Na₂CO₃. The mixtures were left to stand for 20 min then the phenolic content was determined by using colorimetry at 765 nm. The total values of the phenolics were expressed as gallic acid equivalents per milligram (mg GAE g⁻¹ DW). The gallic acid solutions were prepared in methanol:water (50:50,v/v) as 0, 50, 100, 150, 200 and 250 mg mL⁻¹ for standard curve (R² = 0.99).

The free-radical scavenging activity of the extracts was determined by the 1,1-diphenyl-2-pireyl-hydrazyl (DPPH) method (Shimada et al. 1992). The centella extracts were added at different concentrations with volumes equal to the methanolic solution of DPPH (100 μM). The mixture was shaken vigorously and was then left to stand in 15 min. The absorbance was measured at 517 nm as a lower IC50 value corresponding to its higher antioxidant activity. This measurement was repeated three times. The IC50 values denote the concentration of the sample, which was needed in order to scavenge 50% of DPPH free radicals.

Analysis of variance (ANOVA) tests were performed using the Statistical Package for the Social Sciences (SPSS) software version 12. In addition, the F-test was applied to test significance and means were compared using the least significant difference (LSD) test at a 5% probability level.

**RESULTS AND DISCUSSION**

**Plant growth and biomass production**

Both plant growth and biomass production were significantly improved by application of organic and inorganic fertilizer in combination or individually (Table 2). Leaves are the economic parts of the plant that are harvested for use as a vegetable, tea and medicine. In the present study, number of leaves was increased significantly by application fertilizer. The highest number of leaves (29.3) was observed in the integrated treatment of organic and inorganic fertilizer. Treatment with inorganic fertilizer (as farmer’s recommendation rate) increased the leaf number by 33.8% compared to the control while application organic fertilizer increased this value by 20%. A similar trend was recorded in leaf area and specific leaf area. The highest leaf area of 31.4 cm² was observed with the combined organic and chemical fertilizer treatment, whereas a leaf area of only 22.7 cm² was obtained from the control. Centella formed thicker leaves with the application of fertilizers. The highest specific leaf area (324.2 cm² g⁻¹) was noted in the control treatments.

Treatment with combined organic and inorganic fertilizer increased significantly the rosette diameter (21.2 cm) and

<table>
<thead>
<tr>
<th>Table 1: Properties of organic fertilizer.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.5</td>
</tr>
<tr>
<td>EC (dS m⁻¹)</td>
<td>6.0</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>58</td>
</tr>
<tr>
<td>P2O5 (%)</td>
<td>2.5</td>
</tr>
<tr>
<td>K2O (%)</td>
<td>2.9</td>
</tr>
<tr>
<td>CaO (%)</td>
<td>6.5</td>
</tr>
<tr>
<td>MgO (%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>12</td>
</tr>
<tr>
<td>Total carbon</td>
<td>28.5</td>
</tr>
</tbody>
</table>
petiole length of centella over the control however the differences among the rest of the treatments were insignificant. The integrated application of manure and inorganic fertilizer produced the highest fresh weight (113.2 g/plant), followed by the inorganic application (108.4 g/plant), while the control treatment gave the lowest fresh weight (72.1 g/plant). A similar trend was observed for dry weight. The combination of manure and inorganic fertilizer treatment increased the dry weight by 56% over the control.

Fertilization is the most important factor affecting the growth, yield and nutritional value of vegetables (Jaswal et al. 2022). In the current research, the increase in growth and yield of centella in treatments with fertilizer could be related to the increase of availability of nitrogen. According to Lawlor (2002), nitrogen increased cytokinin production, which subsequently affected cell wall elasticity, number of meristematic cells and cell growth. The combined organic and chemical fertilizer treatment showed the greatest fresh and dry yield and promoted the other growth parameters. This effect may relate to the association of microorganisms and the presence of essential nutrients in the organic fertilizer (Table 1). Previous research reported the application of organic fertilizer reduced soil acidification, increased soil organic matter contents, improve soil physicochemical properties and microbial community and enhanced soil metabolism (Song et al. 2022; Jiang et al. 2022). These changes often resulted in improving plant growth and yield (Wang et al. 2022; Li et al. 2022). This result opened up a potential to use of compost as an alternative of inorganic nitrogen application to reduce the greenhouse gas emissions in cultivation (Qaswar et al., 2019).

**Total chlorophyll content**

Total chlorophyll content was significantly effect by the treatments (Fig 1). Highest total chlorophyll content was observed under treatment with combined organic fertilizer and inorganic fertilizer, followed by of the plants grown under treatment inorganic fertilizer.

