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

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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 famer'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 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 (Bylka *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 safer alternative source tochemical 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 fertilizersincreases 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.

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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_e of 0.250 dS m^{-1}, 40 mg NO_3^- kg $^{-1}$, 160 mg K kg^{-1}, 162 mg P kg^{-1}, 7.57 meq Ca^{2+}/100 g and 1.67 meq Mg^{2+}.

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 famer'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 EC_w (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*. (1998). 1 mL of solutions of different extracts was mixed with 5 mL diluted Folin-Ciocalteu reagent and 4 mL of 1 M aqueous Na_2CO_3. 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 equivalens 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-picryl-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 famer'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 cm^2 was observed with the combined organic and chemical fertilizer treatment, whereas a leaf area of only 22.7 cm^2 was obtained from the control. Centella formed thicker leaves with the application of fertilizers. The highest specific leaf area (324.2 cm^2 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 1: The physiochemical properties of organic fertilizer.

pH	6.5
EC (dS m^{-1})	6.0
Organic matter (%)	58
P2O5 (%)	2.5
K2O (%)	2.9
CaO (%)	6.5
MgO (%)	0.4
Moisture (%)	12
Total carbon	28.5

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

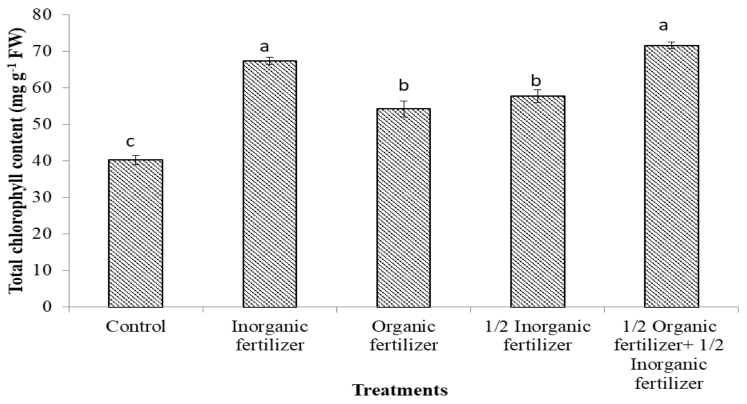
Total chlorophyll content was significantly effect by the treatments (Fig 1). Highest total chlorophyll content was observed under treatment with combined organic fertilizer and inorganic fertilizer, followed by of the plants grown under treatment inorganic fertilizer.

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.

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

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



Values with a common letter in the same column are not significantly different using LSD at 5% level (FW: Fresh weight).

Fig 1: Total chlorophyll content (mg g^{-1} FW) of *Centella asiatica* L. as affected by organic and inorganic fertilizer level.

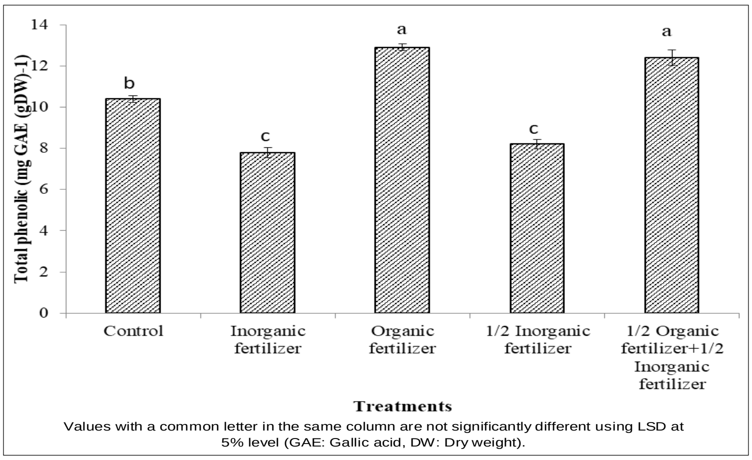


Fig 2: Total phenolic content of *Centella asiatica* L. as affected by organic and inorganic fertilizer level.

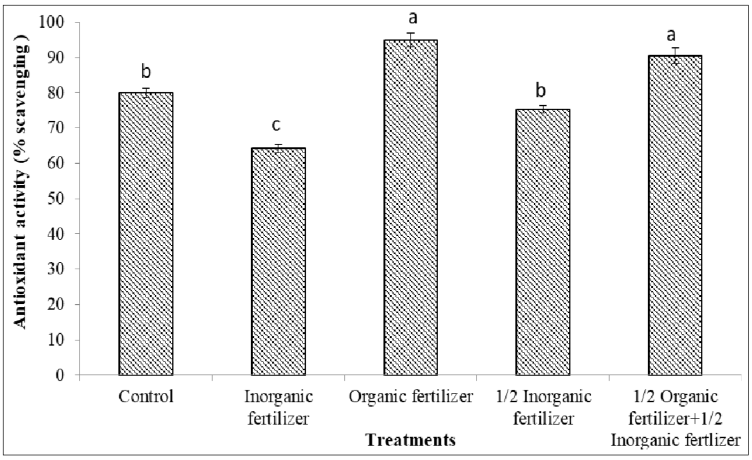


Fig 3: Antioxidant activity of *Centella asiatica* L. as affected by organic and inorganic fertilizer level.

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 fertilizerproduced 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.

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Conflict of interest: None.

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