Chlorophyll content is an important factor in assessing photosynthetic activity in plants (Taïbi et al. 2016). Therefore, in this study, the effect of nutrient sources on plant photosynthesis was evaluated by determining the photosynthetic pigment content. The increase in total chlorophyll content was due to the increase in nitrogen content in the fertilization treatments. Previous studies have shown a close relationship between chlorophyll content and nitrogen content in fertilizers (Hoang et al. 2023). Nitrogen is a factor in chlorophyll biosynthesis because the chlorophyll molecule contains nitrogen. The content of nitrogen supply may be associated with an increase in stromal and thylakoid protein in leaves (Heidari et al. 2012) to induce the synthesis of chlorophyll pigment.

**Table 2:** Growth and yield of *Centella asiatica* L. as affected by organic and inorganic fertilizer.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of leaves</th>
<th>Leaf area (cm²)</th>
<th>Rosette diameter (cm)</th>
<th>Specific leaf area (cm² g⁻¹)</th>
<th>Petiole length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.5d*</td>
<td>22.7d</td>
<td>13.5c</td>
<td>324.2a</td>
<td>6.3c</td>
<td>72.1d</td>
<td>16.42c</td>
</tr>
<tr>
<td>Inorganic fertilizer</td>
<td>26.1b</td>
<td>29.8b</td>
<td>20.5ab</td>
<td>253.2b</td>
<td>8.1ab</td>
<td>108.4b</td>
<td>19.5b</td>
</tr>
<tr>
<td>Organic fertilizer</td>
<td>23.5c</td>
<td>24.8c</td>
<td>19.8ab</td>
<td>190.4c</td>
<td>9.4ab</td>
<td>90c</td>
<td>19.8b</td>
</tr>
<tr>
<td>1/2 Inorganic fertilizer</td>
<td>24.6c</td>
<td>27.1c</td>
<td>20.3ab</td>
<td>182.7c</td>
<td>9.0ab</td>
<td>98.4c</td>
<td>19.6b</td>
</tr>
<tr>
<td>1/2 Organic fertilizer +</td>
<td>29.3a</td>
<td>31.4a</td>
<td>21.2a</td>
<td>180.4c</td>
<td>10.2a</td>
<td>113.2a</td>
<td>25.7a</td>
</tr>
</tbody>
</table>

*Value with the common letter in the same column are not significantly different using LSD at the 5% level.

**Fig 1:** Total chlorophyll content (mg g⁻¹ FW) of *Centella asiatica* L. as affected by organic and inorganic fertilizer level.
Phenolic Content and Antioxidant Activity of *Centella asiatica* L. in Response to Organic and Chemical Fertilizer

**Phytochemical content**

Various fertilizer sources affected total phenolic and antioxidant activity of centella (Fig 2). The highest total phenolic contents were obtained in the plants treated with organic fertilizer, followed by the combined organic and inorganic fertilizer. Organic fertilizer treatment increased total phenolic content by 19.5% over the control whereas inorganic fertilizer treatment reduced this value by 25%. Similar response was also obtained in antioxidant activity (Fig 3). The greatest antioxidant activity was obtained at organic fertilizer treatment with a 15% increase compared to the control. The decrease of antioxidant activity in treatments with inorganic fertilizer was 19.8%.

According to the carbon/nutrient balance hypothesis, excess photosynthetic carbon is channeled into secondary phenolic compounds under low nutrient status and poor environment conditions (Clemensen et al., 2019). It was noticed that the highest values of total phenolic content was observed in treatment with organic fertilizer. Organic fertilization stimulated the accumulation of phenolics in centella leaves by inducing the acetate shikimate pathway, resulting in higher production of phenolics (Sousa et al., 2008). In addition, the higher photo-pathogen stress in organic farming may cause abiotic stress for plants and they induced the accumulation of phenolics (a non-enzyme antioxidant) as a sign of plant defense system (Chowdhary et al., 2022). Total phenolic was found to be highly correlated with antioxidant activity. Phenolic compounds can scavenge free radicals and other oxidative species (Zhang et al. 2022) and this contributes to the antioxidant activity of centella.

**CONCLUSION**

The inorganic fertilizers increased significantly plant growth and yield however it caused a reduction in the phytochemical content of *Centella asiatica*. The integrated application of organic and inorganic fertilizer produced maximum biomass production and increased phytochemical content of the herb. This treatment should be considered as the optimal amount for reconciling limited yield loss and maintaining the quality of centella. This could be suitable to reduce inorganic application in centella production. However, further study needed to evaluate the effect of fertilizer treatments on the field over a long period of time.
ACKNOWLEDGEMENT
We are grateful to Ministry of Education and Training (Grant numbers B2020-DHH-03) for the financial support to conduct this research.

Conflict of interest: None.

REFERENCES